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USING MODELS OF CARBON ISOTOPE FRACTIONATION DURING PHOTOSYNTHESIS TO UNDERSTAND THE NATURAL FRACTIONATION RATIO

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ABSTRACT. The fractionation correction *b* is used to correct for the fractionation of ^{14}C by using information from ^{13}C in samples. This value is assumed to have a value of 2, where the $^{14}C/^{12}C$ ratio is double that of the $^{13}C/^{12}C$ ratio. While natural and laboratory fractionation are usually not considered separately, this article explores the differential fractionation of ^{14}C and ^{13}C during the process of photosynthesis. Values of $\delta^{13}C_p$ can be used to calculate $\Delta^{13}C_p$ values, which in turn can be used to calculate $\Delta^{14}C_p$, the discrimination against $^{14}CO_2$ during photosynthesis. Models can then be built of $\Delta^{14}C_p/\Delta^{13}C_p$, an approximation for the natural fractionation ratio. This approximation suggests that for C_3 plants the ratio is ~1.90 and for C_4 plants the ratio is more variable. While error introduced by the natural fractionation is small, it is also possibly systematic, as $b = 2.0$ does not seem physiologically possible following these models of carbon fractionation during photosynthesis. The central aim of this study is to illustrate that *b* derives not from a natural constant, but rather from a variable natural process.

INTRODUCTION

Radiocarbon measurements are corrected for isotopic fractionation using a value of 2.0 to correct for differences between $^{14}C/^{12}C$ and $^{13}C/^{12}C$ fractionation (Craig 1957; Stuiver and Polach 1977). It has been suggested in the past that this value may be closer to 1.9 (Stuiver and Robinson 1974). Deviations in this value add only a small amount of error to resulting probable ages derived from ^{14}C dating (Wigley and Muller 1981). While Wigley and Muller (1981) concluded that variation in *b* from 2.0 would not significantly impact ^{14}C dates, Southon (2011) has recently suggested that the combination of anomalies in the literature and increasing accuracy and precision in ^{14}C dating necessitate a reappraisal of the assumed value of 2.0 (Southon 2011).

In past consideration of the effect of the fractionation ratio on ^{14}C dating, it has been suggested that natural and laboratory fractionation do not need to be considered separately (Wigley and Muller 1981). Given recent discussion regarding the fractionation ratio (Southon 2011) it may be worthwhile to investigate natural fractionation effects in isolation. The present article uses common models of carbon isotope fractionation used in plant biochemistry to model natural variation in carbon isotope fractionation during photosynthesis. Models developed by Farquhar et al. (1982, 1989) express carbon isotope fractionation as a result of different biological processes in photosynthesis. These models identify potential sources of variability that may influence the dating of plants with different carbon fixation pathways. The scope of this work is limited; these models do not estimate changes in fractionation due to laboratory procedures and they cannot account for all variation in the fractionation ratio. Nonetheless, estimates of *b* may benefit from distinction between C_3 and C_4 plants due to natural differences in fractionation against carbon isotopes.

C_3 PLANTS

Plants that exhibit the C_3 carbon fixation pathway are the most abundant in nature. They are characterized by highly variable $\delta^{13}C_p$ values in photosynthate and product tissues (Farquhar et al. 1982). This is the consequence of kinetic and thermodynamic discrimination processes that occur within the plant. The first is the diffusion of CO_2 through the stomata of the plant. The second results from carboxylation, primarily through Ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco). The third effect is the difference between ambient (c_a) and intracellular (c_i) pressures of CO_2 at the leaf level. Farquhar et al. (1982) developed an expression to approximate these effects of internal $\delta^{13}C$ after carbon fixation in C_3 plants:

$$\delta^{13}C_p = \delta^{13}CO_2 - a_p - (b_p - a_p)c_i/c_a \quad (1)$$

where $\delta^{13}CO_2$ represents atmospheric CO_2 $\delta^{13}C$ values; $\delta^{13}C_p$ is the isotopic carbon product of photosynthesis; a_p is change brought by diffusion of $\delta^{13}CO_2$ (4.4‰) through stomata; b_p is the effect of Rubisco (25–30‰); and c_i/c_a the ratio between the CO_2 partial pressure in the intercellular leaf space and the surface of the leaf, respectively. The fractionation process is driven in part by changes of the carbon source ($\delta^{13}CO_2$) and limitations in water availability. When a plant has sufficient water, carbon is more likely to flow freely through the plant and discrimination is primarily the product of carboxylation. When water is limited, the water use efficiency of the plant goes up as stomatal conductance is reduced. This limits the flow of carbon and the plant's discrimination against carbon approaches the fractionation due to stomatal diffusion. Just as stomatal closure reduces carbon intake and increases the incorporation of ^{13}C in plant tissues, it will also increase the incorporation of ^{14}C in the same tissues.

A model for photosynthetic discrimination in leaves was developed (Farquhar et al. 1982, 1989) to analyze whole plant processes in the same terms as chemical processes. This measure of discrimination factors in variation in source carbon ($\delta^{13}CO_2$) and thus provides a an approximation of plant response to environmental variation:

$$\Delta^{13}C_p = a_p + (b_p - a_p)\, c_i/c_a = (\delta^{13}CO_2 - \delta^{13}C_p)/(1 + \delta^{13}C_p) \quad (2)$$

The resulting discrimination value ($\Delta^{13}C_p$) directly expresses the results of plant photosynthesis, whereas raw $\delta^{13}C_p$ records both source (atmospheric $\delta^{13}CO_2$) and plant biological processes. To build an accurate model for ^{14}C discrimination ($\Delta^{14}C_p$) during the process of photosynthesis, the CO_2 partial pressure ratio within and outside the plant surface (c_i/c_a) must be known. This is the primary source of variation in $\Delta^{13}C_p$ in C_3 plants due to closure of the stomata in response to changes in water availability:

$$c_i/c_a = (\Delta^{13}C_p - a_p)/(b_p - a_p) \quad (3)$$

The calculation of the c_i/c_a ratio is important because it affects the strength of the fractionation effect of Rubisco. Lower values of the c_i/c_a ratio weaken the fractionation effect of Rubisco, while higher values strengthen it.

C_4 PLANTS

C_4 plants, while not as abundant as C_3 plants, nonetheless account for 18% of global productivity (Ehleringer et al. 1997). The first step in the C_4 photosynthetic pathway is the conversion of CO_2 to HCO^-, which in turn is fixed by Phosphoenolpyruvate carboxylase (PEPCase) to form oxalacetate. C_4 plants have taxa-dependent transformations that vary from plant to plant, but all produce CO_2 which is then released into the bundle sheath cells, where Rubisco then becomes the primary carboxylation agent for CO_2 that has not leaked away. These differences are reflected in stable carbon isotope ratios. PEPCase has a lower fractionation effect (5.7‰) than Rubisco (25–30‰). Some leakage occurs in the bundle sheath cells, which also contributes to the resulting fractionation effect. A similar expression to Equation 1 was developed (Farquhar 1983; Farquhar et al. 1989) to model composition of $\delta^{13}C$ of C_4 plants:

$$\delta^{13}C_p = \delta^{13}CO_2 - a_p - (c_p + b_p\varphi - a_p)c_i/c_a \quad (4)$$

$$\Delta^{13}C_p = a_p + (c_p + b_p\varphi - a_p)c_i/c_a = (\delta^{13}CO_2 - \delta^{13}C_p)/(1 + \delta^{13}C_p) \quad (5)$$

$$c_i/c_a = (\Delta^{13}C_p - a_p)/(c_p + b_p\varphi - a_p) \quad (6)$$

where c_p represents the isotopic shift due to carbonic anhydrase and PEP (–7.9 + 2.2 = –5.7‰) and

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φ represents the fraction of CO_2 returned to the mesophyll from the bundle-sheath cells. C_4 plants have much lower discrimination against $\delta^{13}CO_2$ due to the replacement of Rubisco with PEPCase as the primary carboxylyzing agent. This results in less deviation from $\delta^{13}CO_2$, resulting in a more reliable record for atmospheric values (Marino and McElroy 1991).

NATURAL FRACTIONATION RATIO

To determine the natural fractionation ratio, the fractionation effects between ^{13}C and ^{14}C must be determined for the diffusion of CO_2 through stomata (a_p) and carboxylation by Rubisco (b_p). Farquhar et al. (1982) suggested that the diffusion of $^{14}CO_2$ through the stomata is $2(a_p) = 8.8‰$ and $1.9(b_p) = 51.3‰$ for carboxylation of ^{14}C by Rubisco, based on values estimated by O'Leary (1981). To come to a closer number for these two variables, the chemical processes are calculated using the transition-state theory for kinetic isotope effects (Melander 1960):

$$v^{\neq}_1/v^{\neq}_2 = \sqrt{m_2/m_1} \tag{7}$$

where $v^{\neq}$ represents the imaginary species and m represents the reduced molar masses (Tcherkez and Farquhar 2005; Westaway et al. 2007). Equation 7 can be used to calculate the difference in carbon bond formation during carboxylation, where reduced mass in the light isotope (^{12}C) is represented by $1/m_1 = 1/12 + 1/12$ and the heavier isotope (^{13}C) by $1/m_2 = 1/12 + 1/13$; following Equation 7 these can be calculated as $\sqrt{(1/12 + 1/12)/(1/12 + 1/13)} = 1.0198$. The same calculation for ^{14}C follows as $\sqrt{(1/12 + 1/12)/(1/12 + 1/14)} = 1.037749$ (G Farquhar, personal communication, 2012):

$$(v^{\neq}_1/v^{\neq}_2)^{14}/(v^{\neq}_1/v^{\neq}_2)^{13} = \ln(1.037749)/\ln(1.0198039) = 1.89 \tag{8}$$

Following Equation 8, discrimination against $^{14}CO_2$ in carboxylation by Rubisco (b_p) can be estimated by multiplying the value for $^{13}CO_2$, 27‰, by ~1.89, resulting in a value of ~51.03‰. The fractionation effect of diffusion of CO_2 through the stomata of a plant can be similarly expressed, where reduced mass in the light isotope ($^{12}CO_2$) is represented by $1/m_1 = 1/12 + 1/12$ and the heavier isotope ($^{13}CO_2$) by $1/m_2 = 1/44 + 1/45$; following Equation 7 these can be calculated as $\sqrt{(1/28.8 + 1/44)/(1/28.8 + 1/45)} = 1.004425$. The same calculation for $^{14}CO_2$ follows as $\sqrt{(1/28.8 + 1/44)/(1/28.8 + 1/46)} = 1.008713$:

$$(v^{\neq}_1/v^{\neq}_2)^{14}/(v^{\neq}_1/v^{\neq}_2)^{13} = \ln(1.008713)/\ln(1.004425) = 1.97 \tag{9}$$

Discrimination against $^{14}CO_2$ following diffusion through the stomata (a_p) can be estimated by multiplying the value for $^{13}CO_2$, 4.4‰, by ~1.97, resulting in a value of 8.67‰ following Equation 9. This approach differs from previous characterizations of the fractionation ratio, which used a value of 2.0 for kinetic processes (e.g. stomatal diffusion) and 1.9 for chemical processes (e.g. carbon fixation by Rubisco) (O'Leary 1981; Farquhar et al. 1982). Once these fractionation ratios are identified, ^{14}C discrimination ($\Delta^{14}C_p$) can be calculated for C_3 plants as

$$\Delta^{14}C_p = {\sim}1.97a_p + ({\sim}1.89b_p - {\sim}1.97a_p)c_i/c_a \tag{10}$$

The value $\Delta^{14}C_p$, which represents ^{14}C discrimination due to biological plant processes, is a separate value from $\Delta^{14}C$, which represents per mil depletion/enrichment with regard to normalization to $\delta^{13}C_p$. Here, the two values are distinguished by the subscript p to avoid misinterpretation between the two metrics. The ratio of $\Delta^{14}C_p$ over $\Delta^{13}C_p$ should be representative of a "natural value" of b ($b_{natural}$) as a consequence of natural, biological processes prior to sample treatment or other laboratory influences on fractionation. The ratio of $\Delta^{14}C_p$ to $\Delta^{13}C_p$ can be expressed as a relationship with c_i/c_a to illustrate the changing influence of carboxylation on the natural fractionation ratio (Figure 1).

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Figure 1 displays a model of the ratio between $\Delta^{14}C_p$ and $\Delta^{13}C_p$ values over different c_i/c_a ratios. The value of c_i/c_a is important because it influences the strength of the Rubisco fractionation effect on the natural fractionation ratio. These models predict that $b_{natural}$ will have values falling between 1.97 and 1.89. This variation can be thought of as a gradient of weak to strong Rubisco influence on the fractionation ratio with progressively large intercellular to ambient CO_2 partial pressure ratios. The physiologically unlikely c_i/c_a ratio of 0 represents one extreme; when internal partial pressure of a leaf is 0, the diffusion of CO_2 through the stomata is the determinative factor in the natural fractionation ratio. On the other physiologically unlikely extreme, when the internal and external partial pressures are equivalent with a ratio of 1, Rubisco exerts the primary fractionation influence. The fractionation ratios at lower partial pressures and decrease with higher partial pressures. Plants from the Irish oak chronology (McCormac et al. 1994) and from a study of US Southwest pine trees (Leavitt and Long 1988) provide multiple $\delta^{13}C_p$ values from both mesic and arid environments. Calculated c_i/c_a ratios from these plants range from 0.4 to 0.7 using Equation 3. Based on Equation 10, most C_3 plants within this range of c_i/c_a ratios will have a natural fractionation ratio of 1.90.

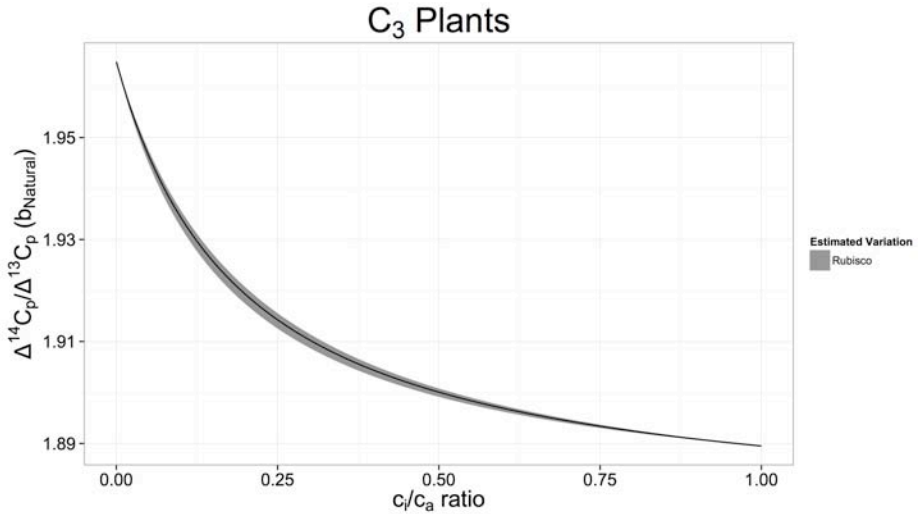

Figure 1 Relationship of $\Delta^{14}C_p$ to $\Delta^{13}C_p$ of C_3 plants as calculated using Equation 10. The model indicates a ratio that varies with the ratio of CO_2 partial pressure within the plant when carboxylation by Rubisco is 27‰. The dark gray error band represents variation in the fractionation effect of Rubisco (25–30‰, dark gray band). When c_i/c_a is low, diffusion through the stomata (a_p) is the dominant influence on the fractionation ratio. When c_i/c_a is higher, carboxylation by Rubisco decreases the discrimination against ^{14}C. Most C_3 plants have a c_i/c_a ratio between 0.4 and 0.7, indicating a natural fractionation ratio near 1.90.

Similarly to C_3 plants, ^{13}C and ^{14}C discrimination rates are calculated by the following formulas for C_4 plants:

$$\Delta^{14}C_p = {\sim}1.97a_p - ({\sim}1.89c_p + {\sim}1.89b_p\varphi - {\sim}1.97a_p)c_i/c_a \quad (11)$$

PEPCase also differs from Rubisco in that an intermediary (HCO_3^-) is used (Farquhar et al. 1982). The kinetic isotope effect is recognized as low with a value of 1.0029 (O'Leary 1981; O'Leary et al. 1981; Chollet et al. 1996), and is smaller than the expected 1.03 value for carbon bond formation (O'Leary et al. 1981). O'Leary et al. (1981) suggested that the carboxylation of PEP occurs by a stepwise mechanism, in which the formation of an intermediate with a divalent metal ion is rate setting. Tovar-Méndez et al. (1998) note that the Mg^+ ion contributes to the intermediate MgPEP, which is the true substrate of PEPCase in absence of activators and the preferred substrate in their presence.

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A value of ~1.89 is adopted for approximating the relative fractionation of $^{14}C/^{13}C$ during carboxylation by PEPCase due to uncertainty, using the same estimate for carbon-carbon bond formation used in Equation 8. While uncertainties remain for estimating discrimination against $^{14}CO_2$ in C_4 plants, Equation 11 can be used to develop a model for the fractionation ratio, illustrated in Figure 2.

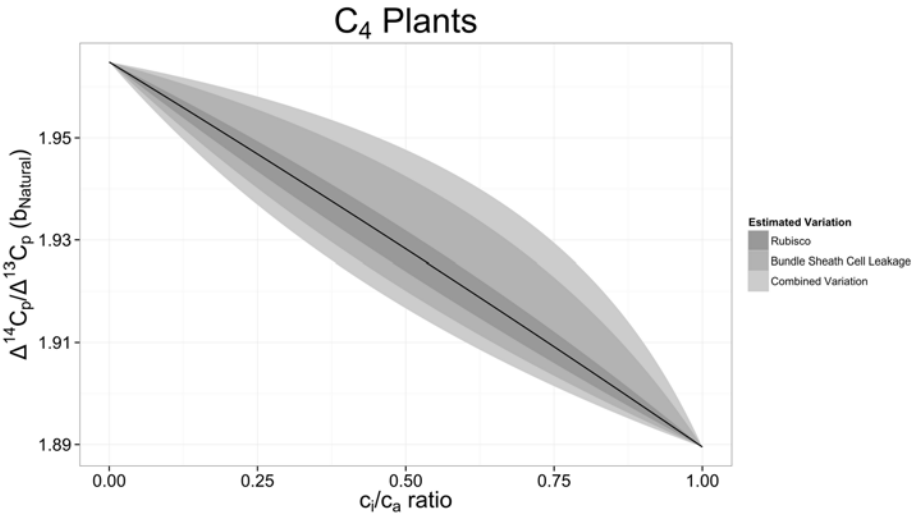

Figure 2 Relationship of the ratio of $\Delta^{14}C_p$ to $\Delta^{13}C_p$ of C_4 plants as calculated using Equation 11. Variation in bundle-sheath cell leakage ($0.28 < \varphi < 0.45$, gray band) and Rubisco ($25‰ < b_p < 30‰$, dark gray band) are represented. The light gray band represents the outer limit of variation in both Rubisco and φ. Variation in φ has an effect on the ratio of $\Delta^{14}C_p$ to $\Delta^{13}C_p$ of C_4 plants relative Rubisco. The variation in C_4 plants may indicate that there is no generalizable single value for the natural fractionation ratio.

The compounding of multiple sources of variation in C_4 plants makes it more difficult to estimate the natural fractionation ratio. In a study of maize, Wang et al. (2012) observed a c_i/c_a ratio of ~0.38 with a range of φ values between 0.25 and 0.53. The model for C_4 plants in Figure 2 would predict a natural fractionation ratio range of 1.93–1.95 based on variation in φ alone. Wang et al. (2012) observed that in maize, φ was the primary source of variation in carbon isotope discrimination. However, the value for φ is difficult to assess in candidate samples for ^{14}C dating, making the analysis of C_4 plants more complicated than for C_3 plants. Nonetheless, C_4 plants may have a slightly higher natural fractionation ratio, closer to 1.93; though changes in φ and c_i/c_a can cause its fractionation to range from 1.90 to 1.96. Ultimately, accurate modeling of the $\Delta^{14}C_p/\Delta^{13}C_p$ ratio in C_4 plants may require information about φ and the c_i/c_a ratio in specific taxa. Perhaps the most salient conclusion from these models is that it is unlikely that a single value for the $\Delta^{14}C_p/\Delta^{13}C_p$ (or $b_{natural}$) ratio can represent both C_3 and C_4 plants.

THE NATURAL FRACTIONATION RATIO AND CALIBRATION CURVES

Of potential importance, these calculations for natural fractionation ratios of ^{14}C and ^{13}C should underly most of the samples used in calibration curves such as IntCal (Reimer et al. 2013). Calibration curves in tree rings are all C_3 plants, and all likely have a natural fractionation ratio of 1.90 based on commonly observed c_i/c_a ratios. Marine calibration curves derived from coral likely have the same natural fractionation ratio. The genus *Symbiodinium*, the photosynthetic endosymbiotic dinoflagellate that lives in the endoderm of coral, has a C_3 carbon fixation pathway (Streamer et al. 1993). The endosymbiot takes in CO_2 and inorganic nutrients and produces lipids and sugars for the coral polyps, which lay down annual bands of calcium carbonate. These bands provide annual records of

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$\Delta^{14}C$ used in marine-based calibration curves (Hughen et al. 2004). Similarly, laminated sediments from the Cariaco Basin feature light bands that contain the remains of *Globigerina bulloides* from past spring upwellings, used to measure past changes in $\Delta^{14}C$ (Hughen et al. 1996). *G. bulloides* is a heterotrophic planktonic foraminifer that consumes both zooplanton and phytoplankton (Lee et al. 1966). The active carbon species for phytoplankton is CO_2 (Swart 1983), though this can differ for other taxa that can utilize HCO_3^- (Rohling and Cooke 2003). Values of $\delta^{13}C_p$ tend to be higher among phytoplankton compared to land plants, –22‰ and –26‰, respectively (Rohling and Cooke 2003). This may be explained by Farquhar et al. (1982) who suggested that a_p is close to zero, though they also note that the incorporation of carbon is more complex than for terrestrial photosynthesis. *G. bulloides* receives carbon from both consumed carbon and its relationship with photosynthetic symbiots (Erez 1978). However, for *G. bulloides*, food plays less of a role in shell $\delta^{13}C$ than temperature's effect on metabolic rate (Ortiz et al. 1996). However, planktonic foraminifera are sensitive to changes in atmospheric $\delta^{13}CO_2$, with an average decrease of 0.63‰ since 1800 (Al-Rousan et al. 2004).

In the case of both coral and foraminifera, the primary carbon input is from phytoplankton with the C_3 carbon fixation pathway. The tree-ring-based calibrations typically come from pine and oak trees, which also employ the C_3 carbon fixation pathway as well. The ubiquity of C_3 carbon fixation in the plants used to form calibration curves indicates that a $\Delta^{14}C_p/\Delta^{13}C_p$ ratio close to 1.90 may be representative of natural fractionation processes for most of these samples.

SIGNIFICANCE TO RADIOCARBON DATING

Time-related errors due to different values of b are relatively small due to variation in $b_{natural}$. To test the effects of these differing ratios, Equations 28–31 derived by Wigley and Muller (1981:183) were used to estimate error due to natural fractionation, laboratory fractionation of oxalic acid, atmospheric $\delta^{13}CO_2$ values, and postdepositional changes (Figure 3). For potential error due to atmospheric $\delta^{13}CO_2$ values, data from the European Project for Ice Coring in Antarctica (Elsig et al. 2009;

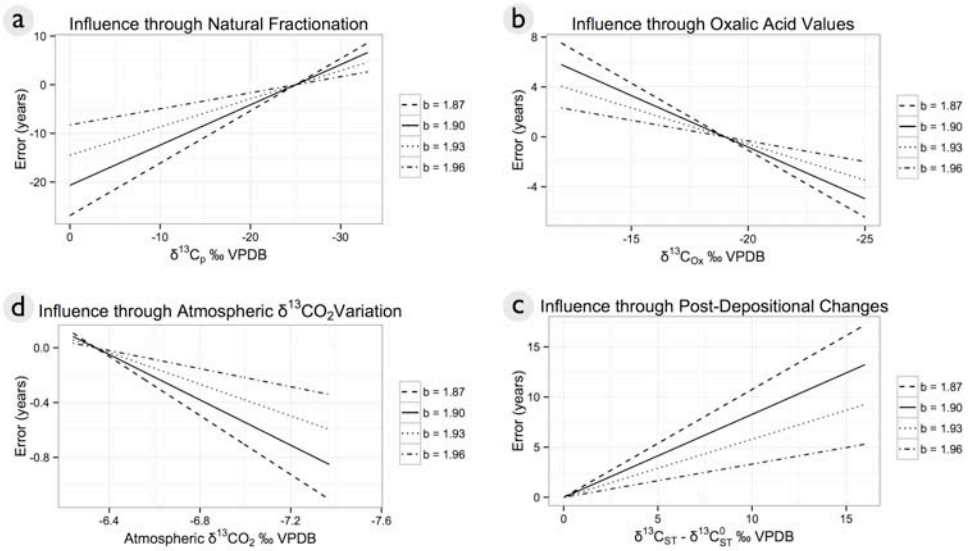

Figure 3 Error resulting from the assumption that $b = 2$ for (clockwise from top left) natural fractionation (a), laboratory fractionation of oxalic acid (b), postdepositional changes (c), and atmospheric $\delta^{13}CO_2$ values (d) based on Equations 28–31 in Wigley and Muller (1981:183), respectively. Potential errors are lower than estimated by Wigley and Muller for $b = 2.0+$, though this is due to the larger range of potential b values in their study.

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Lourantou et al. 2010), Taylor Dome (Indermühle et al. 1999; Smith et al. 1999), and Law Dome (Francey et al. 1999) were used in calculations. Error rates are modest, and their total range of variation is less than 50 yr, though most error introduced by $b < 2.0$ will be closer to 10–20 yr (Figure 4).

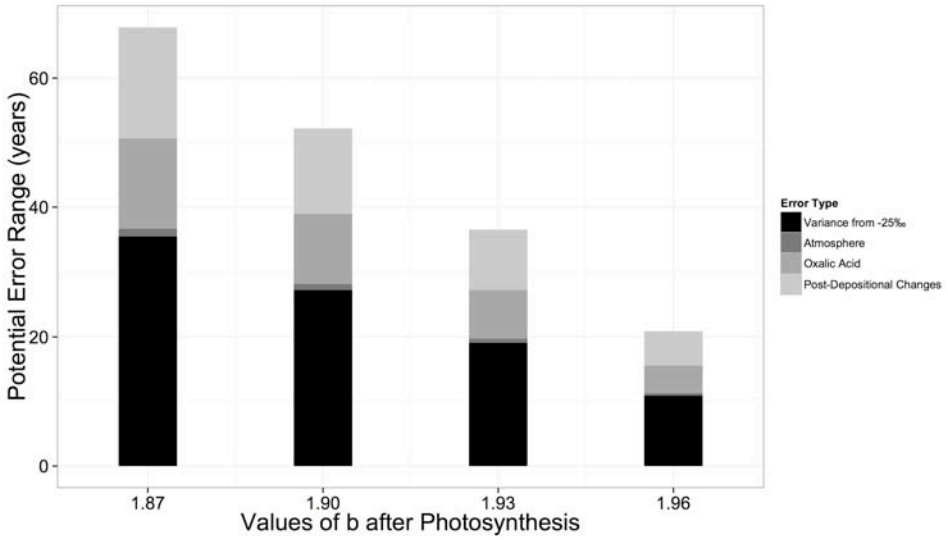

Figure 4 Range of potential errors for natural fractionation, laboratory fractionation of oxalic acid, atmospheric $\delta^{13}CO_2$ values, and postdepositional changes for each value of $b_{natural}$, assuming no further fractionation effects. Actual error is likely less than 20 yr for C_3 plants and less than 10 for C_4 plants.

Critically, these calculated errors, while modest, do not include any information about laboratory fractionation. The significance of this data, however limited, results not in the magnitude of the potential error; these time differences can be demonstrated to be small using the equations derived from Wigley and Muller (1981:183). Rather, they may be important due to the nature of the error. The upper limit for the fractionation ratio for photosynthesis is determined by the fractionation difference due to diffusion of CO_2 through the stomata (~1.97), though this itself is a physiologically impossible number as it assumes no influence due to carboxylation. Nonetheless, this implies that the current fractionation ratio used, $b = 2.0$, is physiologically impossible for organisms using either the C_3 or C_4 carbon fixation pathways based on current fractionation models. As the likely natural value based on these models of photosynthesis is approximately 1.90 for C_3 plants, it introduces the possibility for systematic error in the event that laboratory fractionation does not introduce further discrimination against ^{14}C. Thus, if $b = 2.0$ is physiologically implausible, it raises the issue of endogeneity. For sigmas of 50 or greater, any systematic error is likely to be subsumed under the general uncertainty of the date, leading to only a small and undetectable trend. However, with increased precision in ^{14}C dating (Southon 2011) such error may be worth further consideration.

Increased use of Bayesian models to refine chronologies, such as those employed by OxCal (Bronk Ramsey 2001), place additional emphases on revisiting the fractionation ratio. For example, a debate regarding the Iron Age settlement in the Levant focuses on whether major destruction levels in sites such as Megiddo and Hazor occurred in the 10th or 11th century BCE (Finkelstein and Piasetzky 2009). Multiple ^{14}C dates have resulted in standard deviations of 10–20 yr for key destruction layers. A small systematic bias of 5–10 yr has the potential to be important in resolving such tight chronological sequences.

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One additional impact on ^{14}C dating is worth consideration, though resolution is not likely in the near future. Photosynthetic tissue tends to be depleted in $\delta^{13}C_p$ relative to non-photosynthetic tissue with no known single cause (Cernusak et al. 2009). Hypotheses for this effect range from variation in the biochemical composition of different plant tissues, differential tissue growth over time, different diurnal use of photosynthate, dark respiration, among others (Cernusak et al. 2009). In many cases, variation in plant $\delta^{13}C_p$ appears to have no general trend. For example, two cores taken from the same *Podocarpus latifolus* tree had substantial differences in $\delta^{13}C_p$ within the same year (~2.0‰) and differences in their significance in relationship to instrumental rainfall and temperature records (Hall et al. 2008). The discrimination factors that affect ^{13}C also affect ^{14}C barring as-yet unknown differential fractionation effects during photosynthate transport within the same plant. The effect that such variable $\delta^{13}C_p$ has within the same organism presumably carries over to uncertainty in dating the same tree. However, the cause of this variation is unknown, and likely varies between taxa and potentially between individual organisms.

CONCLUSION

The natural fractionation ratio, $b_{natural}$ or $\Delta^{14}C_p/\Delta^{13}C_p$, results not from a constant in nature but rather from variable biological processes related to photosynthesis. For C_3 plants, the natural fractionation ratio is approximately 1.90. C_4 plants have the potential to have a more diverse range of natural fractionation ratios, though these are likely to be higher than the ratio for C_3 plants. The error introduced by a lower ($b < 2.0$) natural fractionation ratio is not high, and may not be detectable in many samples. Nonetheless, the present work does indicate the possibility of the final value of b (after laboratory fractionation) being systematically less than the current estimate of 2.0, introducing a potential endogeneity problem.

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