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Long-term trends in intrinsic water-use efficiency and growth of subtropical *Pinus tabulaeformis* Carr. and *Pinus taiwanensis* Hayata in central China

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Abstract

Purpose This study analysed tree-ring stable carbon isotope composition ($\delta^{13}C$) of *Pinus tabulaeformis* Carr. and *Pinus taiwanensis* Hayata, from a subtropical forest located in central China for the last 130 years, to obtain the long-term trends of $\delta^{13}C$, carbon isotope discrimination by plants (Δ), leaf internal carbon dioxide (CO_2) concentration (c_i) and intrinsic water-use efficiency (iWUE) in response to elevated atmospheric CO_2 concentration (c_a) and climate change and explore how environmental changes affected long-term tree physiological responses and growth.

Materials and methods Tree-ring cores were taken in Dabie Mountains, the border of Hubei, Henan and Anhui Provinces of China. $\delta^{13}C$ was undertaken at every 3-year interval, and Δ, c_i, iWUE and basal area increment (BAI) were determined. Regression analysis was used to quantify the trends in climate and the relationships of c_i, iWUE and BAI with elevated c_a and climate. Partial correlation analysis was used to distinguish the effects of c_a and climate on c_i and iWUE.

Results and discussion $\delta^{13}C$ of *P. tabulaeformis* and *P. taiwanensis* decreased in the past 130 years, but Δ had no obvious change over time for the two tree species. Both c_i and iWUE increased significantly with the calendar year. BAI of *P. tabulaeformis* continuously increased during 1897–1993, but decreased slightly in the recent 20 years. However, BAI of *P. taiwanensis* did not present obvious change in the period 1882–2010. The c_i and iWUE of *P. tabulaeformis* and *P. taiwanensis* also increased linearly with elevated c_a in the past 130 years and with mean annual temperature during 1960–2007. Partial correlation analysis showed that elevated c_a, not temperature, induced the changes in c_a and iWUE. BAI of *P. tabulaeformis* since 1897 and that of *P. taiwanensis* during 1975–2010 responded quadratically to elevated c_a. Warming-deduced drought in the study area in the recent 30 years resulted in increases in iWUE and decreases in BAI.

Conclusions This study showed that while iWUE increased in the past 130 years, tree growth of two subtropical tree species in central China responded to rising c_a non-linearly. Negative effects of some factors on tree growth, such as climate change (particularly warming-induced drought), nutrient limitation and physiological long-term acclimation to elevated c_a, have overridden the CO_2 fertilization effects in the past 30 years.

Keywords Basal area increment · Carbon isotope discrimination · Elevated CO_2 concentration · Intrinsic water-use efficiency · Subtropical trees

1 Introduction

The elevated atmospheric carbon dioxide (CO_2) concentration (c_a) not only makes a significant contribution to current global warming (Mann et al. 1998; Allen et al. 2009; Lacis et al. 2010; Montzka et al. 2011) but also plays an important role in tree physiological changes which might be associated with long-term trends in tree growth and forest productivity. Higher c_a generally increases intrinsic water-use efficiency (iWUE),

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the ratio between the carbon assimilation (A) and stomatal conductance (g_s) (Francey and Farquhar 1982; LaMarche et al. 1984), and the increases in iWUE have been well-established in the literature in both controlled experiments (Morison 1993; Overdieck and Forstreuter 1994; Picon et al. 1996; Leavitt et al. 2003) and natural environments around the world (Bert et al. 1997; Feng 1999; Tang et al. 1999; Saurer et al. 2004; Liu et al. 2008; Gomez-Guerrero et al. 2013). Compared with short-term experimental results from studies of plants in growth chambers, the findings of long-lived trees growing in natural forests are more reliable and reveal more authentic behaviour of mature trees adjusting their physiological responses over time to gradually increasing c_a.

Tree iWUE has received much attention due to the well-known theory of stable carbon isotope composition (δ^{13}C), the relationships between leaf δ^{13}C and leaf internal CO_2 concentration (c_i), and the effects of g_s and A on c_i (Farquhar et al. 1982). As one of the most consistent responses of plants to elevated c_a is the decrease in g_s (Morsion 1987; Medlyn et al. 2001; Wullschleger et al. 2002; Long et al. 2004; Ainsworth and Long 2005; Zeppel et al. 2012), this water-saving mechanism can explain the improvement of iWUE. When the g_s limitation of photosynthesis is strong, leaves or needles exhibit a low c_i and a reduced carbon isotope discrimination by plants (Δ), along with improved iWUE (Saurer et al. 2004). Therefore, δ^{13}C and Δ technologies in tree rings have been widely applied to examine long-term physiological changes, because changes of c_i and Δ over time can be assessed for natural forests by measuring δ^{13}C, and then potential changes of iWUE due to elevated c_a and climate change can be assessed.

Long-term trends in tree-ring δ^{13}C and iWUE have been described across biomes (Peñuelas et al. 2011; Silva and Horwath 2013). However, the tropical and subtropical results are much under-represented in the field of the forest responses to rising c_a, comprised only 11 % of all studies (K rner 2009). One of the reasons is that trees growing in the non-seasonal tropical environment cannot display well-defined tree rings, which makes cross-dating between trees and between sites impossible. Hence, it has been generally assumed that regions, where climate does not exhibit a strong seasonal control on tree growth, are unsuitable for dendrochronology (Whitmore 1990). In spite of this, some tree species in tropical forests are able to generate the identified tree rings annually; therefore, some research has been done on the long-term iWUE variations in tropical and subtropical forests by δ^{13}C analysis for trees in Brazil, Mexico and Thailand (Hietz et al. 2005; Silva et al. 2009; Brienen et al. 2011; Nock et al. 2011), and the iWUE changes of trees in these regions comply with the widespread increasing trends. In China, Sun et al. (2010) have evaluated long-term tree growth and iWUE of Masson pine (*Pinus massoniana* L.) in response to global climate change for subtropical forests, a very valuable supplement for tree-ring stable isotope studies in China, where most analogous research has been conducted in arid and semi-arid areas (e.g. Liu et al. 2007; Liu et al. 2008; Wang et al. 2012; Xu et al. 2013). Recently, two subtropical tree species, Chinese red pine (*Pinus tabulaeformis* Carr.) and Huangshan pine (*Pinus taiwanensis* Hayata) from the Dabie Mountains, central China, have been certified that they do produce annual rings (Zheng et al. 2012b) and can be used as proxy data to reconstruct the past climate (Zheng et al. 2012a). Here, we analysed δ^{13}C of *P. tabulaeformis* and *P. taiwanensis* for the last 130 years, aiming to obtain the long-term trends of δ^{13}C, Δ, c_i and iWUE with elevated c_a and climate change, and explore how environmental changes affected long-term tree physiological responses and growth.

2 Materials and methods

2.1 Study area and climate

The study area is located in Dabie Mountains, the border of Hubei, Henan and Anhui Provinces of China (30° 10′~32° 51′ N, 112° 40′~117° 10′ E). The average elevation of Dabie Mountains is 500~800 m.a.s.l., and the highest peak is about 1,792 m.a.s.l. The East Asian monsoon reaches this region from the North Pacific Ocean, resulting in abundant rainfall and high temperature during the summer (Ou and Qian 2006). At the meteorological station in Macheng (31° 11′ N, 115° 01′ E, 59 m.a.s.l.), the wettest and driest months were July (mean precipitation of 223.3 mm) and December (23.8 mm), while January (mean temperature of 3.29 °C) and July (28.2 °C) were the coldest and warmest months, respectively, based on the instrumental data from 1959 to 2009 (Fig. 1a). The average of annual mean temperature was 16.4 °C, and the average of annual rainfall was about 1,216.9 mm in this region from 1959 to 2009, with 43.6 % of the annual rainfall falling during the summer (June to August). An apparent warming trend was observed since 1980 in the study area (Fig. 1b); however, no significant increasing or decreasing trends could be detected for the annual mean temperature from 1959 to 1979 (Fig. 1b, P=0.636) or annual rainfall since 1959 (Fig. 1c, P=0.303).

2.2 Sampling and preparation

P. tabulaeformis and *P. taiwanensis* were sampled at two different sites in August 2010, the former from Shuidongwan (SDW) and the latter from Zherenfeng (ZRF), because these two tree species are not co-occurring at the same study site, but they are the dominant conifer species at each site. The soils of both SDW and ZRF are yellow brown earth although they are at a distance of about 18.5 km. Table 1 shows the locations and soil characteristics of the two samplings sites. Sixty increment cores from 25 *P. tabulaeformis* and 43 increment cores from 16 *P. taiwanensis* were sampled in total. Two thin cores were taken at breast height (1.3 m) for

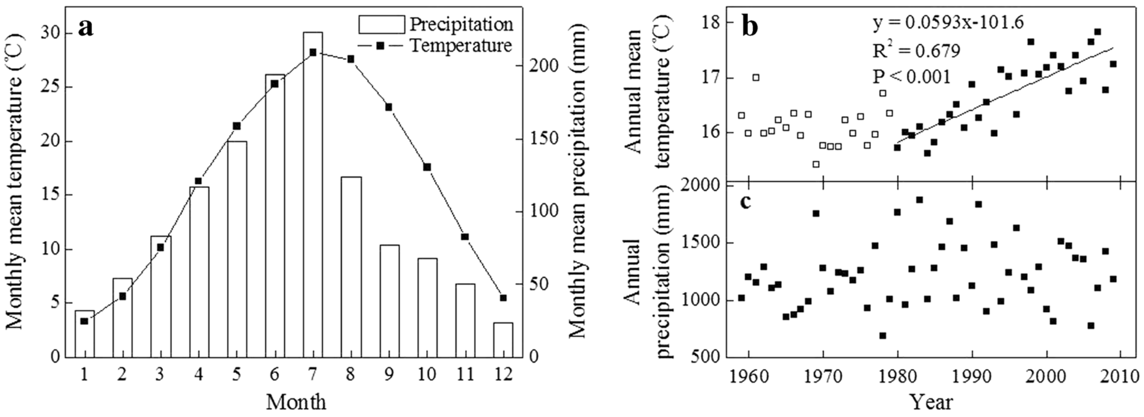

Fig. 1 Variations of monthly mean temperature and monthly precipitation (**a**), annual mean temperature (**b**) and annual precipitation (**c**) in Macheng meteorological station from 1959 to 2009. The *line in* **b** represents the linear regression analysis between annual mean temperature and the year from 1980 to 2009

each tree using an increment borer (4.3 mm). In addition, 10 *P. tabulaeformis* and 10 *P. taiwanensis* were randomly selected from them, and a thicker core was taken from each tree by an increment borer (10 mm) and used for undertaking isotope analysis.

All the cores were air-dried, sanded and polished until wood cells were clearly visible under the microscope (Stokes and Smiley 1968). Then, they were visually cross-dated following the procedures described by Yamaguchi (1991). After a rigorous cross-dating, ring widths were measured at a resolution of 0.01 mm using a semi-automatic device Lintab 6 High Resolution digital positioning table and TSAP-Win Scientific Software. The quality of the cross-dating and ring-width measurements was verified using the COFECHA program (Holmes 1983).

2.3 δ^{13}C analysis

Five intact thick cores of each species were selected for δ^{13}C analysis. To minimize noise from short-term variations, rings from each 3-year period were pooled from the outer to pith (e.g. 2002–2004, 2005–2007 and 2008–2010). Wood samples representing 3-year increment for each core were carefully split out in sequence with a sharp blade. All the samples were oven-dried at 65° to constant weight and ground to fine powder with a ring grinder. The δ^{13}C values were analysed by weighing an amount of 2 to 3 mg in tin capsules, using a Sercon Hydra 20-22 isotope ratio mass spectrometer coupled with a Europa EA GSL sample prep system in Stable Isotope Laboratory, Griffith University, Brisbane, Australia. The spectrometer for δ^{13}C analysis was calibrated with sucrose (IAEA-CH-6) supplied by the Australian National University, and the mean standard deviation of δ^{13}C analysis of the sucrose reference standards was 0.16‰. δ^{13}C was calculated as follows:

$$\delta^{13}\mathrm{C} = \left(R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1\right) \times 1000 \tag{1}$$

where R_{sample} is ^{13}C/^{12}C ratio of a sample and R_{standard} ^{13}C/^{12}C ratio of the PeeDee Belemnite international standard (Xu et al. 2000).

2.4 Δ and iWUE

According to Farquhar et al. (1982), ^{12}C was preferentially used over ^{13}C during the plant's photosynthesis, which resulted in the carbon isotope discrimination (Δ), and Δ was defined as follows:

$$\Delta = \left(\delta^{13}\mathrm{C}_{\mathrm{air}} - \delta^{13}\mathrm{C}_{\mathrm{plant}}\right)/\left(1 + \delta^{13}\mathrm{C}_{\mathrm{air}}/1000\right) \tag{2}$$

where $\delta^{13}\mathrm{C}_{\mathrm{air}}$ and $\delta^{13}\mathrm{C}_{\mathrm{plant}}$ were the carbon isotope ratios in the atmospheric CO_2 and plants, respectively. Farquhar et al. (1982) also described the dependence of Δ on plant physiological properties, in particular on c_i/c_a:

$$\Delta = a + (b - a)(c_{\mathrm{i}}/c_{\mathrm{a}}) \tag{3}$$

where the constant a (4.4 ‰) referred to the enrichment during CO_2 diffusion, and b (27.0 ‰) was the fractionation by Rubisco against $^{13}CO_2$. iWUE was calculated by the following (Ehleringer and Cerling 1995):

$$\mathrm{iWUE} = A/g_s = (c_a - c_i)/1.6 = c_a(b - \Delta)/1.6(b - a) \tag{4}$$

Annual c_a was obtained from Law Dome Ice Core 2000-Year CO_2, CH_4 and N_2O data set (Etheridge et al. 1996; Etheridge et al. 1998; MacFarling 2004; MacFarling et al. 2006), available at http://www.ncdc.noaa.gov/paleo/icecore/antarctica/law/law_data.html.

Table 1 Locations of two sampling sites and characteristics of soils (0–10 cm and 10–20 cm)

Site	Longitude (E)	Latitude (N)	Elevation (m.a.s.l.)	pH[a]	Total carbon (%)	Total nitrogen (%)	δ^{13}C (‰)
SDW	115° 43′ 19.9″	31° 10′ 48.8″	880	4.69; 4.77	4.69; 3.02	0.26; 0.16	−26.8; −26.1
ZRF	115° 45′ 32.3″	31° 06′ 49.0″	1,500	4.74; 4.92	1.08; 0.27	0.07; 0.02	−27.3; −24.7

[a] The first value is for the surface soils at 0–10 cm and the second for 10–20 cm

2.5 Tree growth

The variation in tree growth could be recorded by the width of the annual rings. However, ring width in mature trees might biologically decline with tree age or size; thus, a declining growth trend may be suspected if using ring width alone as growth decline detector (Phipps and Whiton 1988). To overcome this problem, ring width could be converted into the basal area increment (BAI), and unlike ring width, age-related trends in unstandardized BAI were generally positive, culminating in a linear level which could be maintained for many decades (Phipps and Whiton 1988; LeBlanc 1990; Pedersen 1998). Therefore, BAI had been suggested as a better means for assessing tree growth, and a negative trend in BAI was a strong indication of a real decline in tree growth (LeBlanc 1990; Pedersen 1998). The conversion from ring width into BAI is based on the assumption that increment was uniform along each ring and used the following formula:

$$\mathrm{BAI} = \pi\left({R_n}^2 - {R_{n--1}}^2\right) \tag{5}$$

where R was the tree radius at breast height, and n was the year of tree ring formation.

2.6 Statistical analysis

Regression analysis was used to quantify the significant temporal trends in climate from Macheng station during 1980–2009 and the significant relationships of c_i, iWUE and BAI with elevated c_a and climate. Partial correlation analysis was used to distinguish whether c_a or climate factors, especially temperature, induced the changes of c_i and iWUE.

3 Results

3.1 Variations of δ^{13}C, Δ, c_i, iWUE and BAI

Overall, the variations of δ^{13}C, Δ, c_i and iWUE over time were very similar for the two tree species in the past 130 years. δ^{13}C of *P. tabulaeformis* and *P. taiwanensis* declined with the calendar year (Figs. 2a and 3a). The former decreased from −24.3 to −26.2‰ during 1897–2010 (Fig. 2a), while the latter from −23.8 to −26.4‰ during 1882–2010 (Fig. 3a). Both of them mainly reflected the trend of $\delta^{13}C_{air}$ in the same period. When the atmospheric δ^{13}C effect on plant carbon isotopic ratios was excluded, Δ was found to have no obvious change over time for *P. tabulaeformis* and *P. taiwanensis* (Figs. 2b and 3b). Conversely, c_i increased significantly over time for the two tree species, and that of c_i of *P. tabulaeformis* increased from 176 to 230 ppm during 1897–2010 (Fig. 2c) while *P. taiwanensis* from 167 to 233 ppm during 1882–2010 (Fig. 3c). Accordingly, iWUE also increased distinctly with the calendar year. In the past 130 years, iWUE of *P. tabulaeformis* increased from 71 to 97 μmol mol^{-1} (Fig. 2d) while that of *P. taiwanensis* from 75 to 94 μmol mol^{-1} (Fig. 3d), and the increases were 37 and 25 %, respectively.

The variations of tree BAI were different for *P. tabulaeformis* and *P. taiwanensis* in the past 130 years. The former firstly increased with the calendar year during 1897–1993, but decreased slightly in the recent 20 years (Fig. 2e). However, BAI of *P. taiwanensis* did not present obvious change in the period 1882–2010 (Fig. 3e).

3.2 Relationships of c_i, iWUE and BAI with c_a and climate

The regression analysis indicated that both c_i and iWUE of *P. tabulaeformis* and *P. taiwanensis* increased linearly with elevated c_a in the past 130 years (Figs. 4a, c and 5a, c) and with mean annual temperature during 1960–2007 (Figs. 4b, d and 5b, d), but there was no significant relationship between c_i or iWUE with annual precipitation. Partial correlation analysis showed that both c_i and iWUE significantly correlated with c_a when mean annual temperature was controlled during 1960–2007 (Table 2); nevertheless, the correlation of mean annual temperature with c_i or iWUE would be negligible when c_a was controlled (Table 2). This demonstrated that elevated c_a, not temperature, induced the changes in c_a and iWUE.

BAI of *P. tabulaeformis* responded quadratically to elevated c_a in the past 130 years (Fig. 6a). BAI increased with rising c_a until it peaked at about 350 ppm (around 1990) and decreased thereafter. Although very unusual BAI variations were present for *P. taiwanensis* in the earlier period (hollow points in Fig. 6b), BAI also responded quadratically to c_a since 1975, when c_a reached about 330 ppm (solid points in Fig. 6b). The coming decrease in BAI of *P. taiwanensis* indicated that tree growth was transforming from increases to decreases in the recent several years. However, BAI of *P. tabulaeformis* and *P. taiwanensis* correlated with neither annual mean temperature nor annual precipitation during 1960–2007.

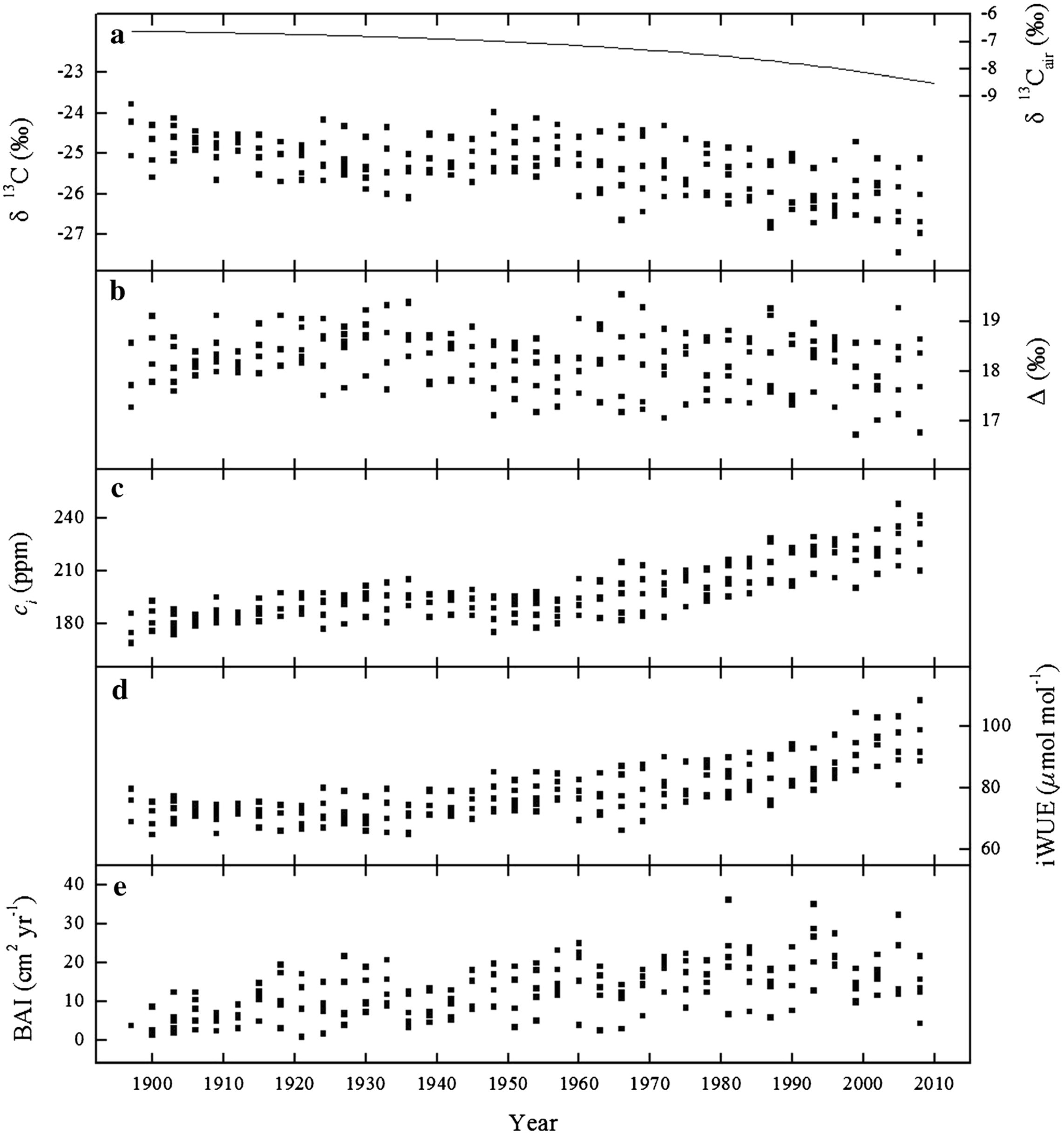

Fig. 2 Variations in carbon isotope ratios (δ^{13}C, **a**), carbon isotope discrimination (Δ, **b**), leaf internal CO$_2$ concentration (c_i, **c**), intrinsic water-use efficiency (iWUE, **d**) and basal area increment (BAI, **e**) for *Pinus tabulaeformis* Carr. from 1897 to 2010. The *upper curve in **a*** is the variation of carbon isotope ratios in the atmospheric CO$_2$

4 Discussion

4.1 Long-term trends in iWUE and BAI

In this study, iWUE of two subtropical tree species from central China were found to increase in the past 130 years. The increases in iWUE have demonstrated a global scale (Peñuelas et al. 2011; Silva and Horwath 2013), especially during the last century, which has been well-established by studies in the boreal (Barber et al. 2000; Saurer et al. 2004; Ivlev and Voronin 2007; Gagen et al. 2008; Kirdyanov et al. 2008; Sidorova et al. 2008), temperate (Bert et al. 1997; Duquesnay et al. 1998; Feng 1999; Waterhouse et al. 2004; Peñuelas et al. 2008; Battipaglia et al. 2013), Mediterranean (Saurer et al. 2003; Peñuelas et al. 2008; Linares et al. 2009; Andreu-Hayles et al. 2011; Maseyk et al. 2011), arid regions (Liu et al. 2007; Liu et al. 2008) and high-altitudinal forests (Gomez-Guerrero et al. 2013). Long-term improved iWUE trends were not unexpected for subtropical and tropical trees (Hietz et al. 2005; Silva et al. 2009; Sun et al. 2010; Brienen et al. 2011; Nock et al. 2011), although only a few studies on this existed to date. Here, we showed more evidence to confirm that iWUE of subtropical trees did increase with elevated c_a in the past century.

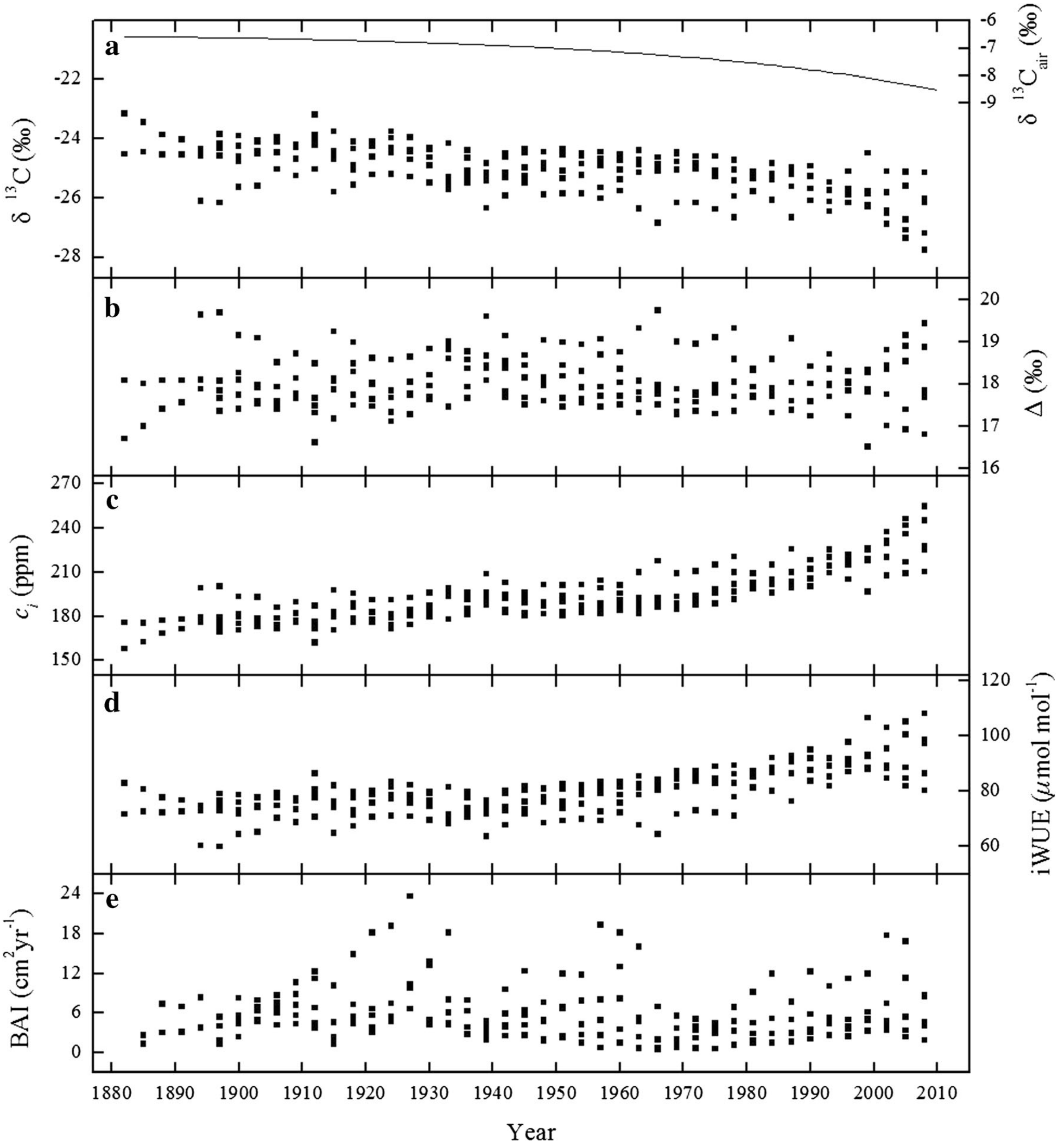

Fig. 3 Variations in carbon isotope ratios ($\delta^{13}C$, **a**), carbon isotope discrimination (Δ, **b**), leaf internal CO_2 concentration (c_i, **c**), intrinsic water-use efficiency (iWUE, **d**) and basal area increment (BAI, **e**) for *Pinus taiwanensis* Hayata from 1882 to 2010. The *upper curve in* ***a*** is the variation of carbon isotope ratios in the atmospheric CO_2

Trees of *Melia azedarch*, *Chukrasia tabularis* and *Toona ciliate* in a tropical monsoon forest in western Thailand showed to have iWUE improved 0.33, 0.38 and 0.38 μmol mol^{-1} per ppm increase in c_a, respectively (Nock et al. 2011). Hietz et al. (2005) reported that iWUE of *Cedrela odorata* and *Swietenia macrophylla* of a tropical moist forest in northern Brazil increased 0.24 and 0.30 μmol mol^{-1} per ppm, respectively, from 1850 to 1990. Another tree species, *Araucaria angustifolia* from southern Brazil was found that the increases of iWUE in forest and grassland ecosystem were 0.47 and 0.33 μmol mol^{-1} per ppm, respectively (Silva et al. 2009). In subtropical forest of southern China, the increase in iWUE of *Pinus massoniana* was 0.44 μmol mol^{-1} per ppm (Sun et al. 2010). In this study, iWUE of *P. tabulaeformis* and *P. taiwanensis* increased about 0.46 and 0.34 μmol mol^{-1} per ppm, within the range (0.33–0.47 μmol mol^{-1} per ppm) of exiting studies on long-term iWUE trends in tropical and subtropical tree species. Higher increase was detected in the recent study (Brienen et al. 2011) that iWUE of *Mimosa acantholoba* from a tropical dry forest in south Mexico increased 0.52 μmol mol^{-1} per ppm. These different increases in iWUE showed that besides tree species difference, divergences in temperature, air humidity and soil water availability were also important factors that caused the

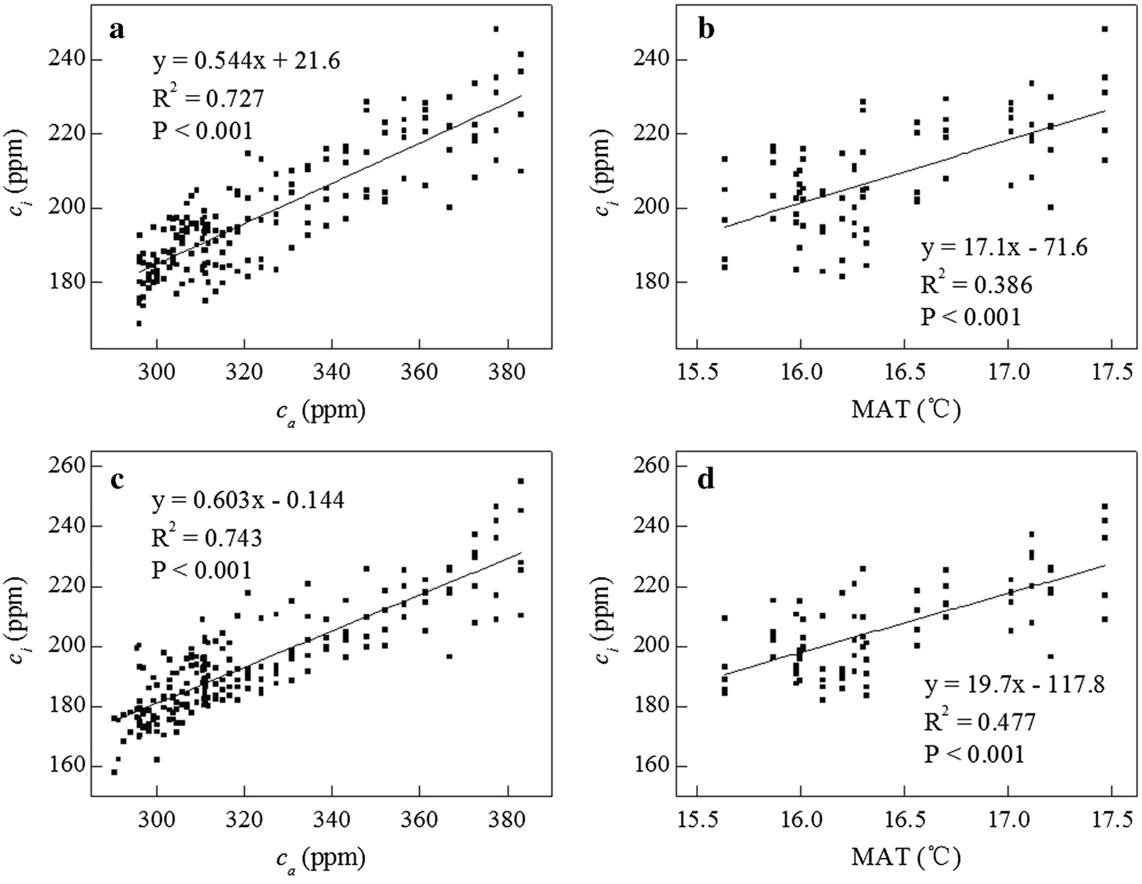

Fig. 4 Relationships between leaf internal CO_2 concentration (c_i) and atmospheric CO_2 concentration (c_a, **a** for *Pinus tabulaeformis* Carr. from 1897 to 2010, **c** for *Pinus taiwanensis* Hayata from 1882 to 2010) and between c_i and mean annual temperature (MAT, **b** for *Pinus tabulaeformis* Carr., **d** for *Pinus taiwanensis* Hayata) from 1960 to 2007

differences in the physiological responses of plants to elevated c_a (Arneth et al. 2002; Saurer et al. 2004; Waterhouse et al. 2004).

Long-term trends in BAI for *P. tabulaeformis* since 1897 and *P. taiwanensis* during 1975–2010 continuously increased for many decades but decreased in the recent decades, which were common either in tropical and subtropical forests (Silva et al. 2009; Sun et al. 2010; Nock et al. 2011) or other terrestrial ecosystems (Peñuelas et al. 2008; Silva et al. 2010; Gomez-Guerrero et al. 2013). Widespread declines in tree BAI in recent years can be attributed to the fact that the negative effects of some factors on tree growth, such as climate change (particularly warming-induced drought) (Silva et al. 2010; Sun et al. 2010; Nock et al. 2011), nutrient limitation and physiological long-term acclimation to elevated c_a, have overridden the potential growth benefits from a CO_2-rich environment (Xu et al. 2009). However, abnormal BAI trends in *P. taiwanensis* in the earlier period could not be explained reasonably at present and a paradoxical cause may be that very anomalous, not concentrically radial growths led to large errors in conversion from ring width to BAI, especially when trees were younger.

4.2 Effects of elevated c_a and climate on iWUE and BAI

The three scenarios of gas exchange responses proposed by Saurer et al. (2004) are useful for better understanding of physiological reactions of trees to elevated c_a: (1) a constant c_i leads to decreasing c_i/c_a and increasing iWUE, (2) a constant c_i/c_a, due to c_i increasing in a proportional way with c_a, indicates that the linkage between g_s and A is largely maintained under elevated c_a (Medlyn et al. 2001) and also causes increasing iWUE, and (3) a constant c_a-c_i, due to c_i increasing at the same rate with c_a, with no improvement in iWUE and no active stomatal responses. The variations in c_i/c_a and c_a-c_i of *P. tabulaeformis* and *P. taiwanensis* (Fig. 7) showed that they experienced two phases in the past 130 years: c_a-c_i remained constant before 1940 (hollow points in Fig. 7b, d), and c_i/c_a were relatively stable after 1940 (solid points in Fig. 7a, c). This indicates that the stomatal responses were converted around 1940, when c_a reached approximately 310 ppm. In the first stage, c_i increased at the same rate with c_a; therefore, no active stomatal responses occurred and iWUE did not present obvious change (Figs. 2d and 3d). After c_a exceeded 310 ppm, the relationship between c_i and

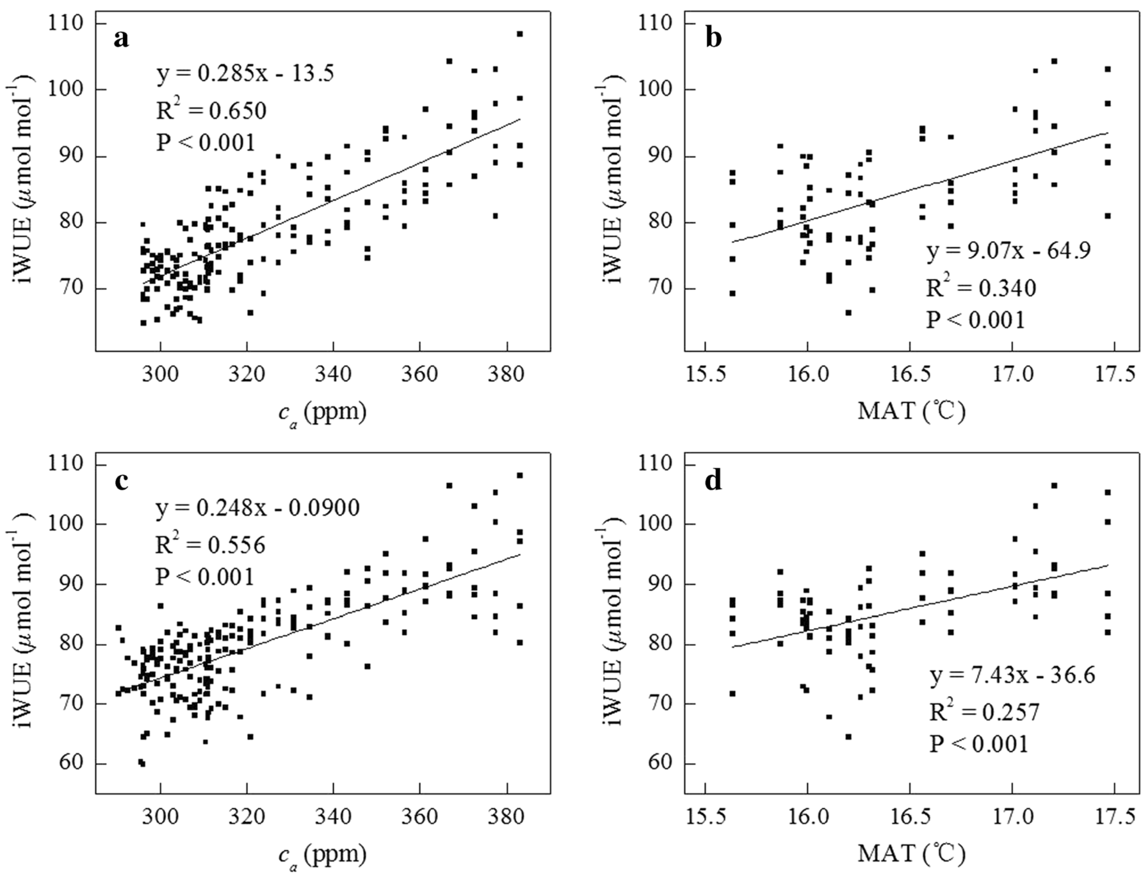

Fig. 5 Relationships between intrinsic water-use efficiency (iWUE) and atmospheric CO$_2$ concentration (c_a, **a** for *Pinus tabulaeformis* Carr. from 1897 to 2010, **c** for *Pinus taiwanensis* Hayata from 1882 to 2010) and between iWUE and mean annual temperature (MAT, **b** for *Pinus tabulaeformis* Carr., **d** for *Pinus taiwanensis* Hayata) from 1960 to 2007

c_a was transformed from constant $c_a - c_i$ to constant c_i/c_a, which meant that stomata began to actively respond to elevated c_a, but c_i could not keep peace with c_a and led to evident increase in iWUE (Figs. 2d and 3d).

Table 2 Partial correlation coefficients of the leaf internal CO$_2$ concentration (c_i) and intrinsic water-use efficiency (iWUE) with atmospheric CO$_2$ concentration (c_a) and annual mean temperature (MAT)

Tree species	Controlled variables	Correlation coefficient	
		Between c_i and uncontrolled variable	Between iWUE and uncontrolled variable
Pinus tabulaeformis Carr.	MAT	0.526*	0.407*
	c_a	−0.039	0.039
Pinus taiwanensis Hayata	MAT	0.506*	0.449*
	c_a	0.080	−0.080

*0.001 level of correlation is significant

Higher iWUE may be caused from reduced g_s, increased A or a combination of both. Although the reason that caused the increasing iWUE could not be determined by δ^{13}C analysis alone, variations in c_i over time may provide some insights into changes of A over time. The c_i of both *P. tabulaeformis* and *P. taiwanensis* increased in the past 130 years (Figs. 2b and 3b), which could prove indirectly that A of two tree species increased over time. For *P. tabulaeformis*, increased A could be considered as the primary factor that resulted in the increase in iWUE during 1940–1990. Nevertheless, A may not increase any more in the recent 20 years because a reduced BAI was detected with elevated c_a, and reduced g_s may contribute to higher iWUE more than increased A. It is not contradictory between reduced g_s and increased c_i, since individual open stomata can absorb more CO$_2$ under very high c_a and keep c_i increasing, even though stomata partially close (Xu et al. 2009). Water deficit is usually regarded as the cause of stomata closing. Although the precipitation did not change in the study area from 1959 to 2009 (Fig. 1c), an obvious warming trend was found during 1980–2009 (Fig. 1b), which could cause the evaporation and transpiration increase. Therefore, the plants need to conserve water by reducing g_s. Similar stomatal responses were applied to *P. taiwanensis*, but A continued increasing until around 2005 and g_s began to decrease about a few years ago. In this study, the sampling site of *P. taiwanensis* is located at a higher elevation than *P. tabulaeformis* by approximately 620 m. As the lower

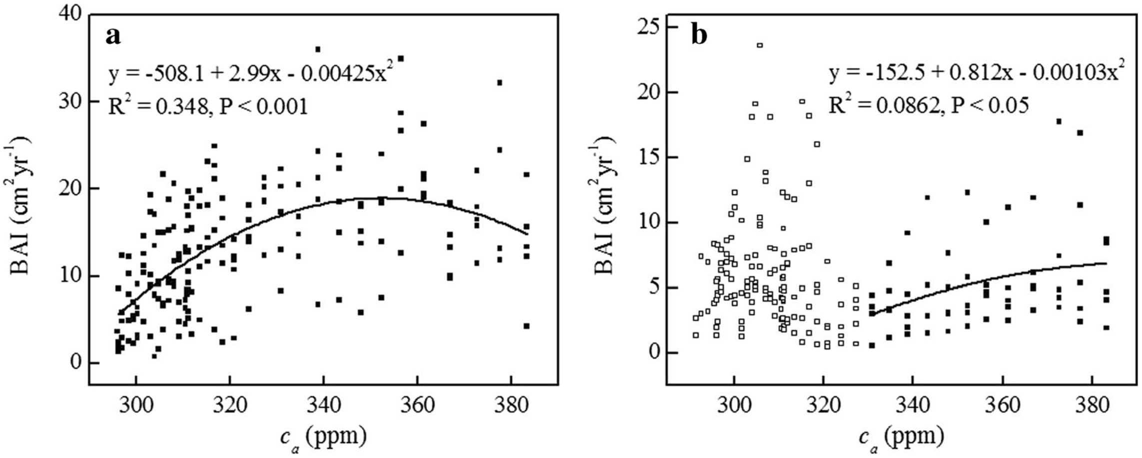

Fig. 6 Relationship between basal area increment (BAI) and atmospheric CO_2 concentration (c_a) for *Pinus tabulaeformis* Carr. (**a**) and *Pinus taiwanensis* Hayata (**b**)

temperature and more rainfall at the higher locations are beneficial to reduce plant evaporation and transpiration, *P. taiwanensis* responded to close parts of stomata later than *P. tabulaeformis*, and correspondingly, the decreases in BAI with c_a lagged behind *P. tabulaeformis*. Overall, our research findings have been consistently with those of Linares and Camarero (2012) and Silva and Anand (2013), which have highlighted that about 66–81 % of the iWUE and 28–56 % of the BAI could be attributed to the rising c_a, with 11–21 % of the BAI explained by the c_a (Linares and Camarero 2012) and that the iWUE increased by 10–60 % with the rising c_a, but tree growth varied widely, from the increases in high latitudes (>40°N), to progressively lower increases towards lower latitudes, as compared with 15–55 and 7–10 % decreases in tree growth for tropical and subtropical forests, respectively, with the mixed tree growth responses for the other regions (Silva and Anand 2013).

5 Conclusions

In this study, similar trends in $\delta^{13}C$, Δ, c_i and iWUE with elevated c_a and climate change were found for *P. tabulaeformis* and *P. taiwanensis* from a subtropical forest

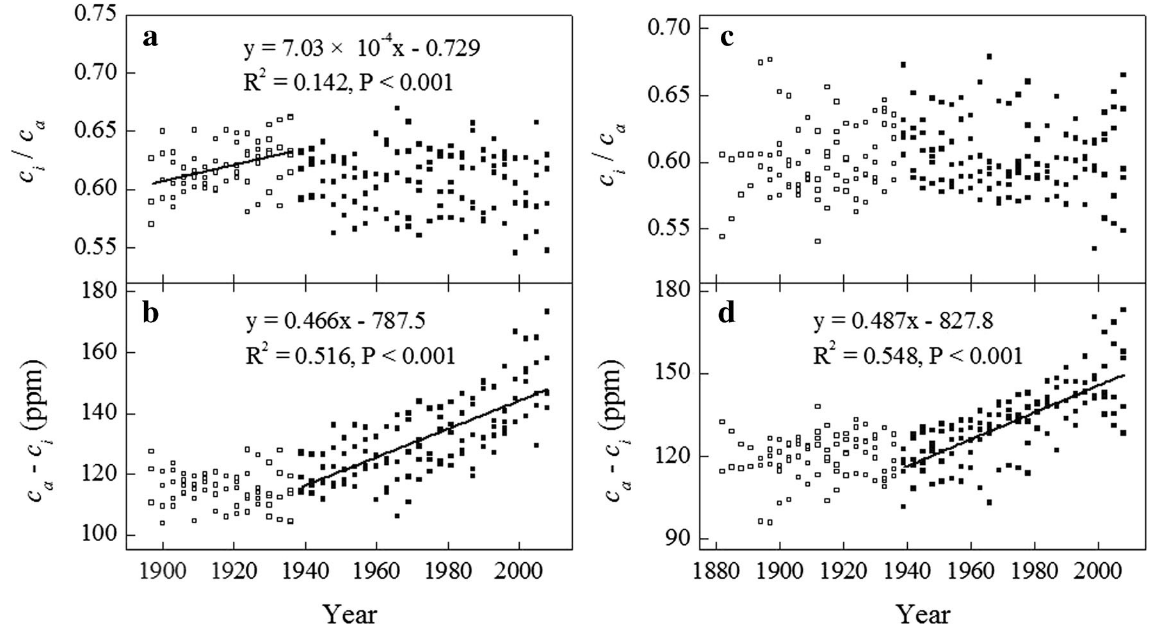

Fig. 7 Variations of leaf internal CO_2 concentration divided by atmospheric CO_2 concentration (c_i/c_a) and atmospheric CO_2 concentration minus leaf internal CO_2 concentration (c_a-c_i) over time, **a** and **b** for *Pinus tabulaeformis* Carr. and **c** and **d** for *Pinus taiwanensis* Hayata

located in central China. Two tree species demonstrated declining trends in BAI in recent years, although the variations of BAI were not uniform in the past 130 years. Continuous increases in iWUE could not prevent the decreases in tree growth in recent years, because negative effects of some factors on tree growth, such as climate change (particularly warming-induced drought), nutrient limitation and physiological long-term acclimation to elevated c_a, have overridden the CO_2 fertilization effects.

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