Oecologia (2003) 136:205–212
DOI 10.1007/s00442-003-1263-y

ECOPHYSIOLOGY

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δ^{13}C and water-use efficiency in Australian grasstrees and South African conifers over the last century

Received: 16 January 2002 / Accepted: 22 March 2003 / Published online: 1 May 2003

Abstract Annual or biannual time courses of plant δ^{13}C ($\delta^{13}C_p$) over the last century (70–100 years) were recorded for leafbases of four grasstrees (*Xanthorrhoea preissii*) at four sites in mediterranean Australia and wood of four conifers (*Widdringtonia cedarbergensis*) at two sites in mediterranean South Africa. There was a strong downward trend of 2–5.5‰ from 1935 to 1940 to the present in the eight plants. Trends were more variable from 1900 to 1940 with plants at two sites of each species showing an upward trend of 1–2.5‰. Accepting that δ^{13}C of the air ($\delta^{13}C_a$) fell by almost 2‰ over the last century, the ratio of leaf intercellular CO$_2$ to atmospheric CO$_2$ (c_i/c_a) rose in five plants and remained unchanged in three over that period. Changes in c_i/c_a rather than $\delta^{13}C_a$ were more closely correlated with changes in $\delta^{13}C_p$ and accounted for 6.7–71.8% (22.6c_i/c_a) and 28.2–93.3% ($\delta^{13}C_a$) of the variation in $\delta^{13}C_p$. We doubt that possible changing patterns of rainfall, water availability, temperature, shade, air pollution or clearing for agriculture have contributed to the overall trend for c_i/c_a to rise over time. Instead, we provide evidence (concentrations of Fe and Mn in the grasstree leafbases) that decreasing photosynthetic capacity associated with falling nutrient availability due to the reduced occurrence of fire may have contributed to rising c_i/c_a. Intrinsic water-use efficiency (W_i) as a function of (c_a–c_i) usually increased linearly over the period, with the two exceptions explained by their marked increase in c_i/c_a. We conclude that grasstrees may provide equivalent $\delta^{13}C_p$ and W_i data to long-lived conifers and that their interpretation requires a consideration of the causes of variation in both c_i/c_a and $\delta^{13}C_a$.

Keywords δ^{13}C · Greenhouse (CO$_2$) effect · Long-term studies · Nutrient availability · Photosynthetic capacity · Rainfall

Introduction

Use of $^{12}CO_2$ is favoured over $^{13}CO_2$ in photosynthetic carbon fixation (Farquhar et al. 1982). Physical discrimination (fractionation) against $^{13}CO_2$ occurs when CO$_2$ diffuses from the atmosphere through the stomates and intercellular spaces to the carboxylation sites within the chloroplasts. Further chemical discrimination favours $^{12}CO_2$ at the carboxylation sites in the chloroplast matrix where reduction of CO$_2$ to phosphoglycerate occurs. Isotopic discrimination measurements can be expressed by the quantity delta, δ. For the plant, $\delta^{13}C_p$ is given by:

$$\delta^{13}C_p = [\delta^{13}Ca + a - (a-b)\cdot c_i/c_a] \qquad (1)$$

(Farquhar et al. 1982), where $\delta^{13}C_a$ is discrimination in air compared with a standard (see below), a is the coefficient of diffusion discrimination (4.4‰), b is the coefficient of carboxylation discrimination (27.0‰), and c_i/c_a is the ratio of intercellular CO$_2$ to atmospheric CO$_2$ concentration (which varies).

There has been a gradual increase in atmospheric CO$_2$ levels (c_a) since the start of the industrial revolution. Since fossil fuels are of plant origin, they are enriched in ^{12}C (Flanagan and Ehleringer 1998). This means that $\delta^{13}C_a$ has been declining (Friedli et al. 1986, Fig. 1a). Work on tree rings in the Northern Hemisphere over the last hundred years or more has shown an overall decline in $\delta^{13}C_p$ (Freyer 1979; Stuiver et al. 1984; Feng 1999). Since the associated decline in $^{13}C_a$ over this time is well established, it is this component of Eq. 1 that has usually been held responsible for the fall in $\delta^{13}C_p$. This assumes that any change in c_i/c_a has been negligible (Fig. 1b). We examined this assumption here.

Where photosynthetic capacity is not limiting, c_i will increase at a lower rate (or remain constant) than the

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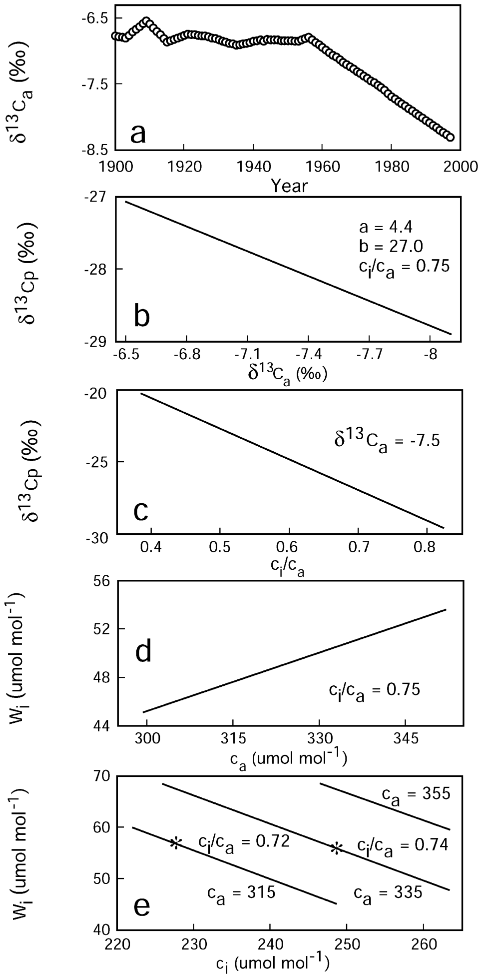

Fig. 1. a $\delta^{13}C_a$ over time from Friedli et al. (1986) and extrapolated from 1977 based on data for 1956–1977, **b** $\delta^{13}C_p$ vs $\delta^{13}C_a$ for constant c_i/c_a, **c** $\delta^{13}C_p$ vs c_i/c_a for constant $\delta^{13}C_a$, **d** W_i vs c_a at constant c_i/c_a, and **e** W_i vs c_i at three levels of c_a, with stars set at W_i = 55 and show how W_i may change as c_a and c_i increase. See Appendix for calculations

increase in c_a, with a reduction in c_i/c_a and a rise in $\delta^{13}C_p$ (Fig. 1c). However, if mineral nutrients for example are in short supply, fixation rates may be limited, c_i/c_a will rise, and $\delta^{13}C_p$ will fall. Thus, c_a has both predictable (on $\delta^{13}C_a$) and unpredictable (on c_i) effects on $\delta^{13}C_p$ over time. Note that at constant c_i/c_a, $\delta^{13}C_p$ increases linearly with increasing $\delta^{13}C_a$ (slope =1, Fig. 1b). At constant $\delta^{13}C_a$, $\delta^{13}C_p$ increases linearly with decreasing c_i/c_a (slope $=a-b$ =22.6, Fig. 1c). These slope values indicate that $\delta^{13}C_p$ is far more sensitive to variation in c_i/c_a than to variation in $\delta^{13}C_a$

A physiological attribute related to isotope discrimination is the ratio of net assimilation rate to transpiration rate, or water-use efficiency (W) (Farquhar and Richards 1984). A rise in W can be due to a fall in stomatal conductance, say from a reduction in water availability (Pita et al. 2001). Here, c_i is drawn down strongly relative to c_a, c_i/c_a declines and thus $\delta^{13}C_p$ rises (Eq. 1). Where stomatal conductance remains constant but c_a increases, W should also increase. Since we are specifically interested in the latter case, there is merit in defining W as a function of ($c_a - c_i$) (Ehleringer et al 1993, see Appendix for estimating c_a, c_i and W). Since this expression assumes that transpiration is constant, it is more correctly termed 'intrinsic' water-use efficiency (W_i). This yields the expected increase in W for an increase in c_a, but constant c_i/c_a (Fig. 1d), and an increase in W for a declining c_i/c_a and fixed c_a (Fig. 1e). Recent tree ring work in the North Hemisphere has shown an increase in W_i over time associated with the increasing c_a (Bert et al. 1997; Duquesnay et al 1998; Feng 1999).

Grasstrees in Australia are arborescent monocotyledons in the genera *Xanthorrhoea, Kingia* and *Dasypogon* (Liliales) that survive for centuries, even in the presence of frequent fire (Lamont and Downes 1979; Staff and Waterhouse, 1981). They consist of a simple or branched caudex (stem) surrounded by dead leaves and a terminal spray of dark green leaves. The needle-like leaves, often exceeding 1 m in length, progressively die off in *Xanthorrhoea* as the grasstree grows and bend downwards to form a skirt that is burnt back to the leafbases during fire. Grinding off the charred surface of the persistent leafbases reveals seasonal banding—regular cream and brown bands 1–2 cm in the vertical plane (spring/summer and autumn/winter growth, respectively), with occasional black bands replacing the cream or brown bands due to the effect of fire (Colangelo et al. 2002). This technique has recently been used to determine the fire history of *X. preissii* in mediterranean Australia over the past 250 years (Ward et al. 2001; Lamont et al. 2003).

We thought that the phenomenon might also be able to provide data on $\delta^{13}C_p$ levels through time, equivalent to tree rings, except that the rings of leafbases run up the stem rather than concentrically; they are much more convenient to sample as they are an order of magnitude larger and on the outside of the stem. We therefore removed leafbases in successive colour bands from 1900 onwards and analyzed their $\delta^{13}C_p$ levels. Since fire rejuvenates the nutrient content of the leaves (Ward et al. 2001) we analyzed leafbases of unburnt and frequently burnt plants for Fe and Mn (required in photosynthesis but immobile and poorly leachable) at the same time. To increase the generality of our study, the grasstree data were compared with $\delta^{13}C_p$ data for annual tree rings of *Widdringtonia cedarbergensis* (Cupressaceae), a conifer endemic to the Cedarberg Mountains near Cape Town, South Africa. This provided equivalent data using the new and traditional techniques, for two mediterranean regions on either side of the Indian Ocean, both in the Southern Hemisphere and subject to the same air circulation system. We interpreted the results in terms of expected (Feng 1999) or observed changes in $\delta^{13}C_a$, c_i/c_a, rainfall, and internal Fe and Mn (as they might affect c_i/c_a) over the last century. We calculated W_i and compared the results with those for North American conifers (Feng 1999).

Materials and methods

Stable carbon isotope ratios ($\delta^{13}C_p$) of leaf bases were determined along a *Xanthorrhoea preissii* stem harvested from a site not burnt since 1931 (Amphion unburnt) near Dwellingup, southwestern Australia, and another in an adjacent block subjected to the usual fire management regime for eucalypt forest (Ward et al. 2001) (Amphion burnt 1931–1997). A third plant was obtained from a regularly burnt site at Gyngoorda between Bannister and Boddington (1900–1987). A fourth also subjected to frequent fires was collected from Boonerring also near Dwellingup (1900–1997). All plants were at least 100 years old by 1900. While *X. preissii* never stops growing, leaf production is least in autumn/winter, and leaves take a year to stop elongating (D. Korczynskyj, personal communication). Since leafbases are the last part of the leaf to mature, we assume that their C arises from both self and previous leaf growth.

The outermost, longitudinal 1-cm sections of leafbases comprising the autumn/winter (brown), and sometimes the spring/summer (cream) and fire (black) bands, were removed sequentially from each stem, after melting the surrounding resin with a heat gun. Each sample contained 3–6 leafbases per colour type. Resin was removed with laquer thinner from the leafbase material that was then dried to constant weight at 70–80°C. The leaf base material was milled with a ball mill (Retsch MM-2, Haan, Germany) and analyzed for $^{13}C/^{12}C$ using a Roboprep connected to a Tracermass isotope ratio spectrometer (Europa Scientific, Crewe, UK) at the Biogeochemistry Centre, University of Western Australia. Samples were compared with a secondary reference sample of *Acacia pulchella* leaves (50.10% C) standardised against primary analytical standards from the International Atomic Energy Agency in Vienna. Analytical variance in the reference never exceeded 0.2‰. Plants were aged relative to the last known fire corresponding to the uppermost black band and all cream-brown bands were treated as annual increments (Ward et al. 2001; Colangelo et al. 2002; Lamont et al. 2003).

In southwestern South Africa, Dunwiddie and La Marche (1980) reported on an annual ring width index chronology for *Widdringtonia cedarbergensis* in the Cedarberg Mountains. This chronology was derived from 52 radii of 32 trees from Die Bos. An average of two cores from each of six of these trees, all of which had previously been aged by Dunwiddie and La Marche (1980), were selected for stable carbon isotope analysis from 1890 to 1976 (February and Stock 1999). For ease of comparison, this is considered as one plant in the rest of the text. Three trees of *W. cedarbergensis* were harvested from Krakadouw (here called 38, 39 and 40) and treated in the same way for 1900–1994. All of the Die Bos trees were 150–250 years old by 1900, while the Krakadouw trees established in 1898. All but two of the conifers were between 150 and 250 years old by 1900. The individual rings were removed from the cores with a scalpel under a stereomicroscope. Samples from corresponding annual rings for the six trees at Die Bos were combined for analysis but the Krakadouw samples were kept separate. *W. cedarbergensis* grows in spring-summer and we assumed that wood C arose from current growth.

The sodium chlorite delignification technique of Leavitt and Danzer (1993) was used to extract holocellulose from wood. See February and Stock (1999) for further preparation details. Stable carbon isotope analysis of the conifer samples was carried out in the Archaeometry laboratory at the University of Cape Town on a Micromass 602E spectrometer. The standard was a laboratory reference gas related to the Chicago PDB marine carbonate standard calibrated against six NBS reference standards (Sealy 1986). The $^{13}C/^{12}C$ ratio was expressed relative to the standard using the formula, $\delta=[(R_{sample}/R_{standard})-1]\times1,000$, where R signifies the isotopic ratio. For three grasstree stems, two ratios per year (autumn/winter and spring/summer) were determined for at least part of the total time sequence along the stem.

$\delta^{13}C_a$ values for 1900–1977 were obtained from Friedli et al. (1986) collated from various parts of the world and the straight part of the relationship for 1955–1977 extrapolated to 1997 (Fig. 1a, Appendix). Rainfall for the wettest and driest 6 months per year was obtained for Dwellingup and Bannister-Boddington since 1930 from the Western Australian Bureau of Meteorology. Annual rainfall was obtained from the Weather Bureau, Department of the Environment, Pretoria for Algeria (1900–1997) and Wupperthal (1900–1977). These were plotted against time and any linear trends noted. Knowing $\delta^{13}C_p$, $\delta^{13}C_a$, a and b, the ratio c_i/c_a was calculated (Appendix). These were plotted against time and any trends noted. The linear correlation coefficients between $\delta^{13}C_p$ and $\delta^{13}C_a$ and $\delta^{13}C_p$ and c_i/c_a were determined. The slopes (b) of the regression lines against time for $\delta^{13}C_p$ and $\delta^{13}C_a$ were compared: 'apparent' contribution of c_i/c_a to $\delta^{13}C_p$ was given by the expression $X=[(b_p-b_a)/b_p]/(a-b)$ and of $\delta^{13}C_a$ by $1-X$. 'Actual' contribution of c_i/c_a to $\delta^{13}C_p$ was given by $(b_p-b_a)/b_p$ and of $\delta^{13}C_a$ by b_a/b_p.

In an attempt to explain variations in c_i/c_a at the level of c_i rather than c_a, concentrations of Fe and Mn [highly immobile once deposited (Marschner 1995) and strongly resistant to leaching from dead grasstree tissues (R. Wittkuhn, unpublished data)] were determined on the same material as for $\delta^{13}C_p$ for the Amphion (unburnt) and Gyngoorda grasstrees. Material was digested with concentrated $HNO_3/HClO_3$ and analyzed by inductively-coupled plasmascan-atomic emission spectroscopy at the Chemistry Centre, Perth. Concentrations were plotted against time and c_i/c_a. W_i was calculated as $0.625(c_a-c_i)$ (Ehleringer et al. 1993), where c_a was obtained from a best-fit curve to data from Mauna Loa, Pacific Ocean (Friedli et al. 1986) and c_i from Eq. 1 (Appendix), plotted against time and any linear trends noted. Finally, all records for which $\delta^{13}C_p$, $\delta^{13}C_a$, rainfall, c_i/c_a and W_i were complete at any time (67 years) were combined for the four grasstrees, and separately for the four conifers. These were subjected to (geometric) partial correlation coefficient analysis as the starting point for principal components analysis (Podani 1995).

Results

$\delta^{13}C$ for the eight plants trended downwards, especially since 1930–1940 (Fig. 2). The Gyngoorda grasstree trended downward from 1900, although the Amphion (burnt) and Boonerring plants and the Krakadouw 40 and Die Bos conifers trended upwards to 1940 before falling. There was no consistent seasonal (colour band) trend for the most comprehensive data sets, although autumn/ winter-initiated leafbases had higher $\delta^{13}C_p$ than spring/summer bases in the Amphion (burnt) grasstree (Fig. 2c).

Wet season rainfall fell substantially over time at one grasstree station but was not significant at the station used for the other three (Table 1). Annual rainfall rose markedly for the station used for the Krakadow plants but there was no trend for the other. There was a rise in c_i/c_a for five of the eight plants while the other three showed no trend (Table 2). Fe and Mn concentrations fell over time in the Amphion (unburnt) grasstree but trends were insignificant (Fe) or negligible (Mn) in the Gyngoorda plant (Fig. 3a, b). The c_i/c_a fell as Fe and Mn concentrations increased for the unburnt grasstree but there were no relationships for the burnt tree (Fig. 3c, d).

All but the die Bos $\delta^{13}C_p$ values were significantly correlated with $\delta^{13}C_a$ (with c_i/c_a as the other independent variable) and the level of correlation was $\geq c_i/c_a$ for six of the eight trees (Table 3). Variation in $\delta^{13}C_a$ accounted for 82.7–99.7% of variation in $\delta^{13}C_p$ and c_i/c_a accounted for 0.5–17.3% of variation in $\delta^{13}C_p$. Since the contribution of c_i/c_a to $\delta^{13}C_p$ is multiplicative [$by(b-a)$=22.6 in Eq. 1] while $\delta^{13}C_a$ is additive, the contribution of c_i/c_a to $\delta^{13}Cp$

Table 1 Best fit lines to annual rainfall (mm) for four closest stations to collection sites for 60–100 years in the period 1900–2000. Dwellingup (closest to Amphion and Boonerring) Bannister-Boddington (Gyngoorda), Algeria (Krakadouw) and Wupperthal (Die Bos)

Station	Slope	Intercept	r^2 (%)	*P*
Dwellingup	–2.48	5, 834	4.0	0.1242
Bannister-Boddington (Gyngoorda)	–2.00	4, 409	10.1	0.0093
Algeria (Krakadouw)	2.99	–5, 125	17.0	<0.0001
Wupperthal (Die Bos)	–0.73	1, 649	3.6	0.0971

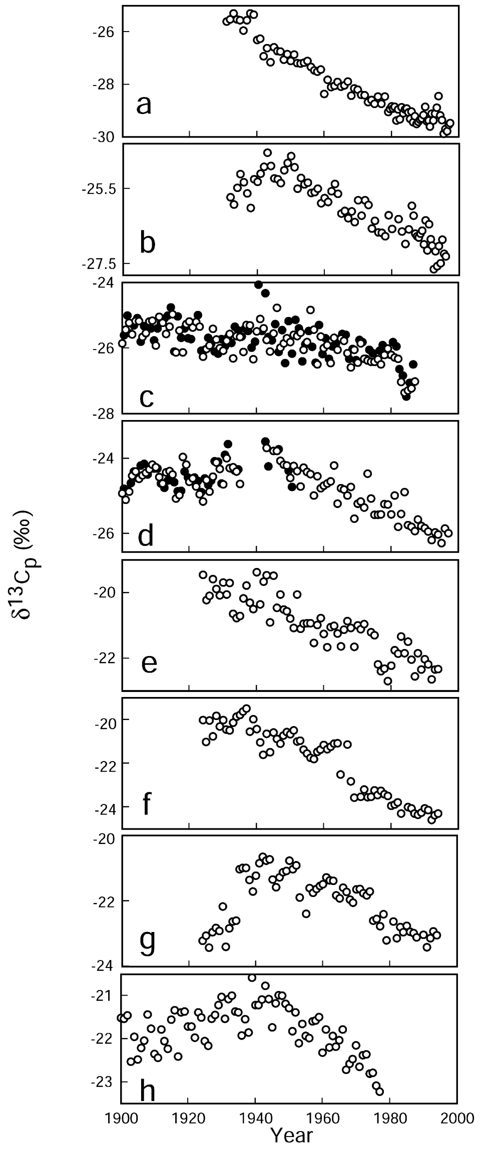

Fig. 2 $\delta^{13}C_p$ vs time for grasstrees at **a** Amphion (unburnt), **b** Amphion (burnt), **c** Gyngoorda (burnt), and **d** Boonerring (burnt) in southwestern Australia, and for conifers at **e** Krakadow (tree 38), **f** Krakadow (tree 39), **g** Krakadow (tree 40), and **h** Die Bos in South Africa. *Open circles* are samples from leaves initiated in autumn-winter, and *filled circles* in spring-summer

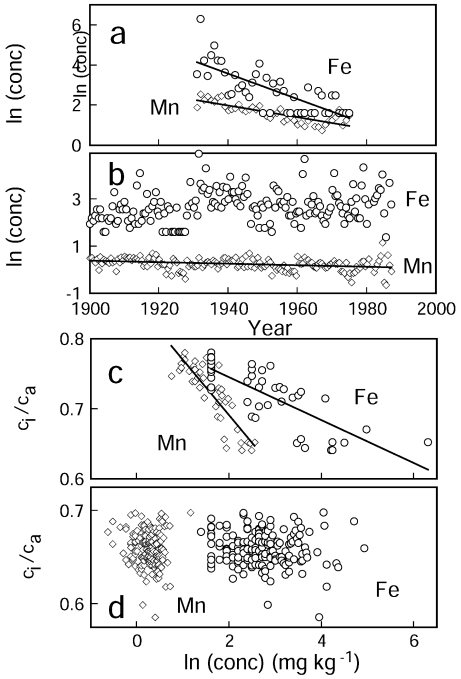

Fig. 3 Log$_e$ Fe and Mn concentration (mg kg^{-1}) vs time for **a** Amphion unburnt grasstree (Fe: Y=113.9–0.057X, r^2=53%, P<0.0001, Mn: Y=46.4 –0.023X, r^2=46%, P<0.0001) and **b** Gyngoorda, (Fe: P>0.05, Mn: Y=8.43–0.004X, r^2=8%, P=0.002), and c_i/c_a vs log$_e$ Fe and Mn concentrations for **c** Amphion (Fe: Y=0.81–0.031X, r^2=62%, P<0.0001, Mn: Y=0.84–0.069X, r^2=59%, P<0.0001) and **d** Gyngoorda (Fe, Mn: P>0.05)

Table 2 Best fit lines to calculate c_i/c_a from Eq. 1, Figs. 1a, 2 and 3. Trees as in Fig. 2

Tree	Slope (×10^2)	Intercept	r^2 (%)	*P*
a	0.17	–2.64	73.9	<0.0001
b	0.04	–0.15	21.5	<0.0001
c	0.01	–0.49	1.4	0.1156
d	0.00	0.65	0.2	0.6613
e	0.09	–1.25	43.4	<0.0001
f	0.24	–4.18	81.8	<0.0001
g	0.09	–1.39	56.5	<0.0001
h	–0.01	0.60	0.4	0.5815

increased to 7.7–71.8% while $\delta^{13}C_a$ fell to 28.2–93.3% (Table 3). W_i rose for five plants, remained unchanged for one, and fell for two (those with the greatest increase in c_i/c_a) over time (Table 4).

The correlation matrix shows that c_i/c_a was more strongly correlated (negatively) with $\delta^{13}C_p$ than was $\delta^{13}C_a$ (positively) (Table 5). Annual winter rain was strongly correlated with that of rainfall summed with the 12-month rainfall on either side (i.e. three winters) and less strongly with year. Both were unrelated to $\delta^{13}C_p$ or c_i/c_a although c_i/

Table 3 Best-fit lines toW_i (μmol mol^{-1}) calculated from Appendix and data for Table 2. Trees as in Fig. 2

Tree	Slope (×10^2)	Intercept	r^2 (%)	P
a	–0.223	492.0	53.1	<0.0001
b	0.081	–87.5	20.5	<0.0001
c	0.099	–125.9	32.1	<0.0001
d	0.144	–202.6	68.0	<0.0001
e	0.073	–28.3	11.1	0.0049
f	–0.256	607.2	57.3	<0.0001
g	0.039	33.1	5.6	0.0476
h	0.171	–230.4	47.8	<0.0001

c_a was weakly correlated with year. W_i showed a strong positive relationship with $\delta^{13}\mathrm{C}_\mathrm{p}$ and strong negative relationship with $c_\mathrm{i}/c_\mathrm{a}$. For the four conifers collectively, $c_\mathrm{i}/c_\mathrm{a}$ was more strongly correlated (negatively) with $\delta^{13}\mathrm{C}_\mathrm{p}$ than was $\delta^{13}\mathrm{C}_\mathrm{a}$ (positively). Annual rainfall was correlated with year but was unrelated to $\delta^{13}\mathrm{C}_\mathrm{p}$ or $c_\mathrm{i}/c_\mathrm{a}$, while $c_\mathrm{i}/c_\mathrm{a}$ was correlated with year. W_i showed a strong positive relationship with $\delta^{13}\mathrm{C}_\mathrm{p}$ and strong negative relationship with $c_\mathrm{i}/c_\mathrm{a}$.

Discussion

From 1935 to 1940 to the present, there was a clear downward trend of 2–5.5‰ in $\delta^{13}\mathrm{C}_\mathrm{p}$ for the eight plants. The trend was less clear for 1900 to 1940, partly because records for five plants only started from 1920–30. Nevertheless, four plants showed an upward trend of 1–2.5‰ from 1900–30 to 1940 and one a slight fall of 0.5‰. These trends agree with many of the conifers reported for Europe and the west coast of North America (Stuiver et al. 1984; Leavitt and Long 1992; Feng and Epstein 1995) although their rates were lower, 1–3‰ (Francey and Farquhar 1982; Feng 1999). However, while most showed a downward trend from 1940, few showed a clear increase for 1900 to 1940. Conifers in Tasmania, SE Australia, showed no overall change in $\delta^{13}\mathrm{C}_\mathrm{p}$ for the period analyzed (Pearman et al. 1976).

All these trends might be explained by local phenomena (e.g. change in precipitation) or a combination of local and global phenomena (e.g. decreasing $\delta^{13}\mathrm{C}_\mathrm{a}$). For those with a dominant downward trend the most obvious likely cause is the $\delta^{13}\mathrm{C}_\mathrm{a}$ component of $\delta^{13}\mathrm{C}_\mathrm{p}$ (Fig. 1a). Only one site (Die Bos) did not show a strong positive correlation between $\delta^{13}\mathrm{C}_\mathrm{p}$ and $\delta^{13}\mathrm{C}_\mathrm{a}$ and this may be significant as it is the mean of six trees. The Friedli et al. (1986) data show a slight decline in $\delta^{13}\mathrm{C}_\mathrm{a}$ up to 1955, followed by a stronger decline (1.5‰) thereafter, but provide no expectation of 1935–40 as a turning point in $\delta^{13}\mathrm{C}_\mathrm{p}$. It has been suggested that warmer summers should increase the level of $^{13}\mathrm{C}$ in the atmosphere, but there is no evidence of this for the Southern Hemisphere in the 1900–40 period (Pearman et al. 1976). Physiological and microenvironmental changes with plant stand age have been used to explain initial increases in $\delta^{13}\mathrm{C}_\mathrm{p}$ (Duquesnay et al. 1998) followed by a decrease (Marshall and Monserud 1996). This cannot explain why the plants peak in our case, and simultaneously at that, as the grasstrees and Die Bos trees would have been already old (100–250 years) for the oldest date analyzed.

$\delta^{13}\mathrm{C}_\mathrm{p}$ is inversely correlated with rainfall and with more direct measures of water availability in woody plants (Tans and Mook 1980; Pita et al. 2001; Lamont et al. 2002). Rainfall data were only available from 1930 for the grasstrees, i.e. covering the downward trend line for

Table 4 Contribution of $\delta^{13}\mathrm{C}_\mathrm{a}$ and $\mathrm{c}_\mathrm{i}/\mathrm{c}_\mathrm{a}$ to variation in $\delta^{13}\mathrm{C}_\mathrm{p}$ over the twentieth century in four grasstrees and four conifers. Rank = ranking of correlation with $\delta^{13}\mathrm{C}_\mathrm{p}$; Apparent contribution = relative slope of lines fitted to $\delta^{13}\mathrm{C}_\mathrm{p}$, with$c_\mathrm{i}/c_\mathrm{a}$ corrected for effect of (a–b) in Eq. 1; actual contribution = relative slope with (a–b) left in. *NS* Non-significant, all other correlations were significant at P<0.05. Trees numbered as in Fig. 2

Origin	Rank		Apparent contribution (%)		Actual contribution (%)	
	$\delta^{13}\mathrm{C}_\mathrm{a}$	$c_\mathrm{i}/c_\mathrm{a}$	$\delta^{13}\mathrm{C}_\mathrm{a}$	$c_\mathrm{i}/c_\mathrm{a}$	$\delta^{13}\mathrm{C}_\mathrm{a}$	$c_\mathrm{i}/c_\mathrm{a}$
a	2	1	93.8	6.2	40.3	59.7
b	1	1	98.1	1.9	69.4	30.6
c	2	1	99.7	0.3	92.3	7.7
d	1	2	99.7	0.3	93.3	6.7
e	2	1	82.7	17.3	52.6	47.4
f	2	1	89.9	10.1	28.2	71.8
g	2	1	95.8	4.2	50.0	50.0
h	2 (NS)	1	98.9	1.1	80.0	20.0

Table 5 Correlation matrix for five attributes measured on the four combined grasstree (n=222) over 67 years (*lower rectangle*) and the four combined conifer (n=279) over 67 years (*upper rectangle*). P<0.001 for r=±0.221 and *df* =200 (2-tailed)

	Year	$\delta^{13}\mathrm{C}_\mathrm{p}$	$\delta^{13}\mathrm{C}_\mathrm{a}$	Rainfall$^\mathrm{a}$	Rainfall$^\mathrm{b}$	$c_\mathrm{i}/c_\mathrm{a}$	W_i
Year		–0.568	–0.856	0.477	–	0.326	0.260
$\delta^{13}\mathrm{C}_\mathrm{p}$	–0.454		0.695	–0.134	–	–0.945	0.606
$\delta^{13}\mathrm{C}_\mathrm{a}$	–0.925	0.457		–0.454	–	–0.421	–0.124
Rainfall$^\mathrm{a}$	–0.037	0.027	0.082		–	–0.038	0.332
Rainfall$^\mathrm{b}$	–0.007	–0.001	0.038	0.870		–	–
$c_\mathrm{i}/c_\mathrm{a}$	0.175	–0.949	–0.153	–0.001	0.015		–0.821
W_i	0.052	0.854	0.066	–0.011	–0.017	–0.973	

[a] Winter rainfall for grasstrees or total rainfall for conifers

[b] 3-winter rainfall for grasstrees

$\delta^{13}C_p$. Winter rainfall showed a slight but non-significant downward trend for the station nearest three of the grasstrees and a significant downward trend for the fourth (i.e., in the 'wrong' direction). Even when combined with the previous summer-winter (to account for a likely source of initial C) and/or the subsequent summer-winter (because the leaves take 12 months to mature, D. Korczynskyj, personal communication) the lack of correlation with $\delta^{13}C_p$ was maintained (Table 5). These factors no doubt explain why there was no consistent seasonal effect on $\delta^{13}C_p$ either (Table 1). While there was a full rainfall record for the conifers, the stations were 7 km away and other nearby stations with less complete records showed quite different patterns and annual totals, as expected in this rugged landscape. There was no overall correlation between rainfall and $\delta^{13}C_p$ for the conifers either (Table 5).

Any rainfall effect on $\delta^{13}C_p$ would have been expressed through c_i/c_a: an increase in water status should increase stomatal conductance and hence c_i. This did occur for the Krakadouw conifers but this may be a coincidence, for their actual rainfall may have been more similar to Wupperthal's with which there was no relationship. There was no overall correlation between rainfall and c_i/c_a for both sets of plants (Table 5). It is postulated that there has been a marked increase of water availability in eucalypt forest due to treefelling (Davison 1997) while grasstree water relations are sensitive to water availability (Crombie 1988). The Amphion (unburnt) block has not been logged since 1931 but showed the most marked increase in c_i/c_a with time for the entire analysis period, while the other grasstrees, with a small or no trend, grew in blocks subjected to selective logging of large eucalypts. The conifers grew in remote nature reserves not subject to logging with three showing a marked rise in c_i/c_a since 1920. Forest management as it might affect water availability therefore seems to have played no role in accounting for the trends in $\delta^{13}C_p$.

Increasing shade could have contributed to the rise in c_i/c_a of the unburnt grasstree (Francey and Farquhar 1982), as mid-level trees gradually overtopped shorter grasstrees. However, the same argument could not hold for the rise in c_i/c_a of the matching Amphion grasstree where fires would have opened up the overstorey every 5–10 years (Ward et al. 2001), more consistent with the other two grasstrees that showed no trend. It also could not apply to the conifers that were by far the largest plants in the landscape and their shoot architecture and scale leaves ensured that self-shading did not increase with time. All sites were at least 80 km from a city and deep within the water catchment so that air pollutants were unlikely to be responsible for any of the rises in c_i/c_a over time (Francey and Farquhar 1982). Decreasing temperatures also provide a mechanism for increasing c_i/c_a. The temperature data were no more comprehensive or reliable than those for rainfall, but it is reasonable to assume that it has been trending upwards as part of global warming (Hughes 2000).

Deserving of more serious consideration is the effect of mineral nutrient availability on photosynthetic capacity and thus c_i/c_a (Ehleringer et al. 1993). We report on Fe and Mn levels here as (1) they are directly involved in photosynthesis, (2) they are not readily absorbed by plants, and (3) they are highly immobile once deposited in the tissues and resist leaching out of the leafbases over time (Marschner 1995; R Wittkuhn, unpublished data). The unburnt grasstree showed a clear downward trend in Fe and Mn concentrations since the fire. This is consistent with a marked decline in Fe and Mn levels in dead leaves within individual grasstrees over 15 years since fire in the same vegetation type (R. Wittkuhn, unpublished data). Thus, the increase in c_i/c_a can be attributed to a declining photosynthetic capacity with time since fire as nutrients become less available for uptake. The same interpretation applies to the three Krakadouw conifers that have escaped fire for over 70 years (the species is killed by fire), but not the Die Bos tree which showed no trend in c_i/c_a. The slight decline for Mn and no trend for Fe over time in the burnt grasstree reflect bouts of increased nutrient availability with each fire (whose frequency has been declining, especially since 1940, Ward et al. 2001; Lamont et al. 2003) and are consistent with the slight increase or no trend in c_i/c_a for the three burnt grasstrees.

Recent research has emphasized the relationship between $\delta^{13}C_p$ and $\delta^{13}C_a$ (February and Stock 1999; Feng 1999). However, our results show that c_i/c_a may individually (Table 3) and collectively (Table 5) be better correlated with $\delta^{13}C_p$. In absolute terms, $\delta^{13}C_a$ still contributes most to $\delta^{13}C_p$, but the fact that c_i/c_a is multiplied by 22.6, i.e. (a–b), in Eq. 1 means that its contribution exceeded 30% in five plants and reached at least 60% in two. Our calculations assume that the $\delta^{13}C_a$ and c_a values we used, gathered from a Pacific Island location in particular (Friedli et al. 1986; Feng 1998), applied to the air around our plants. Both locations were in indigenous vegetation remote from industry so must have had similar values to these as well as to each other. Clearing for agriculture within 10 km of the grasstree stands may have released CO_2 at a faster rate than we assumed (Freyer 1979). Underestimating the rate of c_a rise will have overestimated the rate of rise of c_i/c_a—this trend was universal in the Cape plants post-1930–1940 where their remote, mountain location suggests such an underestimate is unlikely. The tendency for $\delta^{13}C_p$ to rise up until then in several trees cannot be related to expected changes in c_a (Fig. 1a). Any cause of this peak must have operated on both sides of the Indian Ocean yet have been unevenly expressed in different trees.

Has the escalating increase in c_a led to a higher level of C fixation as expressed by a higher W_i? Overall, there has been no trend with time (Table 5). For five plants the increase in W_i has occurred at an overall rate of 7–17 μmol mol^{-1} decade^{-1}. For the two plants with a marked increase in c_i/c_a there has been a decrease in W_i of 22–26 μmol mol^{-1} decade^{-1}. Thus, a marked decline in $\delta^{13}C_p$ can imply a decrease in W_i when c_i increases at a much faster rate than c_a (Fig. 1e, Tables 2, 4). W_i increases when c_i/c_a remains constant (Fig. 1d), increases slowly (e.g. Tables 2, 4), or decreases (not observed here). This is because W_i is a function of $c_a(1–c_i/c_a)$. The increases in W_i for our five trees (3–18.5%) were somewhat less than those recorded by Feng (1999) over the same period (7.5–25.5%) for 21 of 23 trees. Underestimating the rate of c_a rise will have underestimated the already clear rises in W_i for six trees, and exaggerated the fall in the other two. For nine grasstrees in the same forest type (J. Eldridge, unpublished data) and these conifers (E. February, unpublished data) we were not able to detect an increase in productivity (band widths) that might be associated with higher W_i as suggested by Feng (1999). Despite marked trends in c_a, and sometimes rainfall and nutrient levels, overall growth rates have been conserved.

Our results show that the leafbases of grasstrees in southwestern Australia are suitable for time-series analysis of $\delta^{13}C_p$, producing similar patterns as for conifer wood in southern Africa over the last 100 years. While grasstrees are much more convenient to use than wood, the technique is limited by plant longevity to the last 250 years. The universal downward trend in $\delta^{13}C_p$ since 1935–1940 is more closely correlated with a rise in c_i/c_a than a fall in $\delta^{13}C_a$. We attribute the unexpected rise in c_i/c_a to declining nutrient availability associated with reduced fire frequency, and the topic deserves further research.

Acknowledgements The work was supported by the Australian Research Council (Linkage), Department of Conservation and Land Management (Western Australia), the South African Water Research Commission and National Research Foundation (South Africa). The assistance of David Ward, Chantal Burrows and Lidia Bednarek, and advice of John Pate, Pauline Grierson and Mark Adams were much appreciated. We thank Christian Koerner and the referees for their comments.

Appendix

Calculation steps

– Step 1. Calculate c_a:

$$c_a = 277.78 + 1.350 \cdot \exp[0.01572 \cdot (\text{year} - 1740)] \qquad (2)$$

(Feng 1998).

– Step 2. Calculate carbon isotope discrimination by the plant:

$$\Delta 13 = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p/1,000) \qquad (3)$$

(Feng 1999, citing Farquhar et al. 1989).

– Step 3. Calculate c_i using $\Delta 13$ and c_a above:

$$c_i = c_a \cdot [(\Delta 13 - a)/(b - a)] \text{ with } a = 4.4,\ b = 27.0 \qquad (4)$$

(Feng 1998).

– Step 4. Finally, calculate W_i using c_a and c_i above:

$$W_i = (c_a - c_i)/1.6 \qquad (5)$$

(Feng 1999, citing Ehleringer et al. 1993).

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