frontiers | Frontiers in Plant Science

TYPE Mini Review
PUBLISHED 12 January 2023
DOI 10.3389/fpls.2022.1037972

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SPECIALTY SECTION
This article was submitted to
Functional Plant Ecology,
a section of the journal
Frontiers in Plant Science

RECEIVED 06 September 2022
ACCEPTED 19 December 2022
PUBLISHED 12 January 2023

CITATION
Ma WT, Yu YZ, Wang X and Gong XY
(2023) Estimation of intrinsic water-use efficiency from $\delta^{13}C$ signature of C_3 leaves: Assumptions
and uncertainty.
Front. Plant Sci. 13:1037972.
doi: 10.3389/fpls.2022.1037972

Estimation of intrinsic water-use efficiency from $\delta^{13}C$ signature of C_3 leaves: Assumptions and uncertainty

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Carbon isotope composition ($\delta^{13}C$) has been widely used to estimate the intrinsic water-use efficiency (iWUE) of plants in ecosystems around the world, providing an ultimate record of the functional response of plants to climate change. This approach relies on established relationships between leaf gas exchange and isotopic discrimination, which are reflected in different formulations of ^{13}C-based iWUE models. In the current literature, most studies have utilized the simple, linear equation of photosynthetic discrimination to estimate iWUE. However, recent studies demonstrated that using this linear model for quantitative studies of iWUE could be problematic. Despite these advances, there is a scarcity of review papers that have comprehensively reviewed the theoretical basis, assumptions, and uncertainty of ^{13}C-based iWUE models. Here, we 1) present the theoretical basis of ^{13}C-based iWUE models: the classical model ($iWUE_{sim}$), the comprehensive model ($iWUE_{com}$), and the model incorporating mesophyll conductance ($iWUE_{mes}$); 2) discuss the limitations of the widely used $iWUE_{sim}$ model; 3) and make suggestions on the application of the $iWUE_{mes}$ model. Finally, we suggest that a mechanistic understanding of mesophyll conductance associated effects and post-photosynthetic fractionation are the bottlenecks for improving the ^{13}C-based estimation of iWUE.

KEYWORDS

water-use efficiency, carbon isotope discrimination, mesophyll conductance, post-photosynthetic fractionation, climate change, photosynthesis

Introduction

During photosynthesis, plant stomata act as a control valve for the diffusion of CO_2 and water vapor, regulating the rates of water and carbon exchange between the biosphere and the atmosphere (de Boer et al., 2011; Adams et al., 2020; Walker et al., 2021). Intrinsic water-use efficiency (iWUE), defined as the ratio of net photosynthetic rate (A_n) to stomatal conductance for water vapor (g_{sw}), plays a key role in quantifying carbon uptake and water loss at leaf to continental scales (Seibt et al., 2008; Keenan et al., 2013). The response of iWUE is fundamental to climate change research since small changes in iWUE can have profound impacts on global carbon and water cycles. Furthermore, iWUE can provide insights into the mechanisms of plant physiological responses to climate change and support the screening and breeding of climate-resilient crops (Farquhar and Richards, 1984; von Caemmerer et al., 2014; Gresset et al., 2014). Central to these research domains is the quantification of iWUE.

Stable carbon isotope discrimination (Δ) can be used as an integrated measure of iWUE in C_3 plants (Farquhar et al., 1989). Plants discriminate against ^{13}C in favour of ^{12}C during photosynthetic CO_2 assimilation in C_3 leaves, and the variation in carbon isotope composition ($\delta^{13}C$) from source CO_2 to photosynthetic products (e.g., bulk leaf organic carbon or sugars) is termed as Δ, following Farquhar et al. (1982b); Farquhar et al. (1989):

$$\Delta = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p} \qquad \text{Equation 1}$$

where atmospheric $\delta^{13}C_a$ is approximately -7~-8‰ during the 20th century. Δ can also be estimated from $\delta^{13}C$ of CO_2 entering (δ_{in} and C_{in}) and leaving (δ_{out} and C_{out}) the cuvette during gas exchange, termed as online $^{12}C/^{13}C$ discrimination (Evans et al., 1986):

$$\Delta_{online} = \frac{\xi(\delta_{out} - \delta_{in})}{1 + \delta_{out} - \xi(\delta_{out} - \delta_{in})} \qquad \text{Equation 2}$$

where $\xi = C_{in}/(C_{in}\text{-}C_{out})$. In this way, Δ can be measured nondestructively to probe real-time responses of photosynthesis at high temporal resolution. Changes in photosynthetic parameters (A_n and g_s) are captured in Δ_{online} and the isotopic signatures are further imprinted on plant tissues during biosynthesis. As such, biomass-based Δ reflects physiological status of plants throughout the growth period of plant tissues (Cernusak et al., 2013; Soh et al., 2019). Different from classical approaches such as gas exchange or growth analysis, biomass-based Δ can be applied retrospectively, providing a useful record of iWUE at large spatial and temporal scales (Frank et al., 2015; Adams et al., 2020; Gong et al., 2022).

Inferring iWUE from isotopic records relies on theoretical models. In the current literature, most studies have utilized the simple, linear equation of photosynthetic discrimination to estimate iWUE. However, it can be problematic to interpret iWUE using this linear model which ignores effects other than diffusion through stomata and carboxylation. For instance, Seibt et al. (2008) suggested that the uncertainty in iWUE-^{13}C models was related to the simplification of mesophyll conductance (g_m). g_m represents the conductance to CO_2 diffusion from the intercellular space to the carboxylation site in chloroplasts, a key limiting factor of photosynthesis in addition to stomatal conductance and biochemical capacity (Tholen et al., 2012; Stangl et al., 2019). However, recent advances in $\delta^{13}C$-based iWUE estimation have not been systematically reviewed. The main objective of this mini review is to concisely summarize the theoretical basis and uncertainties of $\delta^{13}C$-based iWUE models. We (i) present different formulations of Δ and the associated assumptions, (ii) present Δ-based iWUE models derived from those formulations: the classical model ($iWUE_{sim}$), the comprehensive model ($iWUE_{com}$), the model incorporating g_m ($iWUE_{mes}$), (iii) discuss the limitations of the widely used $iWUE_{sim}$ model; and make suggestions on the application of the $iWUE_{mes}$ model.

Comprehensive model of photosynthetic ^{13}C discrimination and simplifications

A comprehensive description of ^{13}C discrimination (Δ_{com}) during C_3 photosynthesis was given by Farquhar et al. (1982b) and extended to include ternary effects of transpiration on CO_2 assimilation by Farquhar and Cernusak (2012):

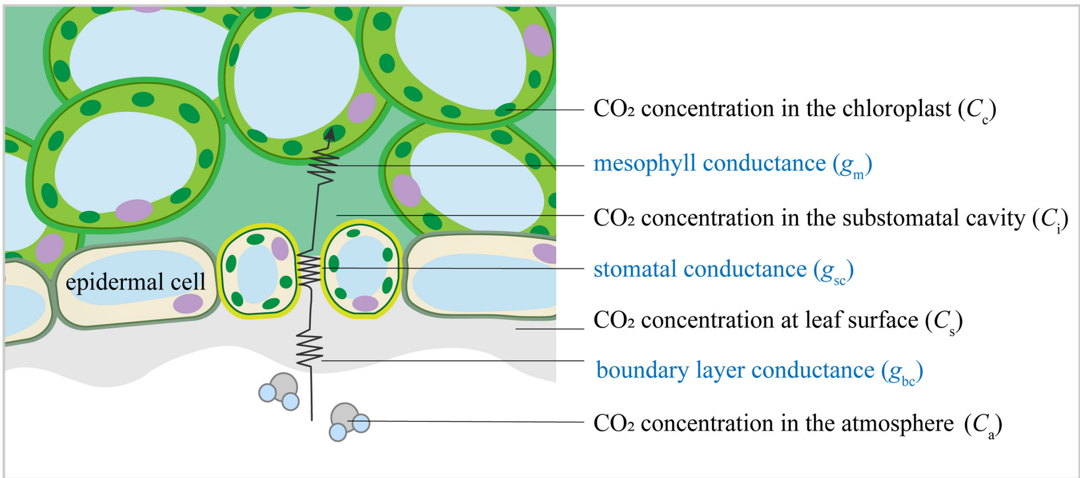
$$\Delta_{com} = \frac{1}{1-t}\left(a_{ac}\frac{C_a - C_i}{C_a}\right) + \frac{1+t}{1-t}\left(a_m\frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - \frac{\alpha_b}{\alpha_e}e\frac{R_d}{V_c}\frac{C_c}{C_a} - \frac{\alpha_b}{\alpha_f}f\frac{\Gamma^*}{C_a}\right) \qquad \text{Equation 3}$$

and

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

$$a_{ac} = \frac{a_b(C_a - C_s) + a_s(C_s - C_i)}{C_a - C_i} \qquad \text{Equation 5}$$

where a_b (2.9‰) and a_s (4.4‰) are fractionations associated with the diffusion of CO_2 through leaf boundary layer and in the air, respectively. a_m (1.8‰) is the fractionation associated with the dissolution and diffusion of CO_2 in mesophyll (see Table S1 for the list of parameters). C_a, C_s, C_i and C_c represent the mole fraction of CO_2 in air, at leaf surface, in the intercellular spaces and chloroplast, respectively (Figure 1). Δ_{com} can be separated into a series of fractionation components of leaf boundary layer conductance



$$\mathrm{iWUE}_{\mathrm{com}} = c_a \cdot \frac{a_{ac}(1-\varepsilon)+(1+t)\left(\frac{\Gamma^*}{c_a}\left(\frac{e'R_d}{A_n+R_d}-f'\right)+\varepsilon\left(b-\frac{e'R_d}{A_n+R_d}\right)\right)-(1-t)\Delta}{\frac{(1+t)\left(b-\frac{e'R_d}{A_n+R_d}\right)-a_{ac}}{\frac{1}{k}+\frac{W}{2}}-(1+t)k\frac{g_{ac}}{g_m}\left(a_m-b+\frac{e'R_d}{A_n+R_d}\right)}$$

With: $e' = e\alpha_b/\alpha_e$ $f' = f\alpha_b/\alpha_f$ and $\varepsilon = \frac{\frac{1}{k}-\frac{W}{2}}{\frac{1}{k}+\frac{W}{2}}$

$$\Delta_{\mathrm{com}} = \frac{1}{1-t}\left(a_{ac}\frac{C_a-C_i}{C_a}\right)+\frac{1+t}{1-t}\left(a_m\frac{C_i-C_c}{C_a}+b\frac{C_c}{C_a}-\frac{\alpha_b}{\alpha_e}e\frac{R_d}{V_c}\frac{C_c}{C_a}-\frac{\alpha_b}{\alpha_f}f\frac{\Gamma^*}{C_a}\right)$$

With: $t = \frac{(1+a_{ac})E}{2g_{ac}}$ $a_{ac} = \frac{a_b(c_a-c_s)+a_s(c_s-c_i)}{c_a-c_i}$

Simplifications:

(1) Meaurements under low to moderate VPD, and g_{bc} is assumed to be infinite (t=0 and C_a=C_s; g_{ac}=g_{sc}):

$$\mathrm{iWUE}_{\mathrm{mes\,R}} = \frac{c_a}{k}\cdot\frac{b-\Delta-f'\frac{\Gamma^*}{c_a}-e'\frac{R_d}{A_n+R_d}\cdot\left(1-\frac{\Gamma^*}{c_a}\right)}{b-a_s+\frac{g_{sc}}{g_m}\cdot\left(b-a_m+e'\frac{R_d}{A_n+R_d}\right)-e'\frac{R_d}{A_n+R_d}}$$

$$\Delta_{\mathrm{mesR}} = a_s\frac{C_a-C_i}{C_a}+a_m\frac{C_i-C_c}{C_a}+b\frac{C_c}{C_a}-\frac{\alpha_b}{\alpha_e}e\frac{R_d}{V_c}\frac{C_c}{C_a}-\frac{\alpha_b}{\alpha_f}f\frac{\Gamma^*}{C_a}$$

(2) Low respiratory fractionation (e≈0 or $R_d/(A_n+R_d)\approx 0$):

$$\mathrm{iWUE}_{\mathrm{mes}} = \frac{c_a}{k}\cdot\frac{b-\Delta-f'\frac{\Gamma^*}{c_a}}{b-a_s+\frac{g_{sc}}{g_m}\cdot(b-a_m)}$$

$$\Delta_{\mathrm{mes}} = a_s\frac{C_a-C_i}{C_a}+a_m\frac{C_i-C_c}{C_a}+b\frac{C_c}{C_a}-\frac{\alpha_b}{\alpha_f}f\frac{\Gamma^*}{C_a}$$

(3) Infinite g_m and low photorespiratory fractionation (C_i=C_c, $f\approx 0$ or $\Gamma^*/C_a\approx 0$):

$$\mathrm{iWUE}_{\mathrm{sim}} = \frac{C_a}{k}\left(\frac{b'-\Delta}{b'-a}\right)$$

$$\Delta_{\mathrm{sim}} = a_s+(b'-a_s)\frac{C_i}{C_a}$$

FIGURE 1

Diagram of the CO$_2$ diffusion pathway in C$_3$ leaves and different formulations of iWUE (iWUE$_{\mathrm{com}}$, iWUE$_{\mathrm{mes\ R}}$, iWUE$_{\mathrm{mes}}$, and iWUE$_{\mathrm{sim}}$) derived from the Farquhar et al. model for photosynthetic ^{13}C discrimination.

(Δ_{gbc}), stomatal conductance (Δ_{gsc}), mesophyll conductance (Δ_{gm}), Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) carboxylation (Δ_b), day respiration (Δ_e), and photorespiration (Δ_f). Note that t is included to account for the ternary effects of transpiration rate (E) on photosynthetic discrimination (Farquhar and Cernusak, 2012). Usually, the effect of t is small and can be omitted under low or moderate vapor pressure deficit (VPD) (Farquhar and Cernusak, 2012; Evans and Caemmerer, 2013). If Δ_{gbc} is also omitted (C_a=C_s and g_{ac}=g_{sc}), the Δ_{com} model is simplified as:

$$\Delta_{\text{mes R}} = a_s \frac{C_a - C_i}{C_a} + a_m \frac{C_i - C_c}{C_a} + b \frac{C_c}{C_a} - \frac{\alpha_b}{\alpha_e} e \frac{R_d}{V_c} \frac{C_c}{C_a} - \frac{\alpha_b}{\alpha_f} f \frac{\Gamma^\star}{C_a} \qquad \text{Equation 6}$$

where the subscript "mes R" indicates that the expression takes mesophyll conductance, day respiration and photorespiration into account.

Δ_e, the respiratory contribution to discrimination is mainly determined by respiratory fractionation (e), and $R_d/(An+ R_d)$. Δ_e has rarely been accurately quantified largely due to the difficulty of estimating R_d (Tcherkez et al., 2017; Gong et al., 2018). Moreover, fractionation of day respiration has rarely been reported, and e estimated from respiration in the dark varies between 0 and -6‰ (Ghashghaie et al., 2003; Tcherkez et al., 2010). Under natural conditions, Δ_e is usually small and negligible (Seibt et al., 2008; Ubierna and Farquhar, 2014). Notably, a significant apparent respiratory fractionation may occur when the CO_2 source used for combined gas exchange and isotopic measurements has a $\delta^{13}C$ differed from that of the ambient air (Gillon and Griffiths, 1997; Gong et al., 2015). Under such conditions, e should be corrected to account for the isotopic disequilibria between photosynthetic and respiratory fluxes (Wingate et al., 2007; Gong et al., 2015). Assuming Δ_e=0, Equation 6 can be simplified as:

$$\Delta_{\text{mes}} = a_s \frac{C_a - C_i}{C_a} + a_m \frac{C_i - C_c}{C_a} + b \frac{C_c}{C_a} - \frac{\alpha_b}{\alpha_f} f \frac{\Gamma^\star}{C_a} \qquad \text{Equation 7}$$

C_c is usually unknown since its calculation requires g_m which cannot be directly measured. g_m is assumed to be infinite in early studies (for a review see Flexas et al., 2012); that is, CO_2 mole fraction in the chloroplast is equal to that in the intercellular space. Assuming C_i=C_c and Δ_f=0, Equation 7 is simplified as:

$$\Delta_{\text{sim}} = a_s + \left(b' - a_s\right) \frac{C_i}{C_a} \qquad \text{Equation 8}$$

Comprehensive model of iWUE and simplifications

The comprehensive model of iWUE which includes all fractionation components of Equation 3 was first derived by Ma et al. (2021):

$$\text{iWUE}_{\text{com}} = c_a \frac{a_{ac}(1-\epsilon) + (1+t)\left[\frac{\Gamma^*}{c_a}\left(\frac{e' R_d}{A_n+R_d} - f'\right) + \epsilon\left(b - \frac{e' R_d}{A_n+R_d}\right)\right] - (1-t)\Delta}{\frac{(1+t)\left(b - \frac{e' R_d}{A_n+R_d}\right) - a_{ac}}{\frac{1}{k}+\frac{W}{2}} - (1+t)k\frac{g_{ac}}{g_m}\left(a_m - b + \frac{e' R_d}{A_n+R_d}\right)} \qquad \text{Equation 9}$$

where $e' = ea_b/a_e$, $f' = fa_b/a_f$ and $\epsilon = (1/k - W/2)/(1/k + W/2)$. This formulation is particularly useful for assessing the contribution of each fractionation component to iWUE estimates. Ma et al. (2021) performed sensitivity tests using theoretical data of the standard photosynthetic scenarios. Their results indicated that ternary correction and Δ_{gbc} had little influence on iWUE_{com} estimates (error< 2 µmol mol^{-1}), which is in agreement with Seibt et al. (2008). Neglecting the contribution of t and Δ_{gbc}, Equation 9 can be simplified as:

$$\text{iWUE}_{\text{mes R}} = \frac{c_a}{k} \cdot \frac{b - \Delta - f' \frac{\Gamma^\star}{c_a} - e' \frac{R_d}{A_n+R_d}\left(1 - \frac{\Gamma^\star}{c_a}\right)}{b - a_s + \frac{g_{sc}}{g_m}\left(b - a_m + e' \frac{R_d}{A_n+R_d}\right) - e' \frac{R_d}{A_n+R_d}} \qquad \text{Equation 10}$$

Δ_e in the $\text{iWUE}_{\text{mes R}}$ model could be ignored as it caused an error of less than 2 µmol mol^{-1} in typical photosynthetic scenarios (Ma et al., 2021). Excluding the contribution of day respiratory, Equation 10 can be simplified as:

$$\text{iWUE}_{\text{mes}} = \frac{c_a}{k} \cdot \frac{b - \Delta - f' \frac{\Gamma^\star}{c_a}}{b - a_s + \frac{g_{sc}}{g_m}(b - a_m)} \qquad \text{Equation 11}$$

The iWUE_{mes} model provided iWUE estimates that are numerically very similar with iWUE_{com} (error< 3 µmol mol^{-1}) (Ma et al., 2021). Neglecting the contribution of g_m and photorespiration, the simplified equation for iWUE is given as:

$$\text{iWUE}_{\text{sim}} = \frac{C_a}{k}\left(\frac{b' - \Delta}{b' - a}\right) \qquad \text{Equation 12}$$

This linear relationship between iWUE and photosynthetic ^{13}C discrimination is the most used to estimate iWUE, however, the limitations of this formulation have been raised (Seibt et al., 2008; Ubierna and Farquhar, 2014; Ma et al., 2021).

Uncertainty in iWUE_{sim} estimation associated with mesophyll conductance

Experimental evidence shows that g_m exerts a significant limitation on CO_2 diffusion and leads to a significant drawdown from C_i to C_c (Loreto et al., 1992; Flexas et al., 2008; Cano et al., 2014). It is apparent from Equation 11 that, assuming an infinite g_m will lead to overestimation of iWUE, this is supported by experimental observations (Barbour et al., 2010; Stangl et al., 2019; Adams et al., 2020). Experimental results showed that the relationship between Δ and water-use efficiency is at least partly a function of g_m (Warren and Adams, 2006). Therefore, it is important to incorporate g_m in the parameterization of the iWUE model. Ma et al. (2021) showed that iWUE_{sim} overestimated iWUE by c. 65%. Importantly, the magnitude of overestimation is dependent on Δ, making correction using empirical relations difficult. These results raise concerns regarding the accuracy of iWUE_{sim} estimations.

The overestimation of the iWUE$_{sim}$ model has also been observed in recent studies using ^{13}C series of environmental archives (Baca Cabrera et al., 2021; Bing et al., 2022). More importantly, iWUE$_{sim}$ model could provide biased estimations of historical iWUE trend. Gong et al. (2022) analyzed tree ring ^{13}C series across the globe using the iWUE$_{mes}$ model, and reported that iWUE$_{sim}$ model significantly overestimated iWUE (by c. 100%) and the rate of iWUE gain with time or C_a (by c. 70%) during the 20th century. This finding has been confirmed by studies carried out in distinct ecosystems (Bing et al., 2022; Mathias and Hudiburg, 2022). Failure to consider g_m must lead to an overestimated historical trend as implied by the partial derivative of iWUE$_{mes}$ (Equation 11) (Gong et al., 2022):

$$\frac{d\text{iWUE}}{dC_a} = \frac{b-\Delta}{k\left(\frac{g_{sc}}{g_m}(b-a_m)+b-a_s\right)} \qquad \text{Equation 13}$$

Given that ^{13}C series of environmental archives (e.g. tree rings) provide a unique proxy for benchmarking the output of land surface models (Frank et al., 2015; Wang et al., 2017; Lavergne et al., 2022), cautions should be paid when iWUE$_{sim}$ model is used to predict historical trend of iWUE.

Uncertainty in iWUE$_{sim}$ estimation associated with *b′*

The value and physiological meaning of *b'* in the equation of Δ_{sim} or iWUE$_{sim}$ remain subjects of debate. Initially, Farquhar et al. (1982a) proposed that *b'* (27‰) could be derived from early *in vitro* estimations of Rubisco carboxylation. As it agreed well with the relationship between measured biomass-based Δ and C_i/C_a, it was interpreted as a fitted value. However, when measured Δ from online instantaneous measurements was used to fit Equation 8, the fitted *b'* appears to be lower than 27‰ (Caemmerer and Evans, 1991; Ma et al., 2021).

b' was also explained as the net fractionation caused by Rubisco and PEPC (phosphoenolpyruvate carboxylase). Farquhar and Richards (1984) described *b'* as a function of relative contribution of Rubisco and PEPC carboxylation:

$$b' = (1-\beta)b + \beta b_4 \qquad \text{Equation 14}$$

where b (29-30‰) and b_4 (usually taken as -5.7‰ at 25°C) are fractionation factors of Rubisco and PEPC carboxylation, respectively. β is the proportion of carbon fixation through PEPC carboxylation. PEPC uses HCO_3^- produced by CO_2 hydration as the substrate for the synthesis of aspartate or malate, which is important for the control of cellular pH (Davies, 1979). Generally, carboxylation by Rubisco contributes a greater fraction of carbon in plants and respiratory substrates. But it is also suggested that the PEPC carboxylation could be important under the conditions of low stomatal conductance or carboxylation in darkness (Ikeda and Yamada, 1981; Gupta et al., 1994; Hibberd and Quick, 2002). Furthermore, several studies have revealed that N source and concentration were potential factors affecting carbon fixation by PEPC, which indicates that *b'* could vary with nitrogen metabolism (Raven and Farquhar, 1990; Douthe et al., 2012; Lian et al., 2021). That is, Equation 14 is not particularly useful for iWUE estimation because β is variable and difficult to quantify.

b' has also been described by Ubierna and Farquhar (2014) as a parameter that included carboxylation, mesophyll conductance, and photorespiration:

$$b' \cong b\frac{C_c}{C_i} + a_m\left(1-\frac{C_c}{C_i}\right) - f\frac{\Gamma^*}{C_a} \qquad \text{Equation 15}$$

According to Equation 15, *b'* is largely dependent on C_c/C_i which is modulated by g_m. It should be noted that the most used *b'*=27‰ is consistent with the C_c/C_i value of 0.9, higher than the common values of 0.7-0.8 (Caemmerer and Evans, 1991; Warren et al., 2003). So far, Equations 14 and 15 have only been used to discuss the potential origin of variation in *b*', but have not been incorporated in the model of iWUE estimation. In short, there is still no consensus concerning the interpretation of *b*', and current discussion on *b'* (Equations 14, 15) illustrated that it should not be treated as a constant value of 27‰.

Uncertainty in iWUE associated with post-photosynthetic fractionation

Post-photosynthetic fractionation (Δ_{post}) includes the discrimination processes that follow photosynthetic carbon fixation, altering δ^{13}C signals in plant organs and leaves at different development stages (Badeck et al., 2005; Vogado et al., 2020). In general, heterotrophic organs (branches, stems and roots) are ^{13}C-enriched compared with autotrophic organs (leaves) (Badeck et al., 2005; Bowling et al., 2008; Cernusak et al., 2009; Lamade et al., 2016), and the immature leaves (heterotrophic phase) are ^{13}C-enriched (by *c.* 2‰) compared to mature leaves (autotrophic phase) in both deciduous and evergreen species (Lamade et al., 2009; Vogado et al., 2020). However, the contribution of Δ_{post} to δ^{13}C of plant tissues and its influence on iWUE estimation are poorly understood.

Several studies has accounted for Δ_{post} to estimate iWUE (Table S2). Gimeno et al. (2021) found an improvement in correlation between iWUE estimated from gas exchange and that from Δ when g_m and Δ_{post} were accounted for. In that study, Δ_{post} was taken as -2.5‰ estimated from the δ^{13}C difference between phloem contents and whole-tree photosynthesis. Similarly, δ^{13}C of wood and cellulose have been corrected by -3.2‰ and -1.3‰, respectively, to account for the offset from leaf δ^{13}C (Thomas et al., 2013; Brownlee et al., 2016). In other studies, iWUE was calculated from tree-ring with a δ^{13}C offset of

-2‰ to account for Δ_{post} (Michelot et al., 2011; Frank et al., 2015). Without correcting a Δ_{post} of about 2.5‰, iWUE estimated from the $\delta^{13}C$ of tree-ring could be overestimated by 20% (Gessler et al., 2009).

The likely mechanisms underlying Δ_{post} include fractionation associated with respiration, transport and mixing of assimilates (Tcherkez et al., 2003; Brüggemann et al., 2011; Bögelein et al., 2019). Respiratory fractionation ranges from -6 to 0‰ in various species (Duranceau et al., 1999; Ghashghaie et al., 2001; Tcherkez et al., 2003). Also, there are some variations in the apparent fractionation during transport processes (e.g., day-night differences in $\delta^{13}C$ of leaf-export organic matter and different leaf-to-phloem $\delta^{13}C$ signatures along vertical canopy gradients) and very few direct measurements of isotopic differences between components at molecule/atom scale (Gessler et al., 2008; Mauve et al., 2009; Gilbert et al., 2011; Gilbert et al., 2012; Bögelein et al., 2019). In addition, post-photosynthetic fractionation is complicated by ontogenic effects (e.g., size, height, and age of individuals) that can confound the relationship between iWUE and environmental factors (Vadeboncoeur et al., 2020). That is, the influence of Δ_{post} could accumulate over time and lead to age-dependent patterns (Cernusak et al., 2009). Therefore, using a constant, empirical value of Δ_{post} could be unreliable. A mechanistic description of Δ_{post} should be very useful to improve the iWUE estimates, which requires further study on the fractionations associated with respiration, transport and allocation of assimilates.

iWUE$_{mes}$, a useful, simplified model for estimating iWUE

iWUE$_{mes}$ takes the influence of mesophyll conductance and photorespiratory fractionation into account. We propose to use the iWUE$_{mes}$ model (Equation 11) since it considers the components that have a significant influence on the iWUE estimation. In particular, it includes g_m effect which is known to affect iWUE prediction.

Parameterizing the iWUE$_{mes}$ model requires g_{sc}/g_m rather than g_m (Ma et al., 2021). Some studies use a constant g_m in the equation to estimate iWUE (Keeling et al., 2017), which makes more sense than disregarding g_m. However, the assumption of constant g_m is not supported by experimental evidence. The positive relationships between g_{sc} and g_m have been reported in many studies (Flexas et al., 2013; Gong et al., 2018), and this relationship is rather conserved across levels of CO_2, irradiance, and drought stress and functionally distinct species (Flexas et al., 2008; Ma et al., 2021; Gong et al., 2022). Incorporating the g_{sc}/g_m ratio improves the predictive accuracy of the iWUE model, as demonstrated in gas exchange experiments (Ma et al., 2021). Without knowing g_m, it is preferable to use the average g_{sc}/g_m of 0.79 (± 0.07) derived from a global synthesis to parameterize the iWUE$_{mes}$ model rather than using the iWUE$_{sim}$ model.

We acknowledge that using a constant g_{sc}/g_m is not always adequate. As more g_m data become available, interspecific differences in g_{sc}/g_m can be identified and should be accounted for in the estimated iWUE. Theoretically, species-specific g_{sc}/g_m is more appropriate to be used in the iWUE$_{mes}$ estimation. It is also noteworthy that short-term responses of g_m are still not well defined, implying that neglecting short-term variation in g_{sc}/g_m might lead to errors in estimating iWUE at nonsteady-states, thus should be addressed in further work. Moreover, the iWUE$_{mes}$ model does not account for the post-photosynthetic fractionation, due to a lack of knowledge on post-photosynthetic fractionation. Therefore, we recommend for biomass-based analyses to distinguish the age of organs to minimize the influence of post-photosynthetic fractionation.

Conclusion remarks

The comprehensive model of photosynthetic ^{13}C discrimination of Farquhar and Cernusak (2012) is a synthesis of current understanding, and provides the theoretical basis for estimating iWUE from the ^{13}C composition of plant materials. The classical iWUE$_{sim}$ model has been shown to strongly overestimate iWUE and its historical trends due to the neglect of g_m associated effect, limiting its use in quantitative studies. iWUE$_{mes}$ is suggested as a useful, simplified model for quantitative estimation of iWUE, which has been included in a standardized, open-source tool (R package) for calculation of iWUE from stable isotope signatures (Mathias and Hudiburg, 2022). Nonetheless, the formulation of iWUE$_{mes}$ could still be further improved. For example, a fixed, empirical g_{sc}/g_m value could be replaced by species-specific values or mechanistic relationships derived from experimental results. ^{13}C discrimination of plant material, combining with appropriate iWUE models, is also an ultimate tool for screening genetic resources to enhance the iWUE of crops under climate change scenarios. One of the primary questions is how g_{sc} and g_m of plants will respond to changes in temperature, water availability, and carbon dioxide concentration. Furthermore, the response of post-photosynthetic fractionation to climate change factors remains unknown. We conclude that mechanistic descriptions of g_m associated effect and post-photosynthetic fractionation are the bottlenecks for improving the ^{13}C-based estimation of iWUE.

Author contributions

XG conceptualized the topic of this review, WM and YY wrote the first draft, and all authors contributed to the writing and revision of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (NSFC 31870377, 32120103005, 32201277).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1037972/full#supplementary-material

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