

Increased water use efficiency does not prevent growth decline of *Pinus canariensis* in a semi-arid treeline ecotone in Tenerife, Canary Islands (Spain)

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Abstract

• **Key message** Intrinsic water use efficiency of *Pinus canariensis* (Sweet ex Spreng.) growing at a semi-arid treeline has increased during the past 37 years. Tree ring width by contrast has declined, likely caused by reduced stomatal conductance due to increasing aridity.

• **Context** Rising atmospheric CO₂ concentration (C_a) has been related to tree growth enhancement accompanied by increasing intrinsic water use efficiency (iWUE). Nevertheless,

the extent of rising C_a on long-term changes in iWUE and growth has remained poorly understood to date in Mediterranean treeline ecosystems.

• **Aims** This study aimed to examine radial growth and physiological responses of *P. canariensis* in relation to rising C_a and increasing aridity at treeline in Tenerife, Canary Islands, Spain.

• **Methods** We evaluated temporal changes in secondary growth (tree ring width; TRW) and tree ring stable C isotope signature for assessing iWUE from 1975 through 2011.

• **Results** Precipitation was the main factor controlling secondary growth. Over the last 36 years *P. canariensis* showed a decline in TRW at enhanced iWUE, likely caused by reduced stomatal conductance due to increasing aridity.

• **Conclusion** Our results indicate that increasing aridity has overridden the potential CO₂ fertilization on tree growth of *P. canariensis* at its upper distribution limit.

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Contribution of the co-authors P. Brito performed all experimental work and co-wrote the manuscript. T.E.E. Grams and R. Matyssek supervised the work on isotope analysis and co-worked in paper writing. M.S. Jimenez and A.M. Gonzalez-Rodríguez participated in data interpretation and provided editorial advice. W. Oberhuber analyzed tree ring data and helped with data processing and statistics with respect to tree growth. G. Wieser participated in data interpretation, paper writing, and coordinated the project.

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1 Introduction

The current rising atmospheric CO₂ concentration (C_a) is a central driver of climate change leading to substantial increase in temperature and altered annual precipitation patterns (Körner 2000). C_a has increased from 303 $\mu\text{mol mol}^{-1}$ in 1920 (McCarroll and Loader 2004) to 391 $\mu\text{mol mol}^{-1}$ in 2011 (IPCC 2013). Increased C_a may stimulate plant growth by reduced water loss upon stomatal closure and enhanced photosynthesis (Morgan et al. 2004; Norby et al. 2005). Consequently, intrinsic water use efficiency (iWUE), being the ratio of net carbon gain (A) versus leaf conductance for water vapor (g_w), should increase (Farquhar et al. 1989). iWUE can be inferred from stable carbon isotope signature

in tree ring wood or cellulose (McCarroll and Loader 2004). Only few studies have integrated long-term trends in climate, C_a , iWUE, and growth (Linares et al. 2009; Linares and Camarero 2012; Granda et al. 2014; Liu et al. 2014). Peñuelas et al. (2011) listed 47 studies related to changes in tree ring-derived iWUE and/or growth of mature trees growing in tropical, arid, Mediterranean, temperate, and boreal biomes, although only seven studies had analyzed iWUE together with growth. These latter studies implied that the observed increase in C_a and iWUE did not translate into tree growth enhancement (Peñuelas et al. 2011), suggesting other factors such as warming-related drought to override potential benefits of rising C_a (Silva and Anand 2013; Levesque et al. 2014; Wu et al. 2014).

Mediterranean forest ecosystems are expected to drastically modify gas exchange and tree growth under rising C_a (Huang et al. 2007; Linares and Camarero 2012; Granda et al. 2014), while drought impact is likely to intensify (Sarris et al. 2013). For the upcoming three decades, modeling predicts increase in surface air temperature by 1 °C and decrease in soil water availability by 15 to 20 % for Mediterranean ecosystems (Sabaté et al. 2002; IPCC 2013), as precipitation may decline by more than 30 % (Giorgi 2006; Somot et al. 2008). Evapotranspiration is expected to increase so that soils may dry, affecting resource acquisition for growth and reproduction (Durante et al. 2009).

Contrasting tree-species specific growth responses to climate change have been reported for continental Mediterranean forest trees. While some studies reported on growth enhancement in response to increasing C_a (Rathgeber et al. 2000; Kuotavas 2008; Linares et al. 2009), other studies show declining growth trends at increasing C_a and iWUE (Tognetti et al. 2000; Maseyk et al. 2011). Such inconsistency may arise from species-related peculiarities in growth and iWUE adjustments, linked to long-term acclimations to increasing C_a or additional competing factors such as drought stress limiting the expected CO₂-induced growth enhancement (Linares and Camarero 2012; Granda et al. 2014).

In the present study, we used tree ring width and stable C isotope analysis to evaluate the effects of rising C_a and drought on growth and iWUE of *Pinus canariensis* (Sweet ex Spreng.) at the semi-arid treeline in Tenerife, Canary Islands, Spain. At present, no other tree species can compete with *P. canariensis* an endemic conifer of the Canary Archipelago, which is well adapted to xeric conditions exemplified by xenomorphic needles (Grill et al. 2004) and tap roots extending down to 15 m belowground (Luis et al. 2005; Climent et al. 2007). Our specific objective were (1) to determine the main limiting climatic factor for radial growth of *P. canariensis* at its upper distribution limit and (2) to test if rising atmospheric CO₂ concentrations and changing environmental conditions (temperature and precipitation) at the semi-arid treeline of Tenerife have caused changes in tree growth and iWUE during the past 37 years (1975–2011).

2 Material and methods

2.1 Study site and climate data

The study was conducted in a reforested even-aged *P. canariensis* forest growing at treeline (2070 m a.s.l.) in Las Cañadas near the Visitors Centre (El Portillo) of Teide National Park (28° 18' 21.5" N, 16° 34' 5.8" W), Tenerife, Canary Islands, Spain. In Las Cañadas, the treeline is formed by sharp line of isolated upright *P. canariensis* trees, and seedling establishment is severely impeded due to topsoil desiccation for about 5 months during the dry summer and frequent night frosts during the winter (Höllermann 1978; Srutek et al. 2002; Wieser et al. 2016). In 2011, the trees were 61-years old. This avoided possible age-dependent differences in growth-climate relationships, which may occur with trees of diverse ages (Carrer and Urbinati 2004).

The climate is typically semi-arid Mediterranean, with an alternation of a warm and dry period from June to September and a cold and wet period from October to May. During the period 1921–2011, mean annual precipitation was 466 mm, with 95 % falling during the cold and wet season and almost no rain in summer. Mean annual temperature was 9.7 °C, with summer maxima of up to 30.5 °C and winter minima down to −9.8 °C. Temperature and precipitation data (annual and monthly means or sums) were obtained from the Izaña weather station, 5 km east of the study site (28° 18' 21.5" N, 16° 30' 35" W; 2367 m a.s.l.; <http://izana.aemet.es/>) for the period 1974–2011. We also calculated an aridity index (AI) as precipitation divided by (temperature + 10) following De Martonne (1926) for the study years, where lower AI values correspond to higher aridity.

The geological substrate is of volcanic origin (basalt), and the soil is classified as a Leptosol, a soil type typical for dry regions at high elevations in Tenerife (Arbelo et al. 2009). The water holding capacity of the topsoil (5–35 cm depth) at saturation (−0.001 MPa) is 0.46 m³ m^{−3}, and the corresponding values for field capacity (−0.033 MPa) and the wilting point (−1.5 MPa; sensu Blume et al. 2010) are 0.23 and 0.09 m³ m^{−3}, respectively (Brito et al. 2014). Due to frequent precipitation during the cold and wet season, soil water potential at 25–30 cm soil depth rarely drops below −0.02 MPa and remains close to the wilting point throughout the dry summer (Brito et al. 2014).

2.2 Sampling and dendrochronological procedure

Dendrochronological methods were used to assess changes in stem radial growth. In fall 2011, we sampled five trees which were previously used for stem CO₂ efflux and sap flow measurements (Brito et al. 2010, 2015). Two cores per tree (S and W exposure) were taken at diameter at breast height (DBH) using a 5-mm-diameter increment borer. For contrast

enhancement of tree ring boundaries, the cores were dried in the laboratory, non-permanently mounted on a holder, and the surface was prepared with a razor blade. Ring widths were measured to the nearest 1 μm using a reflecting microscope (Olympus SZ61) and the software package TSAP WIN Scientific. Tree ring chronologies of the single cores were plotted and cross-dated visually and statistically, respectively. The TSAP software was used for statistically cross-dating by assessing the Gleichläufigkeit (=synchronicity between time series). Gleichläufigkeit is the percent agreement in the signs of the first difference of time series data (Eckstein and Bauch 1969). Agreement was also quantified parametrically using the product–moment correlation coefficient, which in turn was adjusted for the amount of overlap between tree ring series using the standard t statistic, whereby the threshold for acceptable statistical quality was suggested to be 3.5 (Baillie and Pilcher 1973). Ring widths of both cores from each sample tree were averaged, and the quality of the chronologies was evaluated with the ARSTAN software (Cook 1987; Holmes 1994) through calculation of the Expressed Population Signal (EPS; Wigley et al. 1984).

2.3 Stable isotope analysis, ^{13}C discrimination, and intrinsic water use efficiency

$\delta^{13}\text{C}$ analyses for the years 1975–2011 were performed on same cores as used for TRW assessment. Preventing juvenile effects on isotopic tree ring signatures (Heaton 1999; but see McDowell et al. 2011), only the most recent 37 years (1975–2011) of ring formation were sampled. Annual rings (early wood plus late wood) were cut exactly at ring boundaries by use of a scalpel and a reflecting microscope (Wild 308700). For each of the five study trees two samples per tree ring were pooled and homogenized with a swing mill (Retsch MM301, Retsch Haan, Germany). In a subsample, we compared isotope signatures in bulk wood with those in cellulose for determining the necessity of cellulose extraction in our study trees. Cellulose extraction was performed using a modified version of the method of Brendel et al. (2000.). This methodological comparison corroborated a significant correlation ($R^2=0.93$; $P<0.001$; Fig. 1). On average, $\delta^{13}\text{C}$ in cellulose was higher by $1.4\pm 0.1\text{‰}$ than in bulk wood, and differences between wood and cellulose $\delta^{13}\text{C}$ were almost constant amongst trees and tree ring age (see Online Resource Fig. OR1) as reported earlier for other conifer species (Borella and Leuenberger 1998; Jaggi et al. 2002; Sohn et al. 2013; Weigt et al. 2015). Therefore, we corrected the isotope offset of 1.4‰ between cellulose and bulk wood, as we used bulk wood instead of extracted cellulose for our isotope analysis (c.f. also Saurer and Siegwolf 2007).

For analyzing $\delta^{13}\text{C}$, 2.0 ± 0.02 mg of homogenized samples were weighed into tin capsules (3.5×5 mm, IVA Analysentechnik e. K., Meerbusch, Germany) and combusted

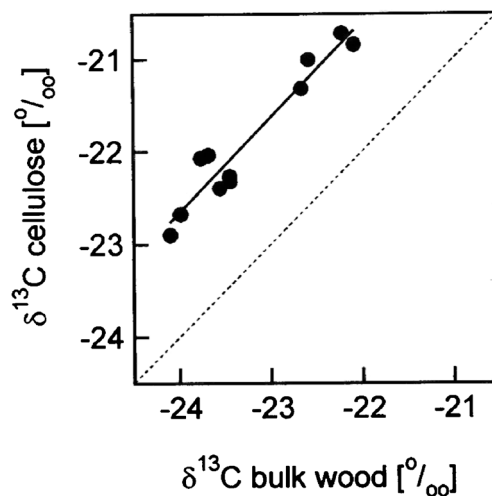


Fig. 1 Comparison of $\delta^{13}\text{C}$ in bulk wood and cellulose of annual growth rings of *P. canariensis*. Points were fit by linear regression analysis: $y=1.04x+2.14$; $R^2=0.93$; $P<0.001$. The dashed line reflects the one-to-one line for comparison

to CO_2 in an elemental analyzer (Eurovector EA3000) connected to an isotope ratio mass spectrometer (Isoprime, Elementar, Hanau, Germany). The isotope signature is expressed in the delta (δ) notation in parts per thousand relative to the Vienna Pee-Dee Belemnite (V-PDB) standard:

$$\delta_{\text{sample}} = \left(R_{\text{sample}} / R_{\text{standard}} - 1 \right) * 1000 \quad (1)$$

where R_{sample} and R_{standard} represent the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the V-PDB standard, respectively. The analytical precision was $<0.12\text{‰}$ (expressed as standard deviation of an internal laboratory standard using identical sample mass). Tree ring specific $\delta^{13}\text{C}$ was corrected for the progressive decline in atmospheric $\delta^{13}\text{C}$ through calculating ^{13}C discrimination ($\Delta^{13}\text{C}$):

$$\Delta^{13}\text{C} = \left(\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{trng}} \right) / \left(1 + \delta^{13}\text{C}_{\text{trng}} / 1000 \right) \quad (2)$$

where $\delta^{13}\text{C}_{\text{atm}}$ and $\delta^{13}\text{C}_{\text{trng}}$ are the $^{13}\text{C}/^{12}\text{C}$ ratios in atmospheric CO_2 and tree rings, respectively. $\Delta^{13}\text{C}$ can also be calculated as follows in order to relate $\Delta^{13}\text{C}$ with physiological responses:

$$\Delta^{13}\text{C} = a + (b-a) * C_i / C_a \quad (3)$$

where a ($=4.4\text{‰}$) refers to the slower diffusivity of $^{13}\text{CO}_2$ relative to $^{12}\text{CO}_2$ in air, b ($=27\text{‰}$) is the isotopic fractionation caused by enzymatic C fixation, and C_i and C_a are the CO_2 concentrations in the intercellular space of the needles and the atmosphere, respectively. The long-term trend in $\delta^{13}\text{C}_{\text{atm}}$ from 1980 to 2011 was obtained from direct atmospheric measurements (www.scrippsco2.ucsd.edu) and extrapolated for the

years 1975 through 1979. C_a was obtained from published data (<http://cdiac.ornl.gov/trends/co2/sio-mlo.html>; 1975–2011) and regional data back to 1991 at Izaña taken from the Earth System Research Laboratory website (www.esrl.noaa.gov/gmd/about/aboutgmd.html; code-IZO). It should be noted that $\Delta^{13}C$ is determined by the ratio of chloroplast to the ambient CO_2 mole fraction (C_c/C_a) rather than C_i/C_a , as used in Eq. 3, making here the calculated value sensitive to mesophyll conductance (g_m ; Seibt et al. 2008). The latter varies in accordance to changes in environmental conditions such as temperature, irradiance, water, and CO_2 availability (Flexas et al. 2008). Consequently, using C_a may be problematic if g_m to CO_2 is not constant (Seibt et al. 2008). However, as information on mesophyll conductance of *P. canariensis* is not available and published means of g_m would not improve results (Cernusak et al. 2013), we chose using the simplified linear model of Farquhar et al. (1982). Hence, intrinsic water use efficiency (iWUE), i.e., the ratio of the net carbon gain (A) versus leaf conductance for water vapor (g_w), was calculated as follows:

$$iWUE = A/g_w = C_a(b - \Delta^{13}C) / (1.6 * (b - a)) \quad (4,)$$

where 1.6 is the ratio between the diffusivities of water vapor and CO_2 in air.

2.4 Data analysis

We assessed Pearson's correlations for assessing the climatic impact on the tree ring variables TRW and $\Delta^{13}C$ throughout the study period (1975–2011). These statistical analyses were based on seasonal and annual calculations of mean air temperature ($^{\circ}C$), total precipitation (mm), and AI for the prior and current growing year using the SPSS 16 software package (SPSS Inc., Chicago, USA). A probability level of $P < 0.05$ was considered as statistically significant. As suggested by Sarris et al. (2013) we did not remove any age-related trend from our tree ring chronologies by conventional detrending procedures, thus, avoiding the risk of removing any environmental signal or trend captured by our tree ring series.

3 Results

3.1 Inter-annual trends in environmental conditions, TRW, and $\Delta^{13}C$

Climate analysis reveal that at our treeline site mean air temperature had increased by $0.3^{\circ}C$ per decade during the period 1975–2011 (Fig. 2a), in particular during spring and summer (see Online Resource Table OR1). Precipitation by contrast declined by 77 mm per decade (Fig. 2b), especially during spring (see Online Resource Table OR1). On an annual scale,

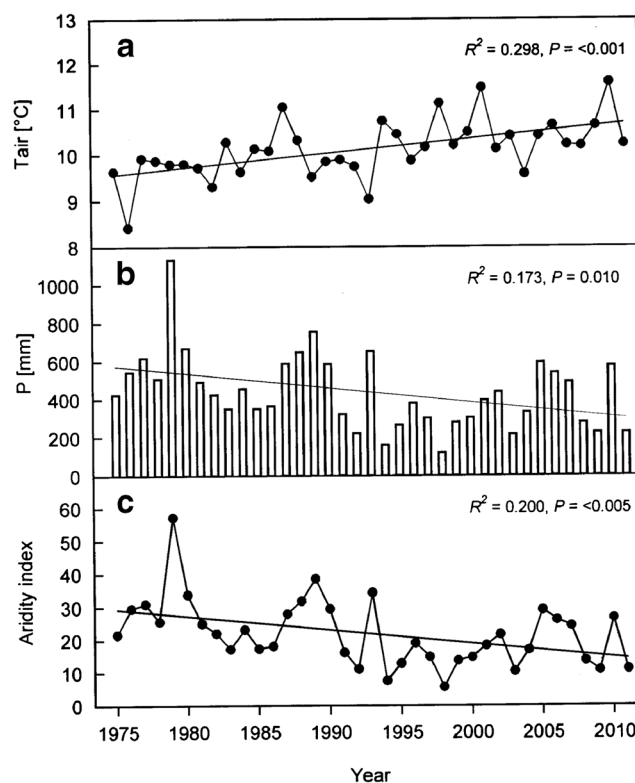


Fig. 2 Temporal variation in **a** mean annual air temperature (T_{air}), **b** total annual precipitation (P), and **c** the aridity index during the period 1975 throughout 2011. Regression analysis: mean annual air temperature: $y = 0.031 \times -52.6$, $R^2 = 0.298$, $P < 0.001$; total annual precipitation: $y = -7.6 \times +15,672$, $R^2 = 0.173$, $P = 0.01$; aridity index: $y = -0.42 \times + 856.5$; $R^2 = 0.20$, $P = 0.005$

increasing temperature coupled with a decrease in precipitation patterns therefore also caused a decline in the aridity index of 4.1 per decade (Fig. 2c; Online Resource Table OR1).

The five study trees were even-aged and the mean ring width was 2.68 ± 0.17 mm. Synchronicity (“Gleichläufigkeit”) between the ring width series reached values $>73\%$, and t values >5.3 (see Online Resource Table OR2). The expressed population signal (EPS) was 0.91, suggesting adequate replications and a strong common climate signal in our treeline chronology.

We detected a significant decline in TRW from 1975 to 2011 ($P = 0.002$) reaching a minimum in 2008 (Fig. 3a). Tree ring $\Delta^{13}C$ did not vary considerably during the past 37 years. $\Delta^{13}C$ averaged $14.2 \pm 0.3\%$ and varied between $14.8 \pm 0.2\%$ in 1976 and $13.2 \pm 0.6\%$ in 1984 (Fig. 3b).

3.2 Climate growth relationships

Pearson's correlations revealed strong links between climatic parameters and the TRW chronology (Table 1; Online Resource Table OR3 and OR4). The main factor controlling TRW was precipitation (Table 1). At a seasonal scale, the

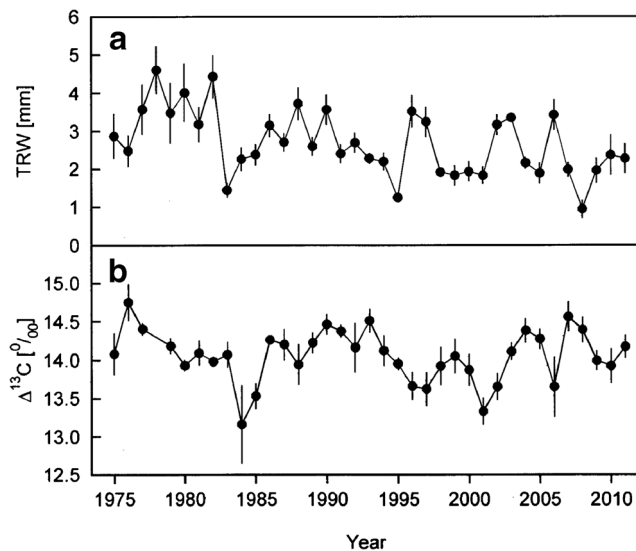


Fig. 3 Chronologies of **a** tree ring width (TRW) and **b** $\Delta^{13}C$ of *P. canariensis* from 1975 throughout 2011. Values are the mean \pm SE of five trees

TRW was significantly favored by winter (previous Dec–Feb; $r=0.396$, $P=0.015$) and spring ($r=0.387$, $P=0.018$) precipitation (Table 1). Interestingly, rainfall of the previous fall had no significant effect on TRW. Correlations between TRW and precipitation, however, improved when we extended the integration period from seasonal to longer time scales, covering (1) winter and spring ($r=0.511$, $P=0.009$), (2) calendar year

Table 1 Pearson’s correlation coefficients calculated between tree ring chronologies (tree ring width, TRW; and ^{13}C discrimination, $\Delta^{13}C$) and integrated periods of precipitation for the period 1975–2011. (–1) indicates the season of the previous year. Spring: March–May, Summer: June–August, Autumn: September–November, Winter: previous year December–February, Calendar year: January–December, hydrological year: October (–1)–September

Season	TRW	$\Delta^{13}C$
Autumn (–1)	0.303	–0.043
Winter	<i>0.396*</i>	0.038
Spring	<i>0.387*</i>	<i>0.351*</i>
Summer	–0.147	0.153
Autumn	–0.057	–0.028
Winter and spring	<i>0.511**</i>	0.191
Calendar year	<i>0.419**</i>	0.180
Hydrological year	<i>0.579***</i>	0.160
Multi-year		
2 years	<i>0.560***</i>	0.198
3 years	<i>0.502**</i>	0.295
4 years	<i>0.513**</i>	0.327

Significant correlations are set in italics (* $P<0.05$, ** $P<0.01$, *** $P<0.001$)

($r=0.419$, $P=0.010$), (3) hydrological year ($r=0.579$, $P<0.001$), and (4) multiple year precipitation (all $r<0.502$, and $P<0.020$), respectively (Table 1). Spring precipitation also significantly favored $\Delta^{13}C$ ($r=0.351$, $P=0.036$; Table 1).

Pearson’s correlation indicated a significantly negative effect of summer temperature on TRW ($r=-0.424$, $P=0.009$), as well as significantly negative effects of autumn ($r=-0.444$, $P=0.007$) and calendar year temperature ($r=-0.400$, $P=0.016$) on $\Delta^{13}C$ (Online resource Table OR3). Pearson’s correlation also indicated a significant positive effect of winter ($r=0.378$, $P=0.021$), spring ($r=0.381$, $P=0.020$), calendar year ($r=0.426$, $P=0.009$), and hydrological year ($r=0.579$, $P<0.001$) aridity index (AI; where lower values correspond to a higher aridity) on TRW, and of spring AI ($r=0.358$, $P=0.032$) on $\Delta^{13}C$ (Online Resource Table OR4).

3.3 Inter-annual trends of C_a , C_i , C_i/C_a , and iWUE

Global atmospheric CO_2 concentration (C_a) increased from $331 \mu mol mol^{-1}$ in 1975 to $392 \mu mol mol^{-1}$ in 2011 (Fig. 4a), being in agreement with local C_a increase as recorded at the Izaña Observatory from 1991 throughout 2011 ($R^2=0.99$; $P<0.001$; see Online Resource Fig. OR2). Paralleling atmospheric CO_2 enhancement, tree ring-derived intercellular CO_2 concentration (C_i) increased significantly ($R^2=0.619$, $P<0.001$) from 1975 through 2011 from 142 to $169 \mu mol mol^{-1}$ (Fig. 4a). The trend of C_i/C_a revealed no

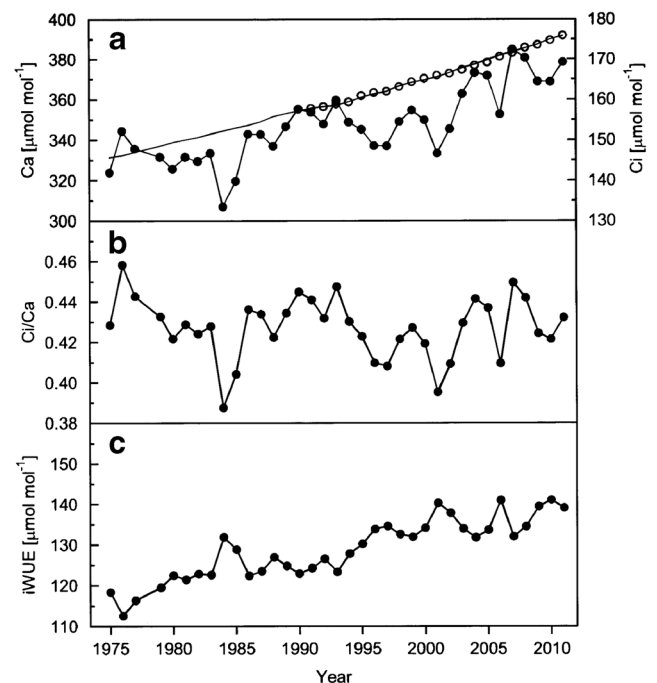


Fig. 4 Temporal variation in **a** global (thin line) and local (open circles) atmospheric CO_2 concentration (C_a), internal CO_2 concentration (C_i ; solid circles); **b** C_i/C_a , and **c** intrinsic water use efficiency (iWUE)

significant trend over the past 36 years ($R^2=0.009$, $P=0.58$), with C_i/C_a averaging 0.43 ± 0.01 (Fig. 4b). *P. canariensis* significantly increased their iWUE ($R^2=0.80$; $P<0.001$) from 1975 through 2011 (Fig. 4c).

4 Discussion

Our results indicate a warming trend at our treeline site for the period 1975–2011, coupled with reduced precipitation, and therefore also increasing aridity (Fig. 2; Online Resource Table OR1), which is in agreement with recent climate change models forecasting similar trends towards 2100 (IPCC 2013). Although we only sampled five even-aged trees, the correlations between the single TRW chronologies were highly significant (Online Resource Table OR2) and the EPS was 0.91. Thus, the EPS for our TRW chronology is within the range of 0.84 and 0.98 estimated for 12 young *P. canariensis* stands of growing between 1120 and 1930 m a.s.l. on the Cordillera Dorsal of Tenerife (Rozas et al. 2013). Our estimated EPS of 0.91 is also considerably above the threshold of 0.85 suggested by Wigley et al. (1984) and thus suggests adequate replications and a strong common climate signal in our treeline chronology with respect to radial growth and the $\delta^{13}C$ signal (c.f. also Borella and Leuenberger 1998 and Levesque et al. 2014). Furthermore, there is also evidence that young *P. canariensis* trees are more sensitive to limiting climatic conditions than older ones (Rozas et al. 2013), as has also been reported for the Mediterranean conifers *Juniperus thurifera* (Rozas et al. 2009) and *Pinus pinaster* (Vieira et al. 2009). In addition, young *P. canariensis* trees have no missing rings, and thus contrasting with mature trees, where missing tree rings are a major limitation for the successful dating of tree ring series (Jonsson et al. 2002).

Stem radial growth variability was mainly controlled by precipitation. The positive responses of TRW to winter, spring, calendar year, and especially to hydrological year and multiple years precipitation (Table 1) are in line with findings from other leeward *P. canariensis* plantations established between 1950 and 1970 between 1130 and 2100 m a.s.l. (Rozas et al. 2013) and indicates that water availability constraints tree growth at treeline in Tenerife (Gieger and Leuschner 2004). The beneficial effect of winter, spring, and hydrological year precipitation is due to a pronounced water deficit at treeline, as 95 % of the annual precipitation falls during the cold and wet season (October–May). The beneficial effect of hydrological year and multiple year precipitation on TRW of *P. canariensis* (Table 1; Jonsson et al. 2002) supports the idea that *P. canariensis* is able to tap water from deeper soil layers originating from years prior the growing season (Brito et al. 2015) as has also been documented for *Pinus halepensis* subsp. *brutia* at a dry low elevation site at Samos, Greece (Sarris et al. 2013). Indeed, net primary

production at our treeline site has been shown to be considerably higher in a hydrological moist as compared to a hydrological dry year (Wieser et al. 2016).

We also observed a negative response of TRW to warm summers (Table 1) which may be due to drought-induced stomatal closure, a loss in photosynthetic efficiency (Brito 2016) and enhanced respiratory carbon losses of aboveground woody tissues (Brito et al. 2010, 2013). Ample soil water availability and a lower evaporative demand as compared to the warm and dry summer (Brito et al. 2015) may help explain the lack of a significant response of TRW to winter and spring temperatures. The lack of any significant positive correlation between TRW and temperature may be attributed to the fact that mean annual air temperature at treeline (Fig. 2; 10.6 ± 0.5 °C) is noticeably higher than the mean air temperature range of 5.5 to 7.0 °C suggested to limit growth in continental treelines worldwide by Körner (2003, 2012). A higher temperature limit for tree growth in Mediterranean climates has also been suggested by Vieira et al. (2013).

Although radial stem increment at treeline in Tenerife terminates round mid-June (Brito et al. 2010), stem radial increment may be prolonged till late fall in hydrological moist years (Brito 2016). Under conditions of severe summer drought when stomata are completely closed (Brito et al. 2014, 2015), stem radial growth however does not extend into the peak of the dry season (Brito et al. 2010). This may help explain the lack of any significant positive correlations between climatic parameters and $\Delta^{13}C$, except for spring precipitation in Table 1 and AI (Online Resource Table OR4), the period when maximum radial growth normally takes place in *P. canariensis* at treeline (Brito et al. 2010; Brito 2016).

It has been shown that in dry years trees photoassimilates accumulate in *Pinus brutia* (Körner 2003), and recent work in drought-exposed *Pinus sylvestris* confirm that nonstructural carbohydrates during periods when cambial activity is close to zero (Gruber et al. 2012), opposite to the often assumed C starvation under drought. Probably, some C was fixed during the dry summer (Brito 2016). As there was commonly no growth during the dry summer, these stored carbohydrates were used for late wood production in autumn when soil water availability permits growth. As we used complete tree rings (early and late wood milled together), the isotopic signal corresponding to summer drought was retained in the annual isotopic signature of the whole tree ring (c.f. also Sarris et al. 2013).

At our treeline site *P. canariensis* showed a constant C_i/C_a ratio over time, leading to a moderate increase in iWUE under rising C_a (Fig. 3). The increase in iWUE observed at our study site is within the range of rising iWUE values of about 8 to 25 % reported for various Mediterranean forest trees since the 1970s (Ferrio et al. 2003; Peñuelas et al. 2011; Linares and Camarero 2012; Granda et al. 2014). Although iWUE increased over

time (Fig. 3c), radial growth has been declining, and thus suggesting that a reduction in stomatal conductance has prevailed which however does not rule out the possibility of changes in C allocation patterns or post-photosynthetic processes (Voltas et al. 2013). The decline in TRW reported here for our treeline site is in agreement with recent studies showing warming-induced growth reductions in spite of increasing iWUE for a variety of tree species at dry sites in the Iberian peninsula (Peñuelas et al. 2008; Linares and Camarero 2012; Granda et al. 2014). Thus, our results suggest that a drought-induced stomatal closure resulting from increasing temperature and aridity has reduced tree transpiration at the price of reducing net assimilation rate, thus overriding the potential CO₂ fertilizer effect. This could have been intensified at our treeline site by low soil water availability resulting from low soil water holding capacity of the topsoil (Brito et al. 2014).

Conversely, soil drying does not necessarily imply *P. canariensis* to suffer from water limitation as shown by Brito et al. (2015). Drought conditions at our study site are related to reduced winter rainfall which typically supplies more than 95 % of the annual precipitation. When winter rainfall is small, tree growth is low as evidenced at our study site by low annual increment in radial growth (Brito et al. 2010). Once topsoil moisture pools are exhausted, the ability to tap water from deeper soil moisture pools determines annual growth (Brito et al. 2010), water loss (Brito et al. 2015), and hence also g_w (Wieser et al. 2016). Moreover, remaining carbon not used for maintenance of metabolic processes during drought may be allocated into roots (Dewar et al. 1994), because during periods of drought stress C investments into below ground growth are of higher priority than aboveground growth (Kotzowski and Palladry 2002) to ensure water acquisition (Saxe et al. 1998).

P. canariensis also adapts to soil drought by developing deep tap roots extending down to 15 m belowground (Luis et al. 2005; Climent et al. 2007) allowing trees to use soil water reserves in deep soil layers when topsoil moisture pools are exhausted (Brito et al. 2015). For the next three decades, climate change and ecophysiological models for Mediterranean ecosystems predict an increase in surface air temperature of 1 °C and a 15–20 % lower soil water availability (Sabaté et al. 2002; IPCC 2013) due to a more than 30 % reduction in precipitation (Giorgi 2006; Somot et al. 2008). In this case growth will primarily depend on the recharge of deep soil water pools, the latter originating from rainfall prior the current year's growth available later in the growing season. Canopy transpiration (Brito et al. 2015) data underpin the significance of deep soil water reserves on the physiological behavior of *P. canariensis* at its upper distribution limit during the dry summer (Brito 2016).

5 Conclusions

Our results indicate that water availability was the main factor controlling TRW of *P. canariensis* at its upper distribution limit. During the past 37 years increasing aridity reduced TRW, while iWUE increased over time. Although it may not completely be ruled out that observed changes in TRW and iWUE are at least partly due to tree aging, our findings agree with recent studies from the Iberian Peninsula (Peñuelas et al. 2008; Linares and Camarero 2012; Granda et al. 2014) indicating reduced stomatal conductance and carbon uptake under xeric conditions despite rising C_a . Finally, our study highlights the importance of deeper soil moisture pools on TRW. Therefore, a solid knowledge on precipitation patterns, soil water pools and source water utilized for tree growth (Sarris et al. 2013; Levesque et al. 2014) is essential for understanding tree response to changes in ambient CO₂ concentration and water availability in semi-arid and arid environments.

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Compliance with ethical standards

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