

RESEARCH ARTICLE

Overestimated gains in water-use efficiency by global forests

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Abstract

Increases in terrestrial water-use efficiency (WUE) have been reported in many studies, pointing to potential changes in physiological forcing of global carbon and hydrological cycles. However, gains in WUE are of uncertain magnitude over longer (i.e. >10 years) periods of time largely owing to difficulties in accounting for structural and physiological acclimation. ¹³C signatures (i.e. $\delta^{13}\text{C}$) of plant organic matter have long been used to estimate WUE at temporal scales ranging from days to centuries. Mesophyll conductance is a key uncertainty in estimated WUE owing to its influence on diffusion of CO_2 to sites of carboxylation. Here we apply new knowledge of mesophyll conductance to 464 $\delta^{13}\text{C}$ chronologies in tree-rings of 143 species spanning global biomes. Adjusted for mesophyll conductance, gains in WUE during the 20th century ($0.15 \text{ ppm year}^{-1}$) were considerably smaller than those estimated from conventional modelling ($0.26 \text{ ppm year}^{-1}$). Across the globe, mean sensitivity of WUE to atmospheric CO_2 was $0.15 \text{ ppm ppm}^{-1}$. Ratios of internal-to-atmospheric CO_2 (on a mole fraction basis; c_i/c_a) in leaves were mostly constant over time but differed among biomes and plant taxa—highlighting the significance of both plant structure and physiology. Together with synchronized responses in stomatal and mesophyll conductance, our results suggest that ratios of chloroplastic-to-atmospheric CO_2 (c_c/c_a) are constrained over time. We conclude that forest WUE may have not increased as much as previously suggested and that projections of future climate forcing via CO_2 fertilization may need to be adjusted accordingly.

KEYWORDS

atmospheric CO_2 , carbon stable isotope, land carbon uptake, mesophyll conductance, photosynthesis, stomatal conductance, tree rings, water-use efficiency

1 | INTRODUCTION

Theories and empirical evidence suggest accumulation of atmospheric CO_2 , mostly caused by anthropogenic emissions, drives increases in plant photosynthesis, growth and terrestrial carbon sinks, leading to a general hypothesis of 'CO₂ fertilization' (Canadell et al., 2007; Ehlers et al., 2015; Le Quéré et al., 2009; Walker et al., 2021; Wang, Zhang, et al., 2020). In addition, leaf conductance to water vapour via stomata (g_{sw}) appears mostly either unchanged or reduced by increases in atmospheric CO_2 (Ainsworth &

Long, 2005; Guerrieri et al., 2019; Mathias & Thomas, 2021; Purcell et al., 2018). Hence, in principle, intrinsic water-use efficiency (WUE) of terrestrial plants (i.e. the ratio of carbon assimilation [A] to stomatal conductance, A/g_{sw} , referred to as WUE_p hereafter) should have increased with CO_2 . Such responses have indeed been observed in multiple types of studies including growth chamber or free-air CO_2 enrichment experiments (FACE; Ainsworth & Long, 2005; Norby & Zak, 2011; Norby et al., 2010), flux tower observations (Belmecheri et al., 2021; Keenan et al., 2013) and isotopic records (Keeling et al., 2017; Mathias & Thomas, 2021; van der Sleen et al., 2015).

Changes in WUE have fundamental impacts on global carbon and water cycles because terrestrial carbon and hydrologic cycles are intimately associated with leaf gas exchange via stomata (Adams et al., 2019, 2020; Frank et al., 2015; Voelker et al., 2016). While ample evidence suggests WUE has increased over time, there remain large uncertainties as to the magnitude of change, hindering projections of future terrestrial carbon and water cycling (Adams et al., 2020; Keeling et al., 2017; Swann et al., 2016; Walker et al., 2021). CO₂ enrichment experiments can provide direct evaluation of changes in WUE in the short-term and under controlled or semi-controlled conditions. WUE estimations from eddy covariance flux towers or satellite proxies are only available for recent decades (Keenan et al., 2013; Wang, Zhang, et al., 2020). The lack of high-resolution and longer term WUE data has limited characterization of decadal to centennial trends in WUE.

It is generally unwise to use results from short-term experiments in making long-term predictions. Plants regulate stomatal conductance dynamically at scales of minutes to hours, and structurally (by producing leaves with altered stomatal densities and pore sizes) at scales of years to decades. Plants thus continuously adapt to long-term increases in atmospheric CO₂ (de Boer et al., 2011; Lammertsma et al., 2011). It is now well-accepted that neglecting long-term changes in nutritional status or acclimation of plants to increased CO₂ can lead to erroneous conclusions (Norby & Zak, 2011; Norby et al., 2010; Oren et al., 2001). Improved understanding of long-term acclimation is essential to improving performance of land surface models (Wang, Atkin, et al., 2020).

Records of carbon isotope composition ($\delta^{13}\text{C}$) in tree rings provide an integrated measure of physiological responses to CO₂ across temporal scales of months to centuries. Dynamic changes in photosynthetic parameters (e.g. A and g_s) are captured in the $\delta^{13}\text{C}$ of photosynthetic products. It follows that this isotopic signature is integrated over time in long-lived plant tissues such as wood. Tree rings thus provide a high-resolution and long-term record of WUE. When considered at the global scale, such records can help elucidate impacts of multiple environmental factors in addition to that of atmospheric CO₂ (Adams et al., 2021; Seibt et al., 2008; Szejner et al., 2018). Ultimately, tree-ring $\delta^{13}\text{C}$ is one of very few formal records of WUE that are of sufficient duration to assess physiological and structural acclimations of trees under climate change.

Water-use efficiency has most often been estimated using a simplified, linear version of Farquhar's model (Farquhar & Cernusak, 2012; Farquhar, O'Leary, et al., 1982). Based on the linear relation of c_i/c_a (ratio of CO₂ mole fractions inside leaves to that of the atmosphere) to photosynthetic $^{12}\text{C}/^{13}\text{C}$ discrimination (Δ ; Farquhar, O'Leary, et al., 1982; Farquhar et al., 1989; Seibt et al., 2008), intrinsic WUE can be expressed as:

$$WUE_{\text{sim}} = \frac{C_a}{1.6} \cdot \frac{b' - \Delta}{b' - a}, \quad (1)$$

where 1.6 is the ratio of CO₂-to-water vapour diffusivity ($g_{\text{sw}} = 1.6g_{\text{sc}}$); a (4.4‰) is a fractionation factor for CO₂ diffusion through stomata,

b' is the apparent net fractionation during carboxylation (27‰ based on early in vitro estimations (Christeller et al., 1976) and estimations using $\delta^{13}\text{C}$ of leaf bulk material (Farquhar, Ball, et al., 1982). WUE_{sim} has provided a useful and convenient way to analyse c_i/c_a since, by definition: $WUE_p = c_a/1.6 \times (1 - c_i/c_a)$.

c_i/c_a is considered a cornerstone of leaf-scale photosynthetic models based on optimization theories as it implicitly reflects the balance between A and g_{sw} (Dewar et al., 2018; Lavergne et al., 2022; Medlyn et al., 2012; Wang, Atkin, et al., 2020). Apparent increases in WUE during the Anthropocene have fuelled a debate about the constancy of c_i , c_i/c_a or $c_a - c_i$ (Feng, 1998; Saurer et al., 2004). Constant c_i (despite rising c_a) implies strong physiological regulation and a rapid increase in WUE. Observations based on eddy fluxes in North American forests (Keenan et al., 2013) supported this hypothesis. In contrast, constant $c_i - c_a$ implies that increases in c_i are commensurate with those in c_a and WUE also remains roughly constant (Belmecheri et al., 2021; Voelker et al., 2016). Constant c_i/c_a (i.e. c_i increases proportionally with c_a) implies greatly reduced physiological regulation of WUE which is instead driven by c_a (Frank et al., 2015; Saurer et al., 2004). Constant c_i/c_a is predicted by stomatal optimization theory (de Boer et al., 2011; Medlyn et al., 2012; Wang et al., 2017). While supported by several tree-ring studies (Frank et al., 2015; Peñuelas et al., 2011), that support is not universal (Belmecheri et al., 2021; Voelker et al., 2016).

This controversy is related to the choice of model for WUE. In particular, use of the simplified model (WUE_{sim}) assumes that mesophyll conductance (g_m) is infinite (i.e. there is no gradient in CO₂ mole fractions from intercellular space, c_i , to carboxylation sites in chloroplasts, c_c). This assumption has been the subject of many studies (Barbour et al., 2010; Flexas et al., 2013; Gong et al., 2018; Ma et al., 2021; Seibt et al., 2008; Warren & Adams, 2006). A common result is that, in fact, g_m limits carbon flux into chloroplasts and influences WUE. Recent studies that used more comprehensive WUE models, considering either finite, constant g_m values ($g_m = 0.29 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, Stangl et al., 2019) or fixed g_m values associated with modelled leaf A/c_a ratios ($g_m = 0.2 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, Adams et al., 2019, 2020, 2021; Keeling et al., 2017), provided improved WUE estimates (derived from Δ) compared to estimations based on the WUE_{sim} model. These results highlight the potential of explicitly accounting for g_m in quantitative assessments of WUE.

Experimental evidence suggests synchronized acclimation of both g_m and g_{sc} to environmental cues (Flexas et al., 2013; Ma et al., 2021). Ma et al. (2021) also showed that g_{sc}/g_m is reasonably well-constrained across a wide range of plant species under moist and drought-stressed conditions. Assumptions of finite or constant g_m are unlikely to produce realistic outcomes when WUE is modelled. Alternatively, if modelling of WUE takes g_m into account via the g_{sc}/g_m ratio (WUE_{mes} , see 'theory'), better estimates (i.e. better fit with experimental data) were obtained for a range of species (Ma et al., 2021). Similarly, incorporating g_{sc}/g_m ratios in a land surface model (Lavergne et al., 2022) helped improve estimations of WUE. The approach of Ma et al. (2021) was applied to estimate long-term

WUE in the Park Grass Experiment at Rothamsted, and resulted in improved estimations of historical WUE (Baca Cabrera et al., 2021).

The WUE_{mes} model of Ma et al. (2021) has not been applied to studies of WUE (or c_i/c_a) in forests across the globe. Likely impacts of g_{sc}/g_m on global WUE trends remain unknown and there remains an open and important question as to the constancy (or otherwise) of c_i/c_a . Here, we address these questions by (i) testing different WUE models for predicting CO₂ responses of WUE, and (ii) exploiting a dataset of annually resolved tree-ring $\delta^{13}\text{C}$ values (see Adams et al., 2020) representing a total of 464 tree-ring $\delta^{13}\text{C}$ chronologies in 143 species distributed across the globe (Figure 1), and (iii) examining long-term trends in WUE and c_i .

2 | MATERIALS AND METHODS

2.1 | Theory

Ma et al. (2021) used the comprehensive version of the Farquhar model describing $^{12}\text{C}/^{13}\text{C}$ isotope fractionation during net photosynthesis (Farquhar & Cernusak, 2012) to provide a full mathematical expression of intrinsic WUE in C₃ species (WUE_{com}), as follows:

$$WUE_{\text{com}} = c_a \cdot \frac{a_{\text{ac}}(1-\varepsilon) + (1+t) \left(\frac{\Gamma^*}{c_a} \left(\frac{e'R_d}{A+R_d} - f' \right) + \varepsilon \left(b - \frac{e'R_d}{A+R_d} \right) \right) - (1-t)\Delta}{\frac{(1+t) \left(b - \frac{e'R_d}{A+R_d} \right) - a_{\text{ac}}}{\frac{1}{k} + \frac{w}{2}} - (1+t)k \frac{g_{\text{ac}}}{g_m} \left(a_m - b + \frac{e'R_d}{A+R_d} \right)}, \quad (2)$$

where Δ is the photosynthetic $^{12}\text{C}/^{13}\text{C}$ discrimination, c_a is ambient CO₂ mole fraction (ppm), a_m (1.8‰) is the fractionation during liquid-phase diffusion and dissolution of CO₂ in mesophyll (0.7‰+1.1‰), b is the fractionation associated with Rubisco-catalysed carboxylation (29‰), R_d is the day respiration rate, A is net CO₂ assimilation rate and Γ^* is the CO₂ compensation point in the absence of day respiration (42.7 $\mu\text{mol mol}^{-1}$). Also, $e' = e\alpha_b/\alpha_e$ and $f' = f\alpha_b/\alpha_f$ where $\alpha_b = 1+b$, $\alpha_e = 1+e$ and $\alpha_f = 1+f$. e (-6-0‰) and f (11‰) represent the fractionations during day respiration and photorespiration respectively. Other parameters are as follows:

ε is given by the relationship:

$$\varepsilon = \frac{\frac{1}{k} - \frac{w}{2}}{\frac{1}{k} + \frac{w}{2}}, \quad (3)$$

where k (1.6) is the ratio of conductance to H₂O to that for CO₂ and w denotes the water vapour drawdown from intercellular spaces to air.

t is the ternary correction factor and a_{ac} is the weighted fractionation for diffusion across boundary layer and stomata. These parameters can be estimated as:

$$t = \frac{(1 + a_{\text{ac}})E}{2g_{\text{ac}}}, \quad (4)$$

$$a_{\text{ac}} = \frac{a_b(c_a - c_s) + a_s(c_s - c_i)}{c_a - c_i}, \quad (5)$$

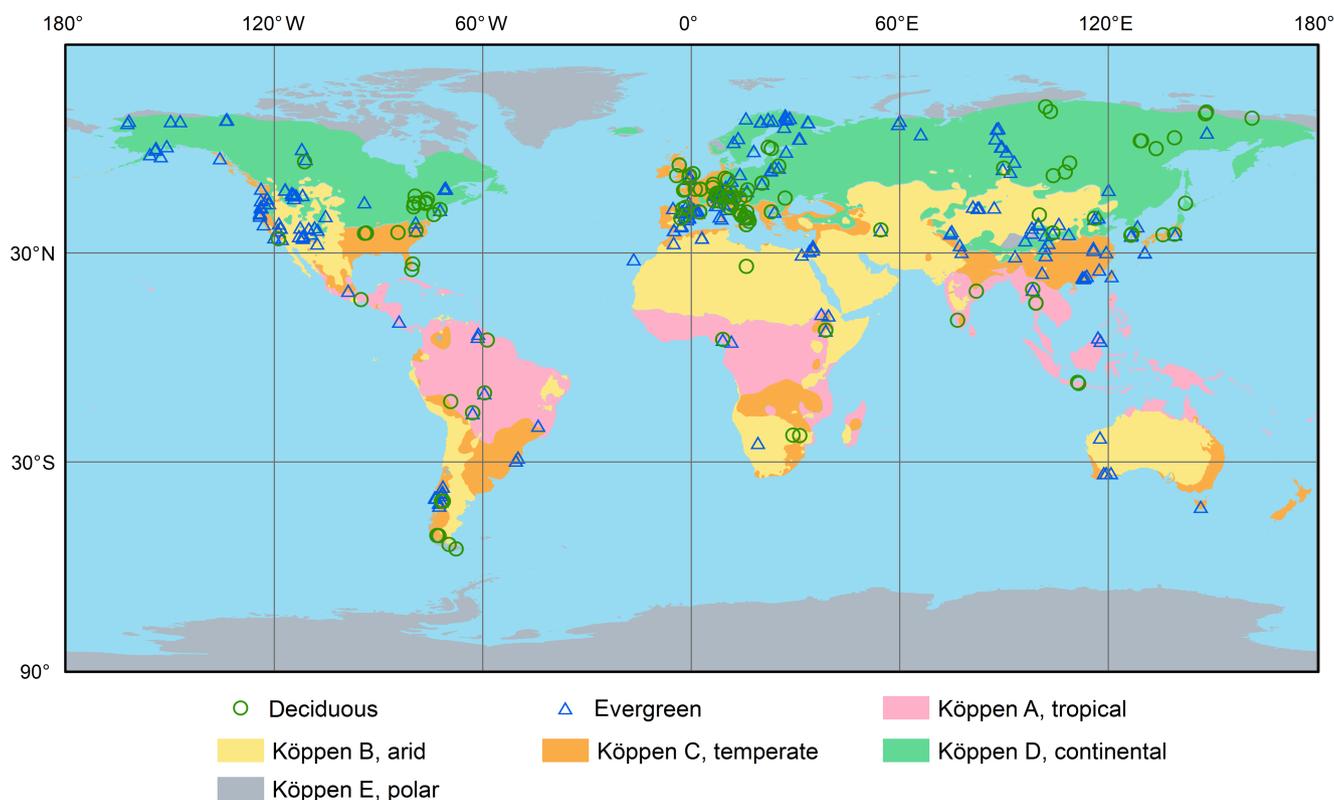


FIGURE 1 Map of geographical locations of 464 tree-ring $\delta^{13}\text{C}$ chronologies of 143 species used in this paper [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

where c_s and c_i denote CO_2 concentrations (mole fractions) at leaf surface and in substomatal cavity respectively. a_b (2.9‰) and a_s (4.4‰) are the discriminations for CO_2 diffusion in air through the boundary layer and stomata respectively. E is the transpiration rate. g_{ac} is the combined boundary layer and stomatal conductance to CO_2 .

WUE_{com} (Equation 2) is a newly derived expression of WUE based on the model of Farquhar, Ball, et al. (1982) and including all components of photosynthetic fractionation. Ma et al. (2021) have shown that Equation (2) can be simplified to:

$$WUE_{mes} = \frac{c_a}{1.6} \cdot \frac{b - \Delta - f' \frac{\Gamma^*}{c_a}}{b - a_s + \frac{g_{sc}}{g_m} \cdot (b - a_m)}, \quad (6)$$

where g_{sc} is stomatal conductance to CO_2 . The justification of simplifications for day respiration and boundary layer conductance are extensively explained in this same reference.

According to the meta-analysis of Ma et al. (2021), g_{sc}/g_m is reasonably well constrained at 0.79 ± 0.07 (mean \pm 95% CI, $n = 198$) across a wide range of plant species of different functional groups (0.87 ± 0.13 for evergreen woody, 0.71 ± 0.11 for deciduous woody and 0.77 ± 0.12 for herbaceous species), under moist and drought-stressed conditions. The median value of g_{sc}/g_m for the same dataset is 0.68 with an interquartile range of 0.44–0.90. In this study, we used the global mean g_{sc}/g_m of 0.79 for the calculation of WUE_{mes} ; we also assessed the uncertainty associated with this parameter by varying g_{sc}/g_m between 0.7 and 0.9.

Using Equation (6), the derivative of WUE_{mes} with respect to c_a gives the response (sensitivity) of WUE to atmospheric CO_2 (mole fraction):

$$\frac{dWUE_{mes}}{dc_a} = \frac{b - \Delta}{1.6 \left[\frac{g_{sc}}{g_m} (b - a_m) + b - a_s \right]}. \quad (7)$$

It is obvious that $dWUE/dc_a$ is negatively correlated with g_{sc}/g_m .

2.2 | Simulating WUE trends under scenarios of constant c_i , constant $c_a - c_i$, and constant c_i/c_a

We used theoretical parameter values (see Table S1) and synthetic data to test the sensitivity of WUE and its relationship to c_a . We used three, ^{13}C -based calculations of WUE (WUE_{sim} , WUE_{com} and WUE_{mes}) and three hypothetical scenarios: constant c_i , constant $c_a - c_i$ and constant c_i/c_a (Saurer et al., 2004). We firstly set a c_a gradient of 300–450 ppm with an interval of 10 ppm, and used $c_i = 250$ ppm, or $c_a - c_i = 80$ ppm, or $c_i/c_a = 0.8$ to derive the corresponding c_i series for each scenario. Using theoretical parameter values, we also calculated photosynthetic $^{12}\text{C}/^{13}\text{C}$ discrimination (Δ) along the c_a gradient for each scenario. ^{13}C -based calculations of WUE were then estimated from each Δ series using the WUE_{sim} , WUE_{com} and WUE_{mes} models, and trends of WUE versus c_a are compared and plotted in Figure 2. We also calculated gas exchange-based WUE (WUE_p) from the pre-set c_i and c_a values as:

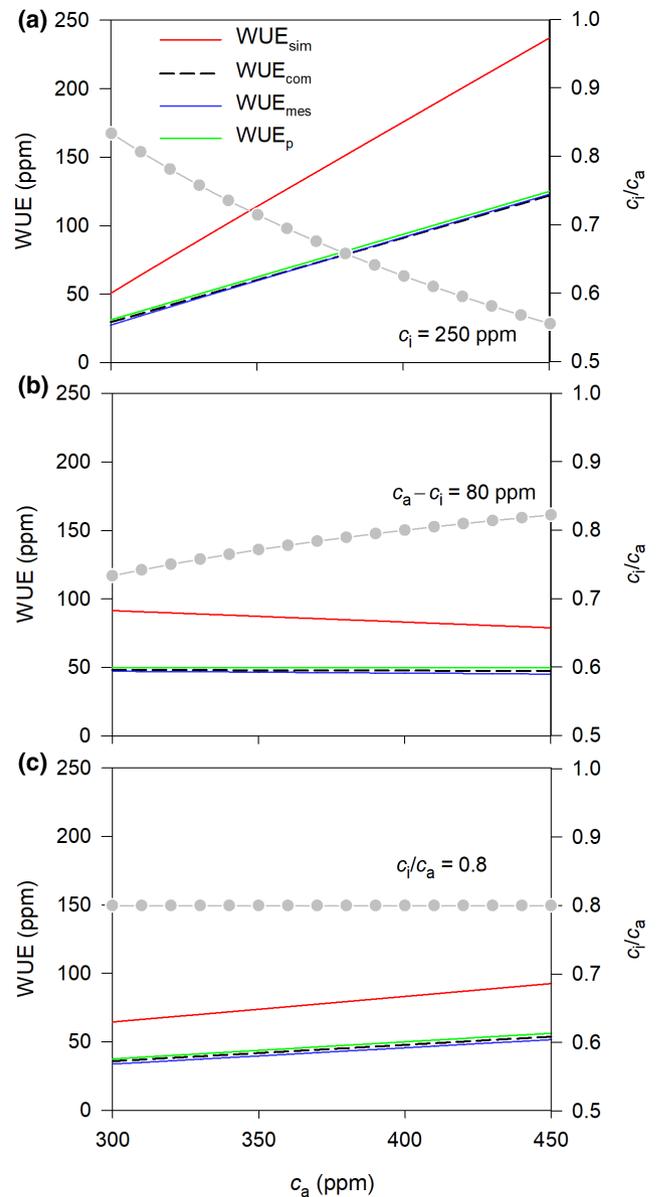


FIGURE 2 Simulated trends in water-use efficiency (WUE) when c_a varies. WUE trends were estimated by assuming a constant c_i of 250 ppm (a), a constant $c_a - c_i$ of 80 ppm (b) or a constant c_i/c_a of 0.8 (c). WUE was calculated from simulated $^{12}\text{C}/^{13}\text{C}$ fractionation (Δ) using three models: the simplified model (WUE_{sim} , Equation 1), the comprehensive model (WUE_{com} , Equation 2) and the mesophyll model (WUE_{mes} , Equation 6). WUE was also directly calculated from c_a and c_i (WUE_p , Equation 8). Grey discs show how c_i/c_a (values on right axis) varied. Note that WUE was expressed in ppm, that is, in $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

$$WUE_p = c_a / 1.6 \times (1 - c_i / c_a). \quad (8)$$

Note that Equation (8) provides a direct estimate of WUE from photosynthetic gas exchange data, with only very small errors due to neglecting boundary layer resistance (Ma et al., 2021). In the simulations based on synthetic data, WUE_p can be treated as the reference WUE ('true' WUE) for comparing the performance of ^{13}C -based WUE models.

2.3 | Tree-ring $\delta^{13}\text{C}$ dataset and WUE calculations

We used the tree-ring $\delta^{13}\text{C}$ chronologies from a previously published database which includes 422 chronologies of 134 species (details of data collection in (Adams et al., 2020)). In short, tree-ring $\delta^{13}\text{C}$ chronologies between 1850 and 2015 were collected by screening search engines of Web of Science and Google scholar using the keywords dendrochron*, cellulose, tree ring, carbon isotope discrimination, ^{13}C , WUE, tropic* (where the asterisk is a wildcard) and by checking citations within these publications (Adams et al., 2020). In addition to the database of Adams et al. (2020), we added 42 chronologies of nine species by searching the literature using the following keywords: WUE, tree ring, cellulose, ^{13}C , carbon isotope, dendrochronology. We mostly applied the same selection criteria as that in (Adams et al., 2020) but included annually resolved tree-ring $\delta^{13}\text{C}$ chronologies of >10 years duration (Adams et al., 2020 limited analysis of rates of change in WUE to chronologies that spanned 100 years). Hence, we used a dataset of 464 tree-ring $\delta^{13}\text{C}$ chronologies for 143 species (Table S2; Note S1) for analyses. When Δ values were reported, WUE was calculated from Δ values. When $\delta^{13}\text{C}$ values were reported, we first calculated Δ as:

$$\Delta = (\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{tree-ring}}) / (1 + \delta^{13}\text{C}_{\text{tree-ring}}/1000). \quad (9)$$

Annual averages of the $\delta^{13}\text{C}$ of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{atm}}$) were taken from McCarroll and Loader (2004) for this calculation, in line with most of the compiled studies that reported Δ values. When WUE_{sim} values were reported, we first calculated Δ by using Equation (1), and then used this calculated value of Δ for further calculations.

Equation (8) is useful for estimating c_i/c_a if WUE values are available. Equating WUE_p in Equation (8) to modelled WUE values, we calculated c_i/c_a based on WUE_{sim} ($c_i/c_{a\text{sim}}$) which is calculated from tree-ring Δ using Equation (1). Similarly, we calculated c_i/c_a based on WUE_{mes} ($c_i/c_{a\text{mes}}$), and this calculation takes g_{sc}/g_m into account.

The isotope discrimination model of Farquhar, Ball, et al. (1982) is applicable for net photosynthesis or primary photosynthetic assimilates of leaves. Downstream post-photosynthetic fractionation associated with processes including respiration, storage and transport of assimilates could further modify $\delta^{13}\text{C}$ of tree-ring cellulose (Badeck et al., 2005; Frank et al., 2015). Therefore, accounting for the isotopic fractionation between tree-ring materials and photosynthetic assimilates (Δ_{post}) is potentially useful to improve estimation of WUE and c_i/c_a . So that Equation (9) can be modified as:

$$\Delta = (\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{tree-ring}}) / (1 + \delta^{13}\text{C}_{\text{tree-ring}}/1000) - \Delta_{\text{post}} \quad (10)$$

Direct comparison of $\delta^{13}\text{C}$ of canopy photosynthesis and that of tree-ring cellulose is not available. Previous studies, including this work, usually assume that Δ_{post} is negligible. In our dataset, $\delta^{13}\text{C}_{\text{tree-ring}}$ data

were mostly measured from tree-ring cellulose (75% of the data) rather than bulk dry matter (25% of the data). Δ_{post} is likely less than 2‰, considering that $\delta^{13}\text{C}$ of leaf cellulose is generally similar to that of leaf sugars (differ by about $\pm 0.5\%$), and the dry matter (carbon) content of woody stems is about 2‰ more enriched in $\delta^{13}\text{C}$ than that of leaves (Badeck et al., 2005; Bowling et al., 2008). We used Equation (10) to test the sensitivity of $d\text{WUE}_{\text{mes}}/dc_a$ (a) and c_i/c_a calculated from WUE_{mes} (Figure S7).

Different studies have used diverse methods to estimate annual c_a , and this would affect calculations of WUE and c_i . We used a standardized c_a series (Figure S1) that includes new compilations of ice core data and direct measurements for all $\delta^{13}\text{C}$ chronologies (Belmecheri & Lavergne, 2020). This standardized c_a series is in close agreement with observations at the Mauna Loa Observatory (gml.noaa.gov/ccgg/trends/) during 1959–2020 and with the reported values for 1850–1958 as reported by McCarroll and Loader (2004; Figure S1).

2.4 | g_{sc}/g_m dataset

We identified articles from the literature that contained paired g_{sc} and g_m data of C_3 species grown under manipulated CO_2 concentrations by screening Web of Science and Google Scholar search engines using the following keywords: mesophyll conductance, internal conductance, CO_2 enrichment, FACE. We selected studies with CO_2 treatments >10 days where g_{sc} and g_m were measured under the physiological steady-state (i.e. eliminating results of short-term manipulation of CO_2 during measurements; Note S2). We used similar (but stricter) criteria to that in Ma et al. (2021) to eliminate outliers: $A/g_{\text{sc}} > 300 \mu\text{mol mol}^{-1}$, $g_{\text{sc}}/g_m < 0.3$, $g_{\text{sc}}/g_m > 1.5$, $g_m > 0.8 \text{ mol m}^{-2} \text{ s}^{-1}$. Ma et al. (2021) showed that the 10%–90% range of global observations in g_{sc}/g_m was 0.3–1.5.

2.5 | Statistical analysis

We calculated $d\text{WUE}/dt$ from linear regression of each tree-ring ^{13}C series, and then performed paired t -tests to compare the mean $d\text{WUE}/dt$ estimated using the WUE_{sim} model (Equation 1) and the WUE_{mes} model (Equation 6). The analysis of variance for $d\text{WUE}/dt$ and c_i/c_a of global forests was restricted to WUE_{mes} -based estimations. Data were grouped into the Köppen climate zones, and evergreen and deciduous species were separated. Compiled $d\text{WUE}/dt$ data were analysed using a general linear model of SPSS Statistics 19 (IBM Corp.) that includes biome, taxa and their interaction as factors (two-way ANOVA). Similarly, we calculated c_i/c_a from linear regression of each tree-ring $\delta^{13}\text{C}$ series, and used a two-way ANOVA that included biome, taxa and their interaction as factors. To analyse the trend of WUE over years or c_a , mean WUE was used for linear regressions, and 95% confidence intervals were used to determine the significance of differences between slopes of linear regression.

3 | RESULTS

3.1 | Sensitivity of ^{13}C -based WUE models with respect to CO_2 mole fraction

We first used mathematical expressions to assess the sensitivity of WUE with respect to c_a (i.e. $d\text{WUE}/dc_a$), using standard parameterization for three ^{13}C -based models: WUE_{sim} (simple model assuming infinite mesophyll conductance), WUE_{com} (full model accounting for both mesophyll conductance and ternary effects) and WUE_{mes} (simplified version of WUE_{com} ; see 'Methods' for further details).

In the constant c_i and constant c_i/c_a scenario, simulated WUE increased with c_a , and the slope of the constant c_i scenario was greater than that observed if c_i/c_a were constant (Figure 2). In the constant $c_a - c_i$ scenario, WUE_p was constant while WUE_{sim} showed an unrealistic decreasing trend (Figure 2b). In all scenarios, WUE_{sim} was always much greater than other WUE estimates and responded more strongly to c_a (larger slope), while WUE_{mes} and WUE_{com} estimates were in close agreement with WUE_p , with errors of less than 5 ppm (Figure 2). It seems likely that WUE_{sim} significantly overestimates true WUE and the rate of change in WUE. Assumptions associated with the simplification of WUE_{com} to yield WUE_{mes} were quantitatively assessed and tested with experimental data (Ma et al., 2021). On the whole, neglecting mesophyll resistance results in overestimation of both WUE and $d\text{WUE}/dc_a$. The origin of systematically greater rates of change ($d\text{WUE}/dc_a$) is clearly illustrated in the mathematical expression of $d\text{WUE}/dc_a$ (Equation 7).

3.2 | Overestimated gains in WUE of global forests

When we calculated WUE_{mes} using the 20th century tree-ring $\delta^{13}\text{C}$ data for global biomes (Figure 1), the change in WUE_{mes} with time ($d\text{WUE}_{\text{mes}}/dt$) was $0.06 \pm 0.004 \text{ ppm year}^{-1}$ for the period 1901–1965, rising to $0.20 \pm 0.004 \text{ ppm year}^{-1}$ during 1966–2000. Average $d\text{WUE}_{\text{mes}}/dt$ for the 20th century was $0.15 \pm 0.01 \text{ ppm year}^{-1}$ (Figure 3a,b). Large year-to-year increases in c_a for the 1966–2000 period were associated with similarly large $d\text{WUE}_{\text{mes}}/dt$ values (Figure S1). In line with our theoretical analysis, mean $d\text{WUE}_{\text{sim}}/dt$ for 1901–2000 was $0.26 \pm 0.01 \text{ ppm year}^{-1}$, that is, 73% greater than that estimated using $d\text{WUE}_{\text{mes}}/dt$ (Figure 3b). This overestimation is evident in all species and biomes. Statistical analysis (ANOVA) further showed no effect of climate zone ($p = .47$), plant taxa ($p = .06$) or their interaction ($p = .23$) on $d\text{WUE}_{\text{mes}}/dt$ (Figure 3c,d). Across climate zones, mean $d\text{WUE}_{\text{mes}}/dt$ of evergreen species ($0.16 \pm 0.01 \text{ ppm year}^{-1}$) was slightly greater than that of deciduous species ($0.13 \pm 0.01 \text{ ppm year}^{-1}$), while median values of $d\text{WUE}_{\text{mes}}/dt$ were similar among taxa. In all species, $d\text{WUE}_{\text{mes}}/dt$ was the greatest in temperate climates ($0.164 \text{ ppm year}^{-1}$), followed by arid climates ($0.158 \text{ ppm year}^{-1}$), the tropics ($0.145 \text{ ppm year}^{-1}$), polar climates ($0.144 \text{ ppm year}^{-1}$) and continental climates ($0.140 \text{ ppm year}^{-1}$).

We also calculated apparent sensitivity of WUE to c_a . For the period 1901–1965, the mean change in WUE ($d\text{WUE}_{\text{sim}}/dc_a$) for

trees was $0.30 \pm 0.02 \text{ ppm ppm}^{-1}$ across the globe. This then fell to $0.23 \pm 0.01 \text{ ppm ppm}^{-1}$ for 1966–2000. Mean $d\text{WUE}_{\text{mes}}/dc_a$ across these periods was $0.15 \pm 0.01 \text{ ppm ppm}^{-1}$ (Figure 4a; $0.18 \pm 0.01 \text{ ppm ppm}^{-1}$ for 1901–1965 and $0.14 \pm 0.01 \text{ ppm ppm}^{-1}$ for 1966–2000). These results confirm slowing rates of change in WUE for the 20th century (cf. Adams et al., 2020), and also confirm that $d\text{WUE}_{\text{sim}}/dc_a$ systematically overestimates the sensitivity of WUE to c_a .

3.3 | c_i/c_a was constant during the 20th century

By equating WUE_p to modelled WUE values, c_i/c_a was then calculated from the WUE dataset. Our analysis suggests c_i/c_a is significantly underestimated if the WUE_{sim} model is used in place of WUE_{mes} (Figure 4b). Based on WUE_{mes} , we analysed c_i and c_a relationships across biomes and taxa. There are consistent linear relationships between c_i and c_a ($r^2 > .9$, with the exception of evergreen trees in the polar zone, Figure S2) across the 20th century. We detected statistically significant effects of plant taxa and climatic zones on c_i/c_a . Also, c_i/c_a of evergreen trees (0.76 ± 0.01) was significantly less than that of deciduous trees (0.80 ± 0.01) in all bioclimatic zones except for the tropics (Figure S2; we note the limited data for the tropics). By pooling all species and biomes, we found a significant linear relationship between c_i and c_a ($r^2 = .996$, $p < .001$) with a mean c_i/c_a of 0.78 ± 0.01 (Figure 4).

4 | DISCUSSION

4.1 | Estimating WUE requires accounting for mesophyll conductance

It would be difficult to overstate the significance of tree-ring $\delta^{13}\text{C}$ to outputs of land surface models and responses of carbon and water cycles to climate change. As far as we are aware, the isotopic composition of tree rings stands as a unique, plant-based and time-integrated measure of key physiological parameters (such as WUE). We took advantage of tree-ring data over 100 year from 1900 to 2000 to analyse changes in WUE in several biomes. The data show that using WUE_{sim} results in strong overestimation of WUE (by c. 100%) and, perhaps even more importantly, in the rate of WUE gain with time ($d\text{WUE}/dt$ and $d\text{WUE}/dc_a$ by c. 70%). Using instead estimates of WUE that account for mesophyll effects results in a 10-ppm gain in WUE during the 20th century (Figure 3a). This is less than the increase of 11–17 ppm (1890–1990) reported by Saurer et al. (2004), and less than the increase of 15 ppm (1960s–2000s) reported by Peñuelas et al. (2011). Most previous studies have used WUE_{sim} and reported values close to those we show here (WUE_{sim} gain of 20 ppm per century). Overestimation of rates of increase in WUE is consistent with theory. Equation (7) derived from the WUE_{mes} model shows that $d\text{WUE}/dc_a$ is eventually negatively correlated with g_{sc}/g_m . Owing to the increase in c_a with time, we expect that $d\text{WUE}/dt$

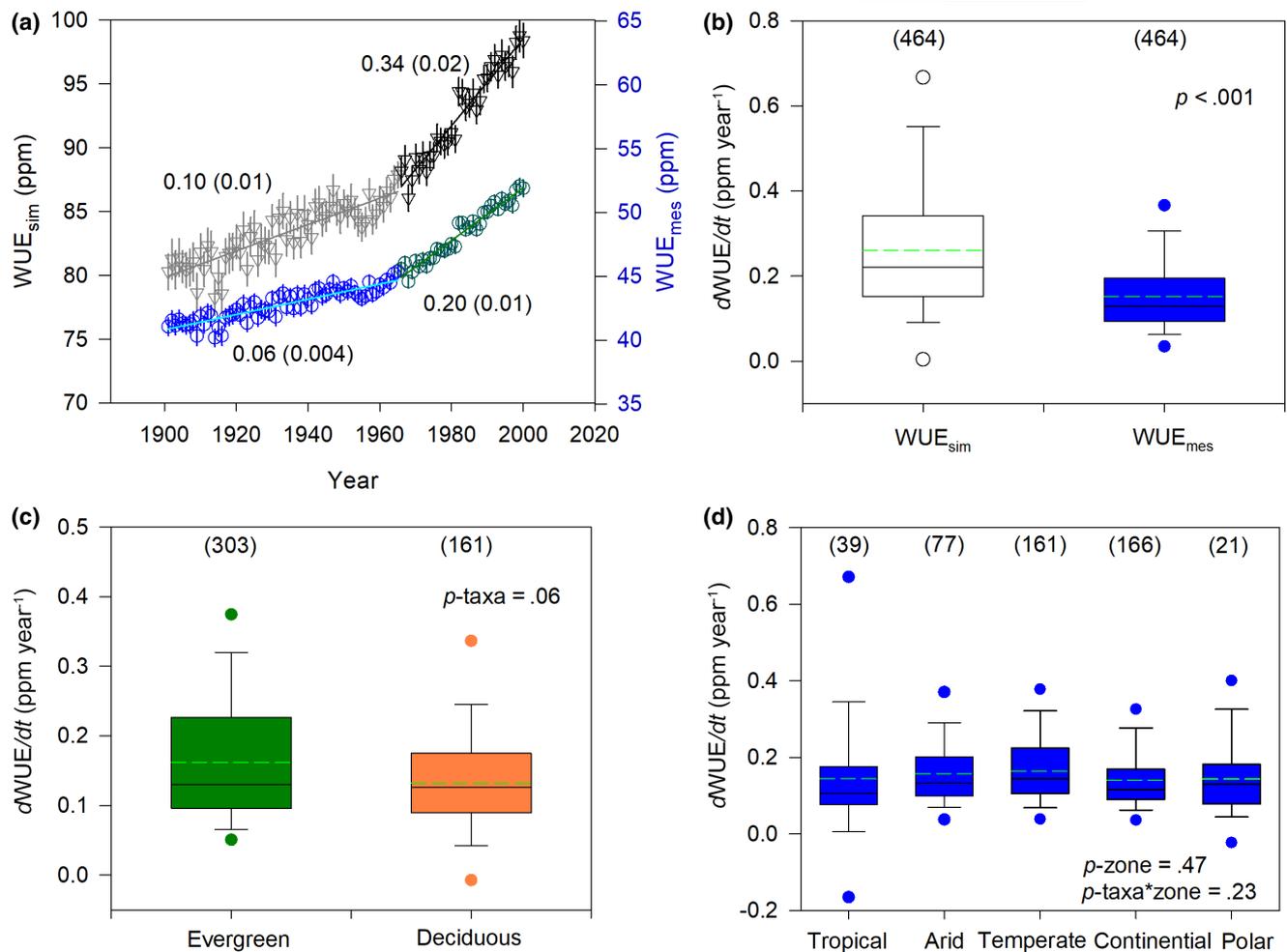


FIGURE 3 Temporal trends in water-use efficiency (WUE) in trees of global biomes. (a) Temporal trends of mean annual WUE_{sim} (grey and black triangles) and WUE_{mes} (blue and green circles, error bars are SE). Numbers indicate means and SE (between parentheses) of slopes ($dWUE/dt$) calculated from two linear regressions, performed in 1901–1965 and 1966–2000 separately. (b) mean $dWUE/dt$ values during the 20th century calculated using WUE_{sim} and WUE_{mes} models. (c) mean $dWUE/dt$ of evergreen and deciduous trees calculated using WUE_{mes} . (d) mean $dWUE/dt$ of trees in Köppen climate zones calculated using WUE_{mes} . Boxplots show median (black centre line), mean (green, dashed centre line), 10%–90% range (whiskers) and 5%–95% range (filled circles); numbers above boxes on top represent the number of $\delta^{13}C$ series for each box [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16221)]

depends also on g_{sc}/g_m . When g_m is assumed infinite (i.e. in WUE_{sim} model), the g_{sc}/g_m term disappears. Consequently $dWUE_{sim}/dc_a$ as well as $dWUE_{sim}/dt$ are inherently larger than equivalent terms derived using the WUE_{mes} model.

The discrepancy between WUE_{sim} and WUE_{mes} estimates cannot be explained by different fractionation factors for carboxylation (i.e. b' values). Manipulating b' between 25‰ and 29‰ still leads to significantly higher WUE_{sim} estimates than that of WUE_{mes} using the data of the constant c_i/c_a scenario. If we force the WUE_{sim} to be equal to WUE_{mes} values, the fitted b' was 22.8‰ (data not shown), which is unreasonable for Rubisco-catalysed carboxylation in higher plants. Note that the b' of 27‰ was originally taken from early in vitro estimations (Christeller et al., 1976) but later treated as a fitted value from $\delta^{13}C$ of leaf organic matter and measured c_i/c_a (Farquhar, Ball, et al., 1982; Ubierna et al., 2018). In the latter case, the WUE_{sim} model should have accounted for all isotopic effects (including g_m and photorespiratory effects). However, to our knowledge, this

empirical relationship (if we treat b' of 27‰ as a fitted value) has not been validated using a broad range of species under diverse environmental conditions. Clearly, the conceptual uncertainty of the WUE_{sim} model cannot be fully addressed using a more precise fractionation factor for net carboxylation.

Despite quantitative differences, $dWUE_{mes}/dt$ and $dWUE_{sim}/dt$ of tree-ring chronologies are tightly, albeit imperfectly related (owing to variable fractionation associated with photorespiration; Figure S3). Hence, previously reported WUE_{sim} remain useful for qualitative studies. A clear example is their use to identify climatic drivers of WUE. Nevertheless, since ^{13}C -based WUE has been used to assess process-based biogeochemical models and their outputs (Keller et al., 2017; Lavergne et al., 2022; Raczka et al., 2016), we suggest caution is required. Errors in WUE or $dWUE/dt$ can be shown to propagate with considerable effects on quantitative estimates of water- and carbon-cycling parameters. For example, gross primary production (GPP) has been estimated from WUE,

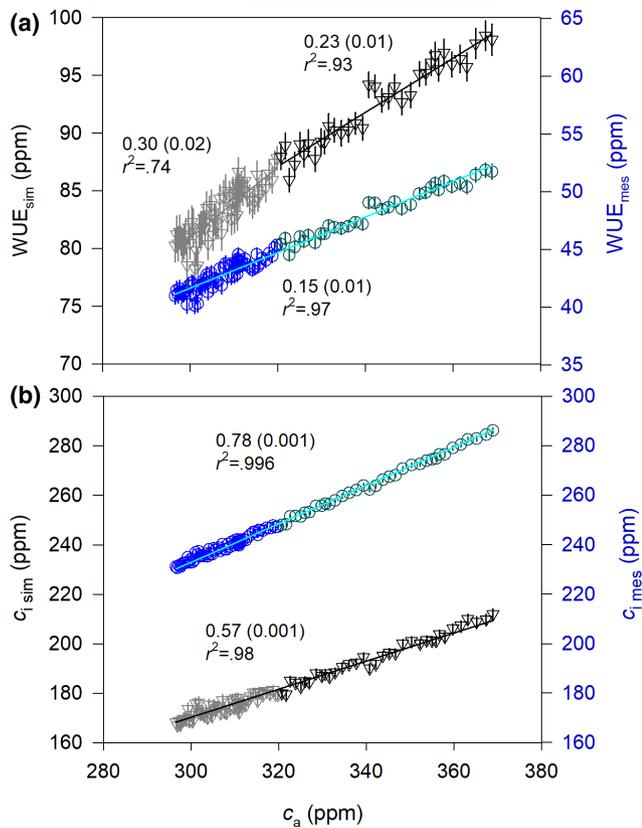


FIGURE 4 Changes in mean water-use efficiency (WUE) and mean c_i in response to c_a based on 464 $\delta^{13}\text{C}$ series across global biomes. (a) Trends in WUE_{sim} (grey and black triangles, $\pm\text{SE}$) and WUE_{mes} (blue and green circles). Linear regressions for WUE_{sim} were conducted separately for the 1901–1965 and 1966–2000 timeframes. Slopes ($d\text{WUE}/dc_a$) mean and SE (between parentheses) are shown along with r^2 values. A single linear regression was conducted for WUE_{mes} over the period 1901–2000. (b) Trends in c_i back-calculated from WUE_{sim} ($c_{i\text{sim}}$) or WUE_{mes} ($c_{i\text{mes}}$) over the last century. Linear regressions through the origin were conducted for the whole period and slopes (c_i/c_a) were shown with SE between parentheses. All regressions are statistically significant ($p < .001$) [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

transpiration rate (Tr) and leaf to air vapour concentration difference (w) as: $\text{GPP} = \text{WUE} \times \text{Tr}/w$ (Klein et al., 2016). Overestimation of the gain in WUE would be directly reflected in predicted gains in GPP (i.e. a 50% overestimate of WUE results in a 50% overestimate of GPP if Tr and w are held constant). The relative ubiquity of WUE in many such quantitative models could lead to large biases in estimated magnitudes of CO_2 effects. The problem remains when dual isotopic ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) approaches (e.g. Mathias & Thomas, 2021) are used to disentangle relative contributions of photosynthetic capacity and stomatal conductance to WUE.

In the specific case of the global ^{13}C budget (sensu Keeling et al., 2017), relationships between photosynthetic $^{12}\text{C}/^{13}\text{C}$ isotope discrimination and estimated WUE ensure that more accurate accounting for mesophyll effects is critical. Keeling et al. (2017) analysed historic trends in $\delta^{13}\text{C}$ of atmospheric CO_2 and suggested a systematic increase in terrestrial photosynthetic discrimination

of 0.014‰ ppm^{-1} . Our data suggest this may not be correct, since $^{12}\text{C}/^{13}\text{C}$ photosynthetic discrimination of global forests mainly decreased or remained unchanged (with the exception of the arid bioclimatic zone; Figure S4). Other analyses, such as long-term $\delta^{13}\text{C}$ series in grasslands do not show increases in $^{12}\text{C}/^{13}\text{C}$ discrimination (Köhler et al., 2010). We suggest that re-evaluating assumptions and theory behind photosynthetic $^{12}\text{C}/^{13}\text{C}$ discrimination models, in particular the assumptions and mathematical expressions of g_m in these models, will improve global ^{13}C budget estimates.

4.2 | c_i/c_a is likely conserved during the 20th century

By definition, temporal trends in WUE are ultimately related to the extent of physiological control on c_i . The report by Ma et al. (2021) and results here suggest c_i increases proportionally with c_a resulting in more or less constant c_i/c_a . This contrasts with interpretations of eddy covariance data (Keenan et al., 2013) and tree-ring $\delta^{13}\text{C}$ series restricted to North American forests (Belmecheri et al., 2021). However, they are in agreement with other comprehensive studies on tree-ring $\delta^{13}\text{C}$ series across major biomes (Frank et al., 2015; Peñuelas et al., 2011). The latter studies provide empirical evidence for modelling based on stomatal optimization theory. That theory predicts optimal c_i/c_a reflects a balance of carbon gain and water costs (Belmecheri et al., 2021; Guerrieri et al., 2019; Wang et al., 2017). Optimal c_i/c_a seems independent of c_a and mainly determined by site-specific air temperature and VPD. Applying such optimality theory, Wang et al. (Wang et al., 2017) predicted lesser c_i/c_a in dry lands but greater c_i/c_a in tropical forest, in agreement with c_i/c_a calculated here from tree-ring $\delta^{13}\text{C}$ records.

A key issue is that because WUE_{sim} overestimates WUE, it underestimates c_i/c_a . This in turn affects photosynthesis-weighted c_i/c_a (and parameters derived therefrom; e.g. optimal c_i/c_a) in process-based land surface models (Belmecheri et al., 2021; Frank et al., 2015; Wang et al., 2017). In other words, accurate c_i/c_a estimates are critical because photosynthetic models (e.g. FvCB model, Farquhar et al., 1980) that remain central to most current land-surface biogeochemical models, estimate enzymatic reaction rates based on c_c that is in turn assumed to be equal to c_i (infinite mesophyll conductance hypothesis; Sun et al., 2014). Our work strongly suggests we need to re-evaluate previously claimed optimal c_i/c_a .

4.3 | Implementing WUE_{mes}

Reliable estimates of g_{sc}/g_m are potential limitations to including effects of mesophyll conductance in estimates of WUE. We used a common g_{sc}/g_m of 0.79 ± 0.07 (95% CI) derived from an analysis of 80+ species. This value was robust to drought stress and plant functional groups (deciduous, evergreen woody and herbaceous

species; Ma et al., 2021). Nonetheless, we sought to ensure that a generic g_{sc}/g_m value at least approximated historic conditions by pairing g_{sc} and g_m data in experiments with elevated CO_2 treatments. We found that elevated CO_2 (>200 ppm) reduced both g_{sc} and g_m , but had no significant effect on g_{sc}/g_m of 10 species (0.82 ± 0.11 at low CO_2 and 0.78 ± 0.10 at elevated CO_2 , Figure S5). Similarly, a mesocosm study (Gong et al., 2017; Tcherkez et al., 2010) showed no difference in g_{sc}/g_m between sunflowers grown at sub-ambient (200 ppm) and elevated CO_2 (1000 ppm). Simulations using a stomatal optimization model also showed well-constrained g_{sc}/g_m across a broad range of CO_2 (Dewar et al., 2018). While all these results support the use of a constant g_{sc}/g_m for estimating long-term WUE responses, we acknowledge that as more data for g_{sc}/g_m become available, and especially as the range of studied plant species expands, there could well be systematic fine-scale variation that we cannot account for here.

Varying g_{sc}/g_m between 0.7 and 0.9 had little effect on estimated $dWUE/dc_a$ (maximum error of $0.02 \text{ ppm ppm}^{-1}$) and c_i/c_a (maximum error of 0.03, Figure S6). We also tested the (unrealistic) scenario of decreasing g_{sc} and constant g_m along c_a gradients, which had no influence on our conclusions (data not shown). Formulation of WUE_{mes} is not sensitive to reasonable variations in g_{sc}/g_m . As we note above, using fixed g_{sc}/g_m may bias WUE estimates for some species given reported variations in g_{sc}/g_m across species and measurement conditions (Flexas et al., 2013; Ma et al., 2021). We also acknowledge that precisely measuring g_m remains a challenge and current methods are at their weakest when estimating dynamic responses of g_m (Gong et al., 2015; Gu & Sun, 2014; Pons et al., 2009). Even so, an assumption of fixed g_{sc}/g_m is more reasonable than the alternate hypothesis that $g_{sc}/g_m = 0$ (infinite mesophyll conductance). The WUE_{mes} model (Equation 6) is easily further improved. For example, a fixed value of g_{sc}/g_m could be replaced by empirical/mechanistic relations based on new knowledge.

Constancy of g_{sc}/g_m despite varying atmospheric CO_2 —as predicted by optimization models (Dewar et al., 2018)—has important implications for photosynthesis. Because g_{sc}/g_m dictates the gradient in CO_2 from atmosphere to chloroplast (c_c/c_a ; $c_c/c_a \approx [1 + g_{sc}/g_m] \times c_i/c_a - g_{sc}/g_m$), if both g_{sc}/g_m and c_i/c_a are largely unchanged over time then c_c/c_a has also been largely unchanged. Assuming global averages values of c_i/c_a (0.78) and g_{sc}/g_m (0.79), we estimate that global c_c/c_a is ~ 0.61 . This opens doors to further optimality models.

Carbon isotope discrimination downstream of photosynthesis (i.e. post-photosynthetic fractionation, Δ_{post}) could cause tree-ring $\delta^{13}C$ to deviate from that of leaf photosynthesis (Gessler et al., 2008, 2014). Previous meta-analyses (Badeck et al., 2005; Bowling et al., 2008) showed that $\delta^{13}C$ of leaf cellulose differed about $\pm 0.5\%$ from that of leaf sugars, and woody stems were about 2‰ more enriched in $\delta^{13}C$ than leaves. A correction of 1–2‰ for Δ_{post} has been suggested (Lavergne et al., 2019, 2020). Accounting for the isotopic fractionation between tree photosynthesis and tree-ring cellulose (Δ_{post}) of $\pm 1\%$, had little effect on simulated $dWUE_{mes}/dc_a$ (maximum

error of 0.03) and c_i/c_a (maximum error of 0.04, Figure S7), and has no influence on our conclusions.

Post-photosynthetic fractionation is also associated with the ontogenic (size, height and age) trends in WUE. Tree-ring $\delta^{13}C$ represents a mixed isotopic signal of leaf photosynthesis at different canopy layers and carbohydrate storage of diverse origins and ages (Hartmann & Trumbore, 2016; McDowell et al., 2011). An important consideration is that WUE mostly increases as trees age, especially during their first few decades (Brienen et al., 2017; Vadeboncoeur et al., 2020). This ontogenic effect has no major influence on absolute changes in WUE over time. Nonetheless, ontogenic effects can confound interpretation of WUE sensitivity to atmospheric CO_2 (or other environmental cues), that is, reported apparent CO_2 sensitivity of WUE cannot be fully assigned to CO_2 . The mechanisms behind ontogenic effects include physiological issues associated with hydraulic limitation and carbon refixation in bark and microclimatic issues including light, humidity and $\delta^{13}C_{air}$ gradient in the canopy (McDowell et al., 2011; Vadeboncoeur et al., 2020). The complexity is also reflected in highly species-specific impacts on WUE, and potential interactions with growth conditions (Brienen et al., 2017). We note that reported WUE trends with tree age are mostly based on the WUE_{sim} model and may thus be overestimated. At present, there are no fully reliable means of correcting for ontogenic effects in a global dataset. We also note that many isotope studies specifically exclude data from the first 20 or so years of tree growth (the so-called 'juvenile period') in recognition of ontogenic impacts (see also Adams et al., 2020). Clearly, estimations of WUE that account for g_m and post-photosynthetic fractionation effect are preconditions when seeking to quantify ontogenic effects.

5 | CONCLUSIONS

Leveraging a more precise, $\delta^{13}C$ -based calculation of WUE, we show that there was a general increase in forest WUE across the globe over the last century. However, our results also indicate that terrestrial WUE has not increased as much as previously suggested, emphasizing the need to re-evaluate historical WUE trends. The increase in WUE is associated with conserved but significantly greater than previously reported c_i/c_a of global forests, providing new insight into mechanisms of optimization of carbon gain to water loss under climate change. Our study further suggests that well-supported g_{sc}/g_m values are central to improving estimates of all of WUE, c_i/c_a and c_c/c_a . Future studies addressing dynamic changes in g_{sc}/g_m should improve the applicability of the WUE_{mes} model for estimating fine-scale variations in WUE. We recognise that to more precisely estimate historic trends in WUE, we would need to measure g_{sc}/g_m in plants fully adapted (field grown) to sub-ambient CO_2 levels (290–370 ppm)—data that do not exist as far as we are aware. In future studies, long-term CO_2 reduction experiments using controlled-environment facilities (cf. Schnyder et al., 2003) or

free-air CO₂ reduction techniques would be desirable to fill this gap in knowledge.

AUTHOR CONTRIBUTIONS

XYG, GT and MAA designed and planned the research; WTM and YZY performed literature survey and data analyses; XYG and WTM wrote the first draft of the manuscript and all authors contributed substantially to the revision.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad (<https://doi.org/10.5061/dryad.c59zw3r9q>).

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