The Intra-Annual Intrinsic Water Use Efficiency Dynamics Based on an Improved Model

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Abstract

The carbon isotope fractionation value (Δ) has been widely used to infer the intrinsic water use efficiency (iWUE) of C3 plants. Currently, the most commonly used iWUE method (expressed as iWUE_{tra}) in tree rings assumes that the mesophyll conductance in plants is infinite. However, many observation-based studies have pointed out that such an assumption leads to overestimating the impact of carbon dioxide (CO₂) on intrinsic water use efficiency in plants. In this study, a constant g_s / g_m ratio (0.79) was introduced for calculating iWUE (expressed as iWUE_{mes}). We applied this iWUE_{mes} model to our newly developed intra-annual (10 samples per ring) $\Delta^{13}\text{C}$ chronology of *Cryptomeria fortunei* tree for 1965–2017 at Gu Mountain Area and our annual $\Delta^{13}\text{C}$ chronology of *Pinus massoniana* tree for 1865–2014 at Niumulin Natural Reserve in southeast China. Using dendrochronology techniques, our analysis revealed that the current iWUE_{tra} model overestimates the iWUE values by approximately 2 times and that the iWUE value of trees inferred from iWUE_{mes} modelling decreased significantly in summer-autumn time, which may indicate that alternative factors play a role in limiting the degree of iWUE improvement under the drought-stressed forest in southeast China.

1. Introduction

Stable isotopes in tree rings could provide palaeoclimate reconstructions with perfect annual resolution and statistically defined confidence limits to study a variety of natural phenomena or events(G, D, Farquhar *et al.*, 1989; Danny McCarroll, 2004; J Huang *et al.*, 2007; Schnyder *et al.*,2012; Lucas C. R. Silva *et al.*, 2013; X Liu *et al.*, 2014; Millicent Smith *et al.*, 2016). For example, the methods involving the stable carbon isotope ratios in tree rings often used to reconstruct past climate and environmental changes(G, D, Farquhar *et al.*, 1989; Ehleringer *et al.*, 1993; Franks *et al.*, 2013) and to study the physiological and ecological changes of trees caused by past climate regimes(Tei *et al.*, 2014).

Intrinsic water use efficiency (iWUE) in plants is a physiological efficiency that represents the ratio of net assimilation (A) and stomatal conductance to water vapor (g_w). It reflects the relationship between plant water consumption and dry matter production, therefore is a comprehensive physiological and ecological index for evaluating the suitability of plant growth (Danny McCarroll, 2004; Schweingruber *et al.*, 2004). The increase in atmospheric CO₂ levels can theoretically increase the intercellular CO₂ concentration of plant leaves, and therefore, can promote the photosynthesis of plants, resulting in a “fertilization effect”(J Huang *et al.*, 2007; Ze’ev Gedalof *et al.*, 2010). In addition, this increase in atmospheric CO₂ concentration also causes a decrease in stomatal conductance(I. C. Prentice *et al.*, 2009; Lucas C. R. Silva *et al.*, 2013). The resulting ratio of the net photosynthetic rate of the plant to the stomatal conductance, i.e. iWUE, shows an increasing trend, which

can alleviate drought to a certain extent and is beneficial to the growth of plants in arid and semi-arid areas (Leavitt *et al.*, 1993; Todd E. Dawson *et al.*, 2002; Andrea Scartazza *et al.*, 2014; X Liu *et al.*, 2014; Pei-Li Fu *et al.*, 2016).

The current iWUE model based on stable isotopes of tree rings as proposed by Farquhar *et al.* (Farquhar *et al.*, 1989), is calculated as follows: $iWUE = A/g_w$
 $= C_a(1 - C_a/C_i) \times 0.625$ (where C_a and C_i are the CO₂ concentrations outside and inside the stomata of plant leaves, respectively. A is the assimilation rate of CO₂ by plant leaves, and g_w is the stomatal conductance of plant leaves). This model has been widely used to infer iWUE based on the $\delta^{13}C$ values in climate archives, such as pasture (Schnyder *et al.*, 2012), tree rings (Franks *et al.*, 2013; Zuidema *et al.*, 2015), or animal tissue (INÊS C. R. BARBOSA *et al.*, 2010). Compared with the instantaneous iWUE measured by gas exchange, the biomass-based Δ provides a time-integrated iWUE and enables time series analysis from days to thousands of years (Schweingruber *et al.*, 2004; McElwain *et al.*, 2019; Adams *et al.*, 2020).

However, some researchers consider that the traditional model's biased prediction of iWUE limits its application to qualitative assessment (Medrano *et al.*, 2010). The main limitation of using the traditional iWUE model ($iWUE_{tra}$) is the simplification of the mesophyll conductance (g_m , the diffusion conductance of CO₂ from the intercellular space to the carboxylation site). Theoretically, this assumption leads to overestimating the iWUE (W Ma *et al.*, 2020), as g_m is not conservatively high (Medrano *et al.*, 2010).

In other words, it may not be appropriate to apply an infinite or species-specific constant g_m in the iWUE model. In general, the wide application of the $^{12}\text{C}/^{13}\text{C}$ isotope fractionation method in calculating the iWUE usually ignores the influence of the mesophyll conductance, either because of technical reasons (difficult to measure mesophyll conductance), or because its impact is assumed to be relatively small (assuming infinite g_m exists in iWUE_{tra}) (Berry *et al.*, 2008; Franks *et al.*, 2013; Zsofa R. Stangl *et al.*, 2019).

In southeast subtropical China, the Western Pacific Subtropical High (WPSH) causes high temperatures and low rainfall during the summer-autumn time (Yong Hua *et al.*, 2013). Li *et al.* (2017) suggested that the contribution of iWUE to the tree growth in southeast China is not significant in most periods (Li *et al.*, 2017). The growth promotion of the increased iWUE on tree growth in southeast China was later determined by Li *et al.* (2019) to not compensate for the growth limitation caused by drought (Y Li *et al.*, 2019). The above results were all based on analyses of the entire rings, and iWUE_{tra} modelling has shown similar conclusions. In such models, the growth was not stimulated, but the iWUE increased significantly, and the “ CO_2 fertilization effect” was observed. This shows that atmospheric CO_2 concentration on the annual scale is probably not the only factor that affects the iWUE of trees in different regions. Yet, the cause and influencing factors of the iWUE changes during the growing season are still unknown.

Here, we hypothesize that, because of higher climate variability and more stable CO₂ concentration, the intra-annual scale can better reflect how the iWUE changes and what affects it. To test this, a higher-resolution intra-annual tree-ring $\delta^{13}\text{C}$ chronology from 1965 to 2017 at Gu Mountain Area (GM), combined with our tree-ring $\delta^{13}\text{C}$ data from Niumulin Natural Reserve (NML) that has previously been published (Li *et al.*, 2017), were established. This study aims to: 1) calculate the iWUE of trees at GM using the higher-resolution iWUE_{mes} model that includes the g_m effect; 2) show the characteristics of iWUE on the intra-annual (representing the drastic changes of climate variables from winter to summer) and annual scales (representing the longer inter-annual change of CO₂ concentration) with two different models; and 3) further disentangle the relationship between C_a , climate, and iWUE under drought-stressed conditions.

2. Data and methods

2.1 Sampling site and climate data

Our study sites were at Gu Mountain Area (GM, 25°20'–26°05'N, 119°22'–119°25'E, about 8 kilometers away from the downtown area of Fuzhou City) and Niumulin Natural Reserve (NML, 25°23'–25°25'N, 117°55'–117°57'E, data has been published) (Li *et al.*, 2017) (Fig. 1). GM is located in the transition zone from the south subtropical zone to the mid-subtropical zone. It is warm and humid with abundant rainfall throughout the year (Zhang *et al.*, 2009). According to the nearest Fuzhou meteorological observation data from 1953 to 2019, the average annual precipitation

is 1366 mm, and the average annual temperature is 19.9 °C. *Pinus massoniana* tree and *Cryptomeria fortunei* tree are constructive species within the well-protected forests. In July of 2018, a total of 186 tree cores were collected from a sample of 87 living *Cryptomeria fortunei* trees with a mean age of 75 years at GM. These were collected using 5 mm diameter increment borers to extract 2–3 cores from each tree at a height of 1.3 m. The climate data of GM and NML sampling sites are obtained from the nearby Fuzhou meteorological station and Yong'an meteorological station, respectively.

In order to obtain more real-time data on intercellular CO₂ concentration during the growing season for trees, we downloaded the monthly global atmospheric CO₂ concentration observation data from the Mauna Loa Observatory in Hawaii (https://scrippsco2.ucsd.edu/data/atmospheric_co2.html), USA, for the period 1958–2017. Furthermore, we calculated additional variables (including meteorological variables and atmospheric CO₂ concentration) corresponding to 10 intra-annual periods based on the length of the tree growing season (April–October) at GM.

2.2 Experimental treatment

2.2.1 Separation of high-resolution intra-annual tree rings

Cores were air dried and hand-sanded with sand paper up to 1200 grit. Visual cross-matching and skeleton plots were used to assign a calendar date to each

ring(Stokes & Smiley, 1968). Using the results from the COFECHA and the moving correlation coefficient between a single sample core and the master sequence, we selected a total of 6 *Cryptomeria fortunei* tree cores with higher correlation coefficients, longer sequences, relatively wide annual rings, no obvious differences in growth, and with the fewer missing rings. These were used to carry out high-resolution experiments of stable carbon isotopes in tree rings. In this study, the average ring width of *Cryptomeria fortunei* tree we used is 5 mm. In order to obtain more accurate and high-resolution intra-annual sub-samples, we measured the width of each annual ring of each tree core in advance and divided it into 10 sub-samples equally. We then cut off each sub-ring using a dissecting scalpel according to the calculated width value.

2.2.2 Chemical treatment and stable isotope measurement

With a slight improvement on the method proposed by Liu Xiaohong(X LIU *et al.*, 2007), we followed the standard method(Leavitt *et al.*, 2008) to mix each intra-annual sub-sample to extract α -cellulose from the wood. We used custom-made hourglass tubes to realize the full reaction of the chemistry reagent, thereby speeding up the further steps and saving experimental time.

We packed 0.08–0.12 mg of α -cellulose in tin capsules for stable isotope measurement using the Flash Elemental Analyzer (Flash 2000) coupled with a Thermo Scientific MAT 253 (Thermo Electron Corporation, Bremen, Germany).

Each sample was repeatedly measured two to four times. The Charcoal Black (standard sample, $\delta^{13}\text{C} = -22.43\text{‰}$) was used to calibrate the values of $\delta^{13}\text{C}$ gained from tree-ring α -cellulose. For convenience, the rate of stable carbon isotope ($^{13}\text{C}/^{12}\text{C}$) was defined in delta (δ) according to the Vienna Pee Dee Belemnite (VPDB) standard(Leavitt & GEOL, 2008), in parts per thousand (‰):

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

where R_{sample} and R_{standard} represent the $^{13}\text{C}/^{12}\text{C}$ ratios of tree-ring α -cellulose sample and VPDB standard, respectively. Our isotopic measurements were stable with a low standard deviation of 0.15‰.

Isotopic discrimination between atmospheric CO_2 carbon and plant carbon (Δ ; see Farquhar & Richards, 1984) in C_3 plants is a result of the preferential use of ^{12}C over ^{13}C during photosynthesis, and it is defined as follows:

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p) / (1 + \delta^{13}\text{C}_p / 1000) \quad (2)$$

where $\Delta^{13}\text{C}$ is the stable carbon isotope discrimination, $\delta^{13}\text{C}_a$ and $\delta^{13}\text{C}_p$ are the stable carbon isotopic ratios ($^{13}\text{C}/^{12}\text{C}$) of ambient air and plant cellulose, $\delta^{13}\text{C}_a$ represents a constant value of -6.4‰ before 1850(Millicent Smith *et al.*, 2016), and the $\delta^{13}\text{C}_a$ value after 1850 is calculated based on ice core bubbles and monitoring data(X Liu *et al.*, 2014).

2.2.3 Stable carbon isotopic analysis

The field tree growth monitoring research conducted at the GM study site shows that

the cambium cells of *Cryptomeria fortunei* begin to expand around April, and the entire lignification process ends around November. Combined with dendrometer data, we determined that the entire growing season of *Cryptomeria fortunei* in the study site is approximately from April 6th to November 5th each year, of which June 15th is the dividing line between earlywood and latewood. To find this, we used the method developed by Berkelhammer and Stott (Max Berkelhammer *et al.*, 2009), combined with the observed growth dates of the earlywood and latewood of the *Cryptomeria fortunei*. We then used the following equation to assign dates to the 10 sub-samples in each ring and recorded the dates of these samples' stable carbon isotope values (Table 1):

$$Date = s_i + (n_s/n_t) \cdot e_s \quad (3)$$

where s_i is the date of the start of growing season, which is taken as April 6 and remains unchanged; e_s is the length of the earlywood growing season (70 days for earlywood; 203 days for latewood); n_s is the sample number; and n_t is the total number of sub-samples in each ring.

2.3 Intrinsic water use efficiency (iWUE)

2.3.1 Traditional intrinsic water use efficiency model (iWUE_{tra})

We assumed that the values of C_a and $\delta^{13}C_a$ at the sampling site are equal to the atmospheric CO₂ concentration and its carbon isotope, respectively, as previous studies have revealed (Li *et al.*, 2017; Y Li *et al.*, 2019). The discrimination of atmospheric CO₂ by plants (the fractionation of carbon isotopes ($\Delta^{13}C$)) is an

important indicator of the intrinsic water use efficiency of plants. After conversion,
the following formula is obtained(G, D, Farquhar *et al.*, 1989):

$$iWUE = \frac{A}{g_w} = \frac{C_a - C_i}{1.6} = \frac{C_a(1 - C_i/C_a)}{1.6} \quad (4)$$

where A is the net photosynthetic rate of the plant; g_w is stomatal conductance to water
vapor; C_a and C_i are the CO_2 concentration outside and inside the stomata of plant
leaves, respectively; and the value of C_i/C_a in the formula can be calculated using Eq.
5(G, D, Farquhar *et al.*, 1989):

$$\frac{C_i}{C_a} = \frac{\Delta^{13}\text{C} - a}{b - a} \quad (5)$$

where a (4.4‰) represents the isotope discrimination of atmospheric CO_2 entering in
the intercellular space and b (27‰) represents the isotope discrimination value due to
the carboxylation(G, D, Farquhar *et al.*, 1989).

Therefore, based on the linear formula between $\Delta^{13}\text{C}$ and C_a , the traditional model
of $iWUE$ is obtained(JAMES R. EHLERINGER *et al.*, 1995):

$$iWUE_{tra} = \frac{C_a(1 - C_i/C_a)}{1.6} = \frac{C_a(b - \Delta^{13}\text{C})}{1.6(b - a)} = \frac{C_a}{1.6} \times \frac{b - \Delta^{13}\text{C}}{b - a} \quad (6)$$

where the subscript “*tra*” represents the traditional simple intrinsic water use
efficiency model.

2.3.2 Improved intrinsic water use efficiency model ($iWUE_{mes}$)

Here, we used an improved model of $iWUE$ that includes the effect of g_m (denoted as
 $iWUE_{mes}$)(Wei Ting Ma, 2020).

$$iWUE_{mes} = \frac{A}{g_w} = \frac{C_a}{1.6} \cdot \frac{b' - \Delta - f' \frac{\Gamma^*}{C_a}}{b' - a_s + \frac{g_{sc}}{g_m} \cdot (b' - a_m)} \quad (7)$$

where the subscript “mes” indicates that this expression accounts for mesophyll conductance effects; C_a is the CO₂ mole fraction in the atmosphere; a_m (1.8‰) is the fractionation associated with CO₂ dissolution and diffusion in the mesophyll; Γ^* is the CO₂ compensation point in the absence of mitochondrial respiration; b' (29‰) and f' (11‰) represent the fractionations due to carboxylation and photorespiration, respectively; a_s (4.4‰) is the ¹²C/¹³C fractionations during CO₂ diffusion through the stomata; an error that can be corrected by using a constant g_{sc}/g_m is a constant ratio (0.79) based on measurements of a wide range of plant species from different functional groups (including grasses and herbaceous legumes), in moist and dry conditions (W Ma *et al.*, 2020).

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According to the iWUE model, the intercellular CO₂ concentration value (Tei *et al.*) in the trees can be inferred using Eq. 8:

$$C_i = C_a - 1.6 \times iWUE \quad (8)$$

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236 3. Results

237 3.1 Tree-ring width chronology and climate-growth relationship

The annual tree-ring width variability of *Cryptomeria fortunei* at the GM site mainly showed three stages: a downward trend of width from 1965 to 1985, an upward trend with the highest width level between 1986 and 2001, and another downward trend

from 2001 to 2017. In general, the tree-ring width across the whole period from 1965 to 2017 showed a "W-shaped" trend (Fig. S1).

The standard chronology of tree-ring width (STD) at the GM site showed the most significant negative correlation with temperature and sunshine hours in the previous July, the current June, and the current June to September, respectively. Meanwhile, the STD at the GM site showed the most significant positive correlation with relative humidity in the current June, July, October, and the current June to September (Fig. 2).

The intra-annual tree-ring width of *Cryptomeria fortunei* at the GM site fluctuated: before 1985, the width was relatively narrow; then it increased rapidly and peaked in 1997; the width then gradually decreased between 1998 and 2007; after which, there followed a slowly increasing trend from 2008 to 2017 (Fig. S2a). After eliminating age-related growth trends (Fig. S2b), we found the STD to be relatively stable from 1965 to 2017, with a weak inter-decadal variability (Fig. S2b). The inter-decadal variability of STD increased steadily with time after 1980, reached a peak value in 2000, then declined slightly and increased again from 2010 to 2017, reaching a high value again in 2017.

3.2 Chronology of tree-ring $\Delta^{13}\text{C}$

For the study period of 1965–2017, we observed that the intra-annual average values

of both tree-ring $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ have clear seasonal patterns (Fig. S3). Overall, the intra-annual tree-ring $\delta^{13}\text{C}$ values decreased from April 18–April 29 to October 01–November 05 (-0.051), reaching the maximum during October 01–November 05 (Fig. S3). In addition, the intra-annual tree-ring $\Delta^{13}\text{C}$ is relatively stable (-0.24‰), reaching the minimum during April 06–April 17 and the maximum during April 18–April 29.

3.3 The relationship between tree-ring $\Delta^{13}\text{C}$ and climate variables at GM study site

We found that the intra-annual tree-ring $\Delta^{13}\text{C}$ at the GM site from 1965 to 2017 was positively correlated with relative air humidity from April 18 to September 30 ($P > 0.05$) and significantly correlated with the humidity for October 01–November 05 ($r = 0.351$, $p < 0.001$). The intra-annual tree-ring $\Delta^{13}\text{C}$ was significantly positively correlated with sunshine hours for April 06–April 17 and August 26–September 30. A significant negative correlation between the intra-annual tree-ring $\Delta^{13}\text{C}$ and the temperature were observed during the summer time (i.e., June 15–July 20, July 21–August 25, and October 01–November 05) (Fig. 4).

3.4 The iWUE_{mes} and iWUE_{tra} at study sites

At GM site, both the iWUE_{mes} (the slope of linear regression being: 0.04, $R^2 = 0.83$, $p < 0.001$) and iWUE_{tra} (the slope of linear regression being: 0.08, $R^2 = 0.83$, $p < 0.001$) chronologies showed significant increasing trends during the period from 1965 to

2017 (Fig. 5a). At the NML site, both the $iWUE_{mes}$ (the slope of linear regression being: 0.12, $R^2 = 0.84$, $p < 0.001$) and $iWUE_{tra}$ (the slope of linear regression being: 0.23, $R^2 = 0.83$, $p < 0.001$) chronologies, calculated with tree-ring $\Delta^{13}C$ series, showed significant increasing trends during the period from 1865 to 2014 (Fig. 5b).

At the GM site, the ratios of $iWUE_{tra}$ to $iWUE_{mes}$ were 1.88, 1.89, 1.90, 1.91, 1.92, 1.93, 1.96, 1.96, 1.94, and 1.91 during the 10 periods studied on the intra-annual scale, which gradually increased from April 06–April 17 to July 21–August 25 (4.6%) and peaked during July 21–August 25. At the NML study site, the average ratio of $iWUE_{tra}$ to $iWUE_{mes}$ was 2.05 on the inter-annual scale, which decreased from 2.08 in 1965 to 1.99 in 2014.

3.5 The intra-annual $iWUE_{mes}$ and $iWUE_{tra}$ at GM study sites

The trend of the intra-annual $iWUE$ values during April 18–May 22 at GM calculated from Eq.5 and Eq.6 is significantly consistent (the rate of decline for $iWUE_{tra}$ and $iWUE_{mes}$ are 2% and 1%, respectively). The notable difference between $iWUE_{tra}$ ($y = -0.5 \times x^2 + 1.57x + 117.74$, $R^2 = 1$) and $iWUE_{mes}$ ($y = 0.115 \times x^2 - 0.505x + 61.17$, $R^2 = 1$) appears during June 15–September 30 (i.e., drought-stressed condition in summer and autumn drawn above) (Fig. 6).

For $iWUE_{mes}$ during June 15–September 30, the values reduced from 60.78 $\mu\text{mol mol}^{-1}$ (June 15–July 20) to 60.62 $\mu\text{mol mol}^{-1}$ (July 21–August 25) (reduced by

approximately 0.3%) and then increased to 60.69 $\mu\text{mol mol}^{-1}$ (August 26–September 30) (increased by approximately 0.1%). During this time, the minimum iWUE_{mes} value occurred during July 21–August 25 (Fig. 6).

Meanwhile, for iWUE_{tra} during June 15–September 30, the value dropped by approximately 0.06% from June 15–July 20 to July 21–August 25 and then increased by approximately 0.78% from July 21–August 25 to August 26–September 30. Here, the maximum iWUE_{tra} value occurred within July 21–August 25 (Fig. 6).

3.6 The relationship between iWUE chronologies and climate variables

Taken as a whole, the relative importance of each climate variables to the intra-annual iWUE_{mes} and iWUE_{tra} values for the period of 1965–2017 is assessed on the basis of stepwise regression results with relaimpo package in R (Fig.7). Referring to R^2 and to the number of retained series (with $p < 0.1$), the climate factor that contributes the most to the iWUE in trees regardless of the model is relative humidity, followed by sunshine hours and temperature. Meanwhile, the least contributing climate factor is precipitation. Specifically, during April 06–April 17, the difference in the contribution of temperature, precipitation, and sunshine hours to the iWUE calculated using the two iWUE models was the greatest.

4. Discussion

4.1 Summer drought stress and tree growth

The correlation analysis revealed a drought stress during summer on tree growth at GM (Fig. 2). The stressed growth patterns caused by such summer drought have also been revealed in nearby regions of eastern Fujian province. For example, Li et al.(Li, 2017) and Li et al.(Yingjun Li, 2019) revealed that the growth of *Pinus massoniana* were mainly controlled by the July–September precipitation in the eastern region of the Fujian province. Chen et al.(Dan Chen *et al.*, 2016) also demonstrated that the July–August precipitation is the major limiting factor for the tree-ring growth of *Pinus taiwanensis* on Daiyun Mountain of the Quanzhou area, southeast of the Fujian province. The presence of the summer drought stress is because the peak temperature in July–August was accompanied by relatively low precipitation during this period. The high temperature, strong light, low precipitation, and thus low relative humidity in the summer months may lead to stomatal closure and an increase in evaporation from the soil. This increased evaporation causes a decrease in water supply for tree growth. In such conditions, a relatively low precipitation and, thus, a low relative humidity can be limiting factors for tree growth.

This study did not find any significant positive response to winter temperatures as observed in other studies in southeastern China(JIANPING DUAN *et al.*, 2013). As our GM sampling site is not located at as high of an altitude as sites used by Chen et al.(Dan Chen *et al.*, 2016), where the winter temperatures are too low to limit tree growth. These summer drought stressed growth patterns found in humid subtropical

China are different from the drought stressed pattern in arid western China, where a significant and negative response to summer temperature was often the case(Feng Chen *et al.*, 2012), as the warming-induced evapotranspiration in the arid region can be more stressful for vegetation growth than in the humid region with relatively abundant precipitation(Keyan Fang *et al.*, 2015).

4.2 Effects of sunshine and relative humidity on tree-ring $\Delta^{13}\text{C}$

In our study area, *Cryptomeria fortunei* tree-ring $\Delta^{13}\text{C}$ had significant negative correlations with temperature during the second half of the year (from June 15 to November 05), and it had a significant positive correlation with sunshine hours from August 26 to September 30 (Fig. 4b). From early June, photosynthesis started to increase because of the relatively high amount of sunshine hours and the sufficient precipitation accumulated in the previous period(Lucas C. R. Silva *et al.*, 2013). Under such conditions with enhanced photosynthesis, intensified consumption of the intercellular CO_2 can be associated with reduced discrimination of the carbon isotope (Eq. 4)(Allison&Francey, 1999; Max Berkelhammer *et al.*, 2009). Meanwhile, the relatively high precipitation and relative humidity could increase the stomatal conductance and thus increase the supply of C_i with more CO_2 , which could promote carbon isotope discrimination (Eq. 4). The high temperature associated enhancement of the evapotranspiration could reduce the stomatal conductance and thus the C_i , leading to reduced carbon isotope discrimination (Eq. 4). For such hot and humid climatic conditions in the GM study site, the temperature may be sufficient for

photosynthesis, while the relative humidity can be insufficient because of the WPSH (West Pacific Subtropical High)(Yong Hua *et al.*, 2013). For example, previous studies in Fujian Province, including Fang Guangyan (25°53'N, 119°11'E) and Niu Mulin (25°26'N, 117°56'E), have also found that the tree-ring $\Delta^{13}\text{C}$ was mainly controlled by the low relative humidity and the high amount of sunshine hours from July to September(Li *et al.*, 2017; Xu *et al.*, 2018).

4.3 The iWUE_{mes} and iWUE_{tra} at the study sites

On the annual scale, both iWUE_{mes} and iWUE_{tra} chronology show significant increasing trends at the study sites of GM (the slope for iWUE_{mes} being: 0.04, $p < 0.001$; the slope for iWUE_{tra} being: 0.08, $p < 0.001$, 1965–2017) and NML (the slope for iWUE_{mes} being: 0.12, $p < 0.001$; the slope for iWUE_{tra} being: 0.23, $p < 0.001$, 1865–2014). During the past 100 years, the CO_2 concentration in the atmosphere has increased from 300 to 380 $\mu\text{mol mol}^{-1}$ (Danny McCarroll, 2004). Such rising atmospheric CO_2 concentration is affecting the gas-exchange metabolism of trees in several ways(KÖRNER *et al.*, 2003). For instance, in controlled experiments, CO_2 assimilation was generally stimulated and stomatal conductance was reduced by increased CO_2 (C. PICON *et al.*, 2010), suggesting that plants are able to increase their intrinsic water-use efficiency as CO_2 levels rise(Bert G. Drake *et al.*, 1997; Ceulemans *et al.*, 2007).

On the intra-annual scale, the difference between $iWUE_{tra}$ chronology and $iWUE_{mes}$ chronology is more apparent during the summer-autumn time (July 21–September 30) (Fig. 6). Analyzing the intra-annual time segment can provide higher resolution and higher rates of change for climate variables (such as temperature and light intensity), therefore reproducing more accurate climate variables in the environment where the trees grew at that time. Theoretically, under the drought limitation in summer and autumn (Fig. 2 and Fig. 6c), the stomata of *Cryptomeria fortunei* trees would close, resulting in the decrease of intercellular CO_2 (Tei *et al.*, 2014) concentrations, and finally leading to the increase of $iWUE$ (Eq. 4). This was not the case in our study, as we observed an opposite trend for the $iWUE_{mes}$ value during July 21–September 30 (Fig. 6a). There are several possible reasons for this difference. First, C_a dropped drastically, especially during July 21 to August 25, which will fundamentally change the C_i of trees. Another reason is that, from July 21 to August 25, the maximum temperature (sunshine) and the insufficient precipitation increased evapotranspiration and exacerbated drought stress (Annika Hofgaard *et al.*, 1999). Under drought stress, trees can respond by closing their stomata, which decreases stomatal conductance (g_w) and increases $iWUE$. However, the drought-induced stoma will constrain the photosynthetic rate and lead to a reduction in carbohydrate synthesis (A) (Bert G. Drake *et al.*, 1997; Norby *et al.*, 2007; Wullschleger *et al.*, 2010; Randerson *et al.*, 2013). Eventually, the decline of the net photosynthetic rate (A) exceeded that of the stomatal conductance, resulting in the decline of $iWUE$ in *Cryptomeria fortunei* trees.

5. Conclusions

Based on the high-resolution $\delta^{13}\text{C}$ of *Cryptomeria fortunei* tree-rings at the GM study site, our analysis shows that the iWUE_{mes} model (Eq. 7) can be used to reasonably estimate the intrinsic water use efficiency of trees. Our study suggests that both of the iWUE s calculated from the two models (iWUE_{tra} and iWUE_{mes}) show a significant increasing trend, while the trend differs on inter-annual and intra-annual scales. On the inter-annual scale, iWUE is overestimated by approximately 2 times, though this overestimation has decreased in the past ten years. On the intra-annual scale, iWUE is overestimated by an average of 2 times, and the degree of overestimation has decreased slightly during July 21 through August 25. The most distinct difference in iWUE s calculated using the two models (iWUE_{tra} and iWUE_{mes}) appears in the summer-autumn time, when the iWUE_{mes} model indicates that the iWUE of trees has decreased significantly rather than increased. As our study shows, the iWUE_{mes} model can more effectively estimate iWUE without these levels of overestimation.

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Figure captions

Fig. 1. Location of the tree-ring sampling site and the meteorological station.

Fig. 2. Correlations between climate variables and the tree-ring standard chronology (STD) for the period of 1965-2017. The blue (black) horizontal lines represent the 95% (99%) confidence level.

Fig. 3. (a) The ratio of tree-ring stable carbon isotope of *Cryptomeria fortunei* and the ratio of atmospheric stable carbon isotope from 1965 to 2017 at GM and (b) the discrimination of tree-ring stable carbon isotope from 1965 to 2017.

Fig. 4. The Pearson correlation between the intra-annual tree-ring $\Delta^{13}\text{C}$ and the climatic variables (average temperature, average precipitation, average relative humidity and average sunshine hours) from 1965 to 2017 at the study site. The black (red) horizontal dash lines represent the 95% (99%) confidence level.

Fig. 5. Intra-annual (a) and (b) annual chronology of iWUE_{mes} and iWUE_{tra} at the

study sites.

Fig. 6. Intra-annual iWUE ($iWUE_{mes}$ and $iWUE_{tra}$) (a), atmospheric CO₂ concentration (C_a) and intercellular CO₂ concentrations (Tei *et al.*) inferred from $iWUE_{tra}$ and $iWUE_{mes}$ (b) and climate variables(c) at GM study site for the period of 1965-2017.

Fig. 7. Relative importance of climate variables to the intra-annual iWUE chronologies ($iWUE_{mes}$ and $iWUE_{tra}$) during the period 1965-2017 based on the Package relaimpo in R. The red numbers represent relative importance, which ranges from 0 to 1.

Table 1. Dates of the 10 sub-samples each ring at GM study site.

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Fig. 2

Fig. 3.

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Fig. 5

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740 **Fig. 7**

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Number of sub-sample	Date of each number	Number of sub-sample	Date of each number
1	Apr 06-Apr 17	6	Jun 02-Jun 14
2	Apr 18-Apr 29	7	Jun 15-Jul 20
3	Apr 30-May 10	8	Jul 21-Aug 25
4	May 11-May 22	9	Aug 26-Sep 30
5	May 23-Jun 01	10	Oct 01-Nov 05

752

753 **Table 1.**

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