Commentary

Advances in measurements and models of photosynthetic carbon isotope discrimination in C_3 plants

The scientific community has long recognized that the study of atmosphere–biosphere interactions is vital to understanding fields as varied as agriculture, ecology and global change. Measurements of CO_2 and H_2O exchange are potentially enhanced by measurements of the minor isotopologues of those species (e.g. $^{13}C^{16}O_2$), because of the extra information revealed about mechanisms limiting the fluxes. In the past, it was difficult to add the minor isotopologues to these ecosystem fluxes because samples had to be analysed in an isotope ratio mass spectrometer in a laboratory setting. Nowadays, optical isotope analysers (lasers and Fourier transform infrared spectroscopy) allow for rapid measurement of isotope ratios with sufficient precision, and in field settings.

This has motivated a series of innovative studies both in the laboratory and in the field that combine measurements and models of photosynthetic fluxes and isotope exchange. Absorption spectroscopy methods have increased the rate at which measurements can be taken in the laboratory, allowing us to gain a better understanding of the dynamic responses of mesophyll conductance (Tazoe *et al.* 2011; Evans & von Caemmerer 2013), value for photorespiratory fractionation (Evans & von Caemmerer 2013), respiratory effects and their impact on photosynthetic discrimination calculations (Barbour *et al.* 2007; Stutz *et al.* 2014) and calculations of leakiness in C_4 species (Ubierna *et al.* 2011). Field applications that traditionally have used isotope records from atmospheric flask samples or tree ring chronologies will benefit from the large data sets that are starting to become available with optical isotope analysers. Example of these are isotope-constrained carbon budgets (Ballantyne *et al.* 2010), partitioning of oceanic and terrestrial fluxes (Ciais *et al.* 1995; Fung *et al.* 1997), or confirmation of simulations of ^{13}C discrimination by the terrestrial biosphere (Suits *et al.* 2005). Some other examples of field applications that have already used optical analysers include partitioning of net ecosystem exchange (Bowling *et al.* 2001; Zobitz *et al.* 2008) and the testing of the predictive ability of different discrimination models (Wingate *et al.* 2007; Bickford *et al.* 2009). In this issue of *Plant, Cell & Environment*, Gentsch *et al.* (2014) collect the largest available field data set of instantaneous measurements of observed discrimination (60 d of records with a half-hour interval) and use it to test different formulations of the ^{13}C photosynthetic discrimination model ($\Delta^{13}C$). They demonstrate that both simple and 'comprehensive' models of $\Delta^{13}C$ were able to predict flux-weighted discrimination equally well. Herein, we build upon the work of Gentsch *et al.*

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(2014), comment on the errors that may result when using different formulations of $\Delta^{13}C$ and give some recommendations about what formulation should be chosen for different applications.

SIMPLIFIED VERSUS 'COMPREHENSIVE' MODELS OF DISCRIMINATION

One common debate in these kinds of studies is the degree of complexity that should be included in the mathematical models of ^{13}C photosynthetic discrimination ($\Delta^{13}C$). The original model for $\Delta^{13}C$ (Farquhar *et al.* 1982b), extended to include ternary effects (Farquhar & Cernusak 2012), includes several parameters that are difficult to measure or estimate and therefore often sacrificed for easier formulations (Fig. 1a). In the last few years, as the reliability of data and of parameter values has improved, detailed studies have shifted from the use of the very simplified formulation to the use of the full equation.

To illustrate the impact of Rubisco fractionation, stomatal conductance, mesophyll conductance, respiration and photorespiration on total $\Delta^{13}C$, we divide the 'com*prehensive*' (thus far) photosynthetic discrimination model for C_3 plants into the following components:

$$\Delta_{com} = \Delta_b - \Delta_{gs} - \Delta_{gm} - \Delta_e - \Delta_f \qquad (1)$$

where the detailed equations for Δ_b, Δ_{gs}, Δ_{gm}, Δ_e and Δ_f are given in Fig. 1b. This division is different from what was applied in Evans & von Caemmerer (2013), which lumped together the first two terms $\Delta_b - \Delta_{gs}$. We prefer to keep them separated because then the magnitude of the stomatal (Δ_{gs}) and mesophyll (Δ_{gm}) contributions to Δ_{com} are directly comparable. We calculated these five components of discrimination in four scenarios resulting from the combination of high or low photosynthetic rate ($A = 20$ or $3 \mu mol\ m^{-2}\ s^{-1}$) with large or small mesophyll conductance ($g_m = 0.5$ or $0.1\ mol\ m^{-2}\ s^{-1}$; Fig. 2). Stomatal conductance (g_s), day respiration (R_d) and transpiration (E) rates were kept constant at $0.3\ mol\ m^{-2}\ s^{-1}$, $1\ \mu mol\ m^{-2}\ s^{-1}$ and $4\ mmol\ m^{-2}\ s^{-1}$, respectively. All other parameters involved in the calculation of $\Delta^{13}C$ from theory were either derived from these previous values or from known constants.

Figure 2 illustrates that the largest moderators of Rubisco fractionation are Δ_{gs} and Δ_{gm}. When both A and g_m were large, the diminutions of Δ_b (Rubisco fractionation) by g_s and g_m were 8 and 3‰, respectively. Under current ambient conditions [21% O_2, $[CO_2] = 400\ \mu mol\ mol^{-1}$], photorespiration represents a constant contribution of 1.1‰ at 25 °C. The effect of day respiration is modulated by the ratio

$R_d/(A + R_d)$. Thus, the respiratory contribution to discrimination is larger whenever R_d is large in proportion to A. The estimated value of fractionation during day respiration, e, varies between 0 and −5‰ (Tcherkez *et al.* 2004, 2010, 2011). In our example with e = −5‰, Δ_e reached a contribution to total $\Delta^{13}C$ of −1.1‰ when A was low. Occasionally, a much larger apparent respiratory fractionation can occur when the substrate for respiration has a very different $\delta^{13}C$ value from that of recent assimilates. This typically occurs in experiments that use a depleted tank for gas exchange/isotope measurements or when there is a shift in respiratory substrates, for example, at dusk or dawn (Wingate *et al.* 2007; Gentsch *et al.* 2014; Stutz *et al.* 2014). Nevertheless when weighted by carbon assimilation, under most conditions the respiratory contribution to daytime discrimination will be small and negligible. Notice (Fig. 2) that photorespiration and respiration contributions to total discrimination partially cancel each other out because of the opposite sign of the fractionation factors f (positive) and e (negative). If the terms Δ_b–Δ_{gs} are lumped together and ternary and boundary layer conductance effects are considered negligible, it results in the familiar, *sim*ple expression of Δ_{sim} [= $a_s + (b - a_s)C_i/C_a$]. If the value of b in Δ_{sim} is reduced (let us call it $\bar{b}$) to account for the drop in discrimination by Δ_{gm}, then Δ_{com} is essentially approximated by $\Delta_{sim-\bar{b}}$, with the remaining error (<1‰) attributed to photorespiration.

WHAT IS THE VALUE OF $\bar{b}$?

It is often suggested that the 'comprehensive' discrimination model Δ_{com} for C₃ plants can be approximated by Δ_{sim} via substituting the Rubisco fractionation factor b (30‰; Roeske & O'Leary 1984) by $\bar{b}$ (27‰, Δ_{sim-27}; Farquhar *et al.* 1982a). The value of $\bar{b}$ = 27‰ was derived from empirical relationships between $\Delta^{13}C$ that were calculated from $\delta^{13}C$ of leaf bulk material and C_i/C_a values (Farquhar *et al.* 1982a). A similar value was obtained by Cernusak *et al.* (2013) from a compilation of records of bulk material $\delta^{13}C$ and C_i/C_a of different species. Because 27‰ was derived from leaf bulk material, it accounts for all the isotopic effects that happen during photosynthetic discrimination, that is, Δ_b, Δ_{gs}, Δ_{gm}, Δ_e and Δ_f, and also includes any post-photosynthetic fractionation (Badeck *et al.* 2005; Cernusak *et al.* 2009). In order to derive a convenient relationship between b and $\bar{b}$, we consider ternary, boundary layer, respiratory and photorespiratory effects negligible. In this case $\Delta_{com} \approx \Delta_b - \Delta_{gs} - \Delta_{gm} \approx \Delta_{sim} - \Delta_{gm} \approx \Delta_{sim-\bar{b}}$. From here we can derive an expression for $\bar{b}$ by subtracting the simplified expressions for Δ_b, Δ_{gs} and Δ_{gm} (Fig. 1b):

$$\bar{b} \cong b\frac{C_c}{C_i} + a_m\left(1 - \frac{C_c}{C_i}\right) \qquad (2)$$

Notice that in Eqn 2, the term $a_m(1 - C_c/C_i)$ ranges from 0.2 to 0.5‰ if C_c/C_i is 0.7–0.9, and thus we could further simplify to:

$$\bar{b} \cong b\frac{C_c}{C_i} = b\left(1 - \frac{C_i - C_c}{C_i}\right) = b\left(1 - \frac{A}{g_m C_i}\right) \qquad (3)$$

which gives an easy way of converting from b to $\bar{b}$ in C₃ plants. In a slightly more detailed form, we could include the effect of photorespiration in the derivation of $\bar{b}$, resulting in:

$$\bar{b} \cong b\frac{C_c}{C_i} + a_m\left(1 - \frac{C_c}{C_i}\right) - f\frac{\Gamma^*}{C_a} \cong b\frac{C_c}{C_i} - f\frac{\Gamma^*}{C_a} \qquad (4)$$

von Caemmerer & Evans (1991) reported C_c/C_i = 0.7 (⇔ $C_i - C_c$ = 84 μmol mol⁻¹ if C_i = 280 μmol mol⁻¹). In a compilation of studies by Warren *et al.* (2003), the slope of g_m versus A was 0.015 mol μmol⁻¹ (⇔ $A/g_m = C_i - C_c$ = 67 μmol mol⁻¹), which translates to C_c/C_i = 0.8 if C_i = 280 μmol mol⁻¹. According to Eqn 2, if C_c/C_i = 0.7–0.8 and b = 30‰, then $\bar{b}$ = 21.5 to 24.4‰. If photorespiration is included in the derivation of $\bar{b}$, it will result in 1.1‰ smaller values at 25 °C and 21% O₂. Clearly, there is discrepancy with $\bar{b}$ = 27‰, which would only be consistent with C_c/C_i of 0.9 or a drop in concentration between C_i and C_c of ≈ 28 μmol mol⁻¹. Throughout this discussion, we use b = 30‰, but note that this value can be modified as a function of the contribution of CO_2 fixation by PEP carboxylation (Farquhar & Richards 1984), which might vary with factors such as N metabolism (Raven & Farquhar 1990).

The inconsistency between reported values of C_c/C_i and $\bar{b}$ = 27‰ can be explained by the combination of two factors: (1) the difference between discrimination derived from $\delta^{13}C$ of plant bulk material (Δ_p) and from *obs*erved instantaneous measurements of gas and isotopic exchange during photosynthesis (Δ_{obs}; Evans *et al.* 1986), and (2) differences in the method of calculation of g_m. Firstly, the value of $\bar{b}$ = 27‰ was developed from observations of Δ_p. However, Δ_p is often larger than Δ_{obs}, for example, by 2‰ in the study by von Caemmerer & Evans (1991). The reason behind the differences is unclear, but may be related to the different integration times of the isotopic signal and to the fact that bulk material contains fractions that are more depleted than sugars, such as lipids or lignin and possibly reflects nocturnal respiratory fractionations that are not captured in daytime Δ_{obs} (Cernusak *et al.* 2009). A 2‰ difference in discrimination would translate into a change in C_c/C_i of ≈ 0.1.

Secondly, values of g_m are obtained with various methods, each of them with different associated errors (Pons *et al.* 2009). For example, the effect of photorespiration is occasionally ignored when using the isotope method to calculate g_m leading to an underestimation of C_c/C_i of ≈ 0.05 at 25 °C and 21% O₂. Apart from methodological differences among studies, the ratio C_c/C_i is likely to vary among species and environmental conditions. It has been demonstrated that the slope of the relationship between A and g_m is species specific (von Caemmerer & Evans 1991; Loreto *et al.* 1992; Hanba *et al.* 2001; Singsaas *et al.* 2004; Ubierna & Marshall 2011).

A USEFUL SIMPLIFIED MODEL FOR $\Delta^{13}C$

Evidently, when g_m can be accurately determined, there is no need to use an approximate value for $\bar{b}$. However, it is difficult to estimate g_m. Initially, g_m was thought to be a static parameter determined by leaf structural characteristics. Now, recent studies appear to show rapid changes of this variable

(a) 'COMPREHENSIVE' $\Delta^{13}C$ MODEL FOR C_3 PLANTS

$$\Delta_{com} = \frac{1}{1-t}\left[a_b \frac{c_a - c_s}{c_a} + a_s \frac{c_s - c_i}{c_a}\right] + \frac{1+t}{1-t}\left[a_m \frac{c_i - c_c}{c_a} + b\frac{c_c}{c_a} - \frac{\alpha_b}{\alpha_e}e\frac{R_d}{A+R_d}\frac{c_c - \Gamma^*}{c_a} - \frac{\alpha_b}{\alpha_f}f\frac{\Gamma^*}{c_a}\right]$$

SIMPLIFICATIONS

1. $\alpha_b, \alpha_e, \alpha_f \cong 1$

2. Measurements under low-moderate VPD ➔ $t = 0$

$$\Delta_1 = a_b \frac{C_a - C_s}{C_a} + a_s \frac{C_s - C_i}{C_a} + a_m \frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - e\frac{R_d}{A+R_d}\frac{C_c - \Gamma^*}{C_a} - f\frac{\Gamma^*}{C_a}$$

3. Infinite boundary layer conductance ➔ $C_a = C_s$

$$\Delta_2 = a_s \frac{C_a - C_i}{C_a} + a_m \frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - e\frac{R_d}{A+R_d}\frac{C_c - \Gamma^*}{C_a} - f\frac{\Gamma^*}{C_a}$$

4. High assimilation flux $[R_d/(A+R_d) \cong 0]$ or small e $[e \cong 0]$

$$\Delta_3 = a_s \frac{C_a - C_i}{C_a} + a_m \frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - f\frac{\Gamma^*}{C_a}$$

5. 2% $[O_2]$ $[\Gamma^*/C_a \cong 0)$ or small f $[f \cong 0]$

$$\Delta_4 = a_s \frac{C_a - C_i}{C_a} + a_m \frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a}$$

6. Infinite mesophyll conductance ➔ $C_i = C_c$

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

(b) RE-WRITING THE 'COMPREHENSIVE' $\Delta^{13}C$ MODEL FOR C_3 PLANTS

$$\Delta_{com} = \Delta_b - \Delta_{gs} - \Delta_{gm} - \Delta_e - \Delta_f \cong \Delta_{sim} - \Delta_{gm} - \Delta_e - \Delta_f$$

SUBDIVISIONS

1. Rubisco fractionation

$$\Delta_b = \frac{1+t}{1-t}b \cong b$$

2. Stomatal contribution

$$\Delta_{gs} = \frac{1+t}{1-t}\left[b - \frac{\bar{a}}{1+t}\right]\frac{C_a - C_i}{C_a} \cong [b - a_s]\frac{C_a - C_i}{C_a}$$

$\Delta_b - \Delta_{gs} \cong \Delta_{sim}$

$\Delta_{sim} - \Delta_{gm} \cong \Delta_{sim-\bar{b}}$

3. Mesophyll contribution

$$\Delta_{gm} = \frac{1+t}{1-t}[b - a_m]\frac{C_i - C_c}{C_a} \cong [b - a_m]\frac{C_i - C_c}{C_a}$$

4. Respiration contribution

$$\Delta_e = \frac{1+t}{1-t}\left[\frac{\alpha_b}{\alpha_e}\frac{eR_d}{A+R_d}\frac{C_c - \Gamma^*}{C_a}\right] \cong \frac{eR_d}{A+R_d}\frac{C_c - \Gamma^*}{C_a}$$

5. Photorespiration contribution

$$\Delta_f = \frac{1+t}{1-t}\frac{\alpha_b}{\alpha_f}f\frac{\Gamma^*}{C_a} \cong f\frac{\Gamma^*}{C_a}$$

Figure 1. (a) 'Comprehensive' model of $\Delta^{13}C$ photosynthetic discrimination (Farquhar & Cernusak 2012) and several simplifications. (b) Division of the 'Comprehensive' model of $\Delta^{13}C$ into the components that account for the major fractionations that ambient CO_2 undergoes until its fixation in recent photosynthate. The term called *stomatal contribution* includes both stomatal and boundary layer effects, and could easily be decomposed into Δ_{gs} and Δ_{gb}, but boundary layer conductance is often large and thus a small contribution to the total discrimination. C_a, C_s, C_i and C_c ($\mu mol\ mol^{-1}$) are the mol fractions in the ambient air, leaf surface, leaf intercellular spaces and chloroplast, respectively. a_b, a_s, a_m, b, e and f are the fractionations associated with diffusion through the boundary layer (2.8‰), in air (4.4‰), in water (1.8‰), by Rubisco carboxylation (30‰), during respiration (0 to −5‰) and photorespiration (8–16‰), respectively. The terms α_b, α_e and α_f are $1 + b$, $1 + e$ and $1 + f$, respectively. A and R_d are the photosynthetic and day respiration rates ($\mu mol\ m^{-2}\ s^{-1}$), respectively. Γ^* is the CO_2 compensation point in the absence of day respiration ($\mu mol\ mol^{-1}$). The ternary effect is $t = \alpha_{ac}E/2g_{ac}$, where E is transpiration rate ($mol\ m^{-2}\ s^{-1}$), g_{ac} is the conductance to diffusion of CO_2 in air ($mol\ m^{-2}\ s^{-1}$) and the fractionation for the isotopologues of CO_2 diffusing in air is $\alpha_{ac} = 1 + \bar{a}$, where $\bar{a} = \dfrac{a_b(C_a - C_s) + a_s(C_s - C_i)}{C_a - C_i}$.

in response to diverse environmental factors. Accordingly, g_m might be a more dynamic trait than initially thought, although the mechanisms of such variation are poorly understood (Flexas *et al.* 2008). When information on g_m is lacking, and it is desired to average over longer periods in which g_m may well have fluctuated, we propose the use of the following expression for C₃ plants:

$$\Delta = \Delta_{sim-\bar{b}} - \Delta_f = a_s + [\bar{b} - a_s]\frac{C_i}{C_a} - f\frac{\Gamma^*}{C_a} \quad (5)$$

We prefer to separate the photorespiratory term, which is not dependent on C_i/C_a, rather than including it in some lower estimation of $\bar{b}$. This is a cleaner approach because it results in $\bar{b}$ only reflecting the contribution of mesophyll conductance. The value for $\bar{b}$ might be ≈26–28‰ if we are attempting to

estimate Δ_p, but somewhat lower if we are modelling Δ_{obs}. For example, in a field study where Δ_{obs} (gas phase) was determined for *Juniperus monosperma*, the bias of $\Delta_{sim-\bar{b}}$ versus Δ_{obs} was reduced by using $\bar{b} = 25‰$ instead of 27‰ (Bickford *et al.* 2009). In a field study with conifer species, Ubierna & Marshall (2011) found that $\Delta_{sim-\bar{b}}$ with $\bar{b} = 27‰$ was a good proxy for Δ_{com} when Δ_p was derived from phloem contents.

Undoubtedly, Eqn 5 does not account for all observations, and clearly a better fit between observations and a particular model of $\Delta^{13}C$ will be found as more fitting variables are used in the model. In model selection, it should be kept in mind that there should be a trade-off between the goodness of fit and the complexity of the model: appropriate statistical procedures should be used to ensure that there is no over fitting. One typical example from field studies where Eqn 5 might not suffice is the large discrimination values measured at dawn and dusk when photosynthetic rates are low. In these situations, the respiratory term is used as a fitting parameter between modelled and measured discrimination (Wingate *et al.* 2007; Gentsch *et al.* 2014). Of course, data collected when fluxes are low have to be interpreted with caution because of the large error associated with the calculation of Δ_{obs}. Despite the fact that models and measurements can be forced to match with this approach, we still know little about the processes that result in these observations.

In order to decide what equation to choose, the user needs to ponder the application. If the objective is to derive g_m or other parameters from measurements and models of discrimination, the detailed equation is required. Alternatively, for crude applications, such as using $\Delta^{13}C$ to correct ^{14}C data (Drake 2014), it would seem pointless going beyond the simplest model. As illustrated by Gentsch *et al.* (2014), the diurnal variation in Δ_{obs} was mostly explained by the contributions of stomatal and mesophyll conductances and photorespiration, which could be approximated by $\Delta_{sim-\bar{b}} - \Delta_f$. For ecosystem or global scale applications such as isotope-constrained C-budgets, or whenever discrimination needs to be forecast but with no large data set of Δ_{obs} available, it will be advantageous to use the simplified equation introduced here. The 'comprehensive' model requires many estimated parameters, some of them with compensating effects (Gentsch *et al.* 2014) and in the absence of data sets to test the model prediction, it will likely result in larger model uncertainty and instability. A simplified model can introduce more error in the prediction of diurnal patterns,
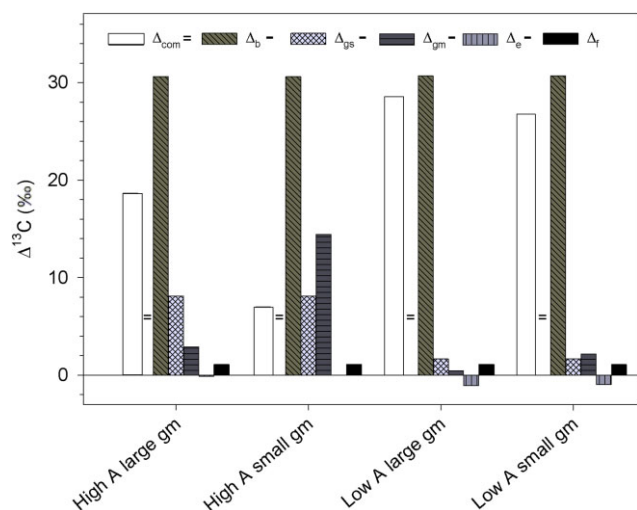

Figure 2. Example of the contribution to total ^{13}C discrimination during photosynthesis of Rubisco fractionation (Δ_b), stomatal and mesophyll conductances (Δ_{gs} and Δ_{gm}, respectively), respiration (Δ_e) and photorespiration (Δ_f). Equations for each component are presented in Fig. 1b. The values used for calculations were high or low photosynthetic rate ($A = 20$ or $3\ \mu mol\ m^{-2}\ s^{-1}$), large or small mesophyll conductance ($g_m = 0.5$ or $0.1\ mol\ m^{-2}\ s^{-1}$), $g_s = 0.3\ mol\ m^{-2}\ s^{-1}$, $R_d = 1\ \mu mol\ m^{-2}\ s^{-1}$ and $E = 4\ mmol\ m^{-2}\ s^{-1}$. All other parameters involved in the calculation of $\Delta^{13}C$ were either derived from these previous values or from known constants (see Fig. 1 caption).

but C cycle models are more concerned with longer term C flux estimates. Technological advances and additional improvements to field studies should help increase the precision of data collected and our understanding of the factors contributing to photosynthetic discrimination at different temporal scales.

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