Quantifying uncertainty of past $p\text{CO}_2$ determined from changes in C_3 plant carbon isotope fractionation

Ying Cui^{1,*}, Brian A. Schubert¹

Affiliations

¹School of Geosciences, University of Louisiana at Lafayette, Lafayette, LA, 70504

*Corresponding author, Email: yxc3845@louisiana.edu

Abstract

Knowledge of the past concentrations of atmospheric CO_2 level ($p\text{CO}_2$) is critical to understanding climate sensitivity to changing $p\text{CO}_2$. Towards this, a new proxy for $p\text{CO}_2$ has been developed based on changes in carbon isotope fractionation ($\Delta^{13}\text{C}$) in C_3 land plants. The accuracy of this approach has been validated against ice-core $p\text{CO}_2$ records, suggesting the potential to apply this proxy to other geological periods; however, no thorough uncertainty assessment of the proxy has been conducted. Here, we first analyze the uncertainty in the model-curve fit through the experimental data using a bootstrap approach. Then, errors of the five input parameters for the proxy are evaluated using sensitivity analysis; these include the carbon isotope composition of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{CO}_2}$) and that of the plant material ($\delta^{13}\text{C}_{\text{org}}$) for two time periods, a reference time ($t = 0$) and the time period of interest (t), and the value of $p\text{CO}_2$ at time $t = 0$. We then propagated the errors on the reconstructed $p\text{CO}_2$ using a Monte Carlo random sampling approach that combined the uncertainties of the curve fitting and the five inputs

for a scenario in which the reference time was the Holocene with a target period for the reconstructed $p\text{CO}_2$ during the Cenozoic. We find that the error in the reconstructed $p\text{CO}_{2(t)}$ increases with increasing $p\text{CO}_{2(t)}$, yet remains $< 122\%$ (positive error) and $< 40\%$ (negative error) for $p\text{CO}_{2(t)} < 1000$ ppmv. The error assessment suggests that it can be used with confidence for much of the Cenozoic and perhaps the majority of the last 400 million years, which is characterized by $p\text{CO}_2$ levels generally less than 1000 ppmv. Towards this, an application of this uncertainty analysis is presented for the Paleogene (52-63 Ma) using published data. The resulting $p\text{CO}_{2(t)}$ levels calculated using this method average $470 \pm 288/-147$ ppmv (1σ , $n = 75$), and overlap with previous $p\text{CO}_{2(t)}$ estimates determined for this time period using stomata, liverwort, and paleosol proxies. The analysis presented here assumes that the paleoenvironment in which the plants grew is unknown and is determined to be the largest source of error in the reconstructed $p\text{CO}_{2(t)}$ levels; errors in $p\text{CO}_{2(t)}$ could be reduced provided independent determination of the paleoenvironmental conditions at the fossil site.

1. Introduction

Various methods have been employed for the reconstruction of ancient atmospheric carbon dioxide levels ($p\text{CO}_2$) using terrestrial substrates [paleosol carbonate (Cerling, 1991; Ekart et al., 1999; Breecker et al., 2010), plant stomatal index (Royer et al., 2001; Beerling et al., 2009), liverworts (Fletcher et al., 2005; Fletcher et al., 2006; Fletcher et al., 2008), sodium carbonate equilibrium (Lowenstein and Demicco, 2006), leaf gas exchange model (Konrad et al., 2008; Franks et al., 2014), weathering profiles (Kanzaki and Murakami, 2015), C_3 terrestrial plants (Schubert and Jahren, 2013, 2015)]

47 and marine substrates [phytoplankton (Pagani et al., 1999; Freeman and Pagani, 2005;
48 Hoins et al., 2015), boron isotope ratios (Pearson and Palmer, 2000; Pearson et al., 2009),
49 changes in seawater chemistry (Demicco et al., 2003), boron/calcium ratios (Tripathi et al.,
50 2009), inverse modeling of benthic oxygen isotope records (van de Wal et al., 2011)].
51 Although sizable scatter in reconstructed $p\text{CO}_2$ exists, especially for intervals of higher
52 $p\text{CO}_2$ (Beerling and Royer, 2011), efforts to improve the accuracy and precision of the
53 $p\text{CO}_2$ estimates (e.g., Breecker et al., 2010; Demicco et al., 2003; Royer, 2014) have led
54 to greater agreement among the diverse techniques. Each proxy has its advantages and
55 disadvantages with respect to the temporal resolution (long-term trends *versus* short-term
56 excursions), applicable time intervals (anthropogenic period, glacial-interglacial cycles,
57 Cenozoic, and Phanerozoic, etc.), and sensitivity across a range of $p\text{CO}_2$ levels (see
58 Royer et al., 2001). Beyond the suitability of each proxy for reconstructing $p\text{CO}_2$ across
59 the time period of interest, recognition for the need to better quantify the precision in
60 individual $p\text{CO}_2$ estimates has led to rigorous statistical analyses (i.e., Monte Carlo error
61 assessment) of the propagation of uncertainty for several of the $p\text{CO}_2$ proxies [e.g.,
62 stomatal index, Beerling et al., (2009); paleosol carbonate, Breecker, (2013); leaf gas
63 exchange model, Franks et al., (2014); phytoplankton, Freeman and Pagani, (2005);
64 liverworts, Fletcher et al., (2008); boron isotopes (Martínez-Botí et al., 2015)]. The C_3
65 plant carbon isotope fractionation based $p\text{CO}_2$ proxy is a novel approach, which has been
66 developed based on the dependence of $p\text{CO}_2$ on carbon isotope fractionation during
67 photosynthesis (Schubert and Jahren, 2012). This relationship has been applied to
68 reconstruct the $p\text{CO}_2$ across the Pleistocene-Holocene transition (Schubert and Jahren,
69 2015) and the Paleocene-Eocene Thermal Maximum (Schubert and Jahren, 2013).

However, no quantitative error assessment has been conducted to evaluate the uncertainty of the reconstructed $p\text{CO}_2$. Here we assess the error associated with this proxy. We first consider the uncertainty of the curve fitting. We then produce a fully propagated assessment of error using a Monte Carlo random sampling approach in order to quantify the uncertainty in this proxy across a wide range of $p\text{CO}_2$ (i.e., ~ 110 to ~ 2000 ppmv) and compare the errors in this new proxy to other published proxies. We also conduct sensitivity analyses on the uncertainty associated with each input parameter individually.

2. Methods

2.1 Curve fitting

Experiments growing plants in controlled chambers across multiple levels of $p\text{CO}_2$ up to 4200 ppmv revealed a hyperbolic relationship between net carbon isotope fractionation $[\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{org}})/(1 + \delta^{13}\text{C}_{\text{org}}/1000)]$ and $p\text{CO}_2$ (Schubert and Jähren, 2012), where $\delta^{13}\text{C}_{\text{CO}_2}$ and $\delta^{13}\text{C}_{\text{org}}$ represent the carbon isotope value of atmospheric CO_2 and plant tissue, respectively. These data, combined with literature data, showed that the change in $\Delta^{13}\text{C}$ value per ppmv of $p\text{CO}_2$ (i.e., S , ‰/ppmv) is consistent across a wide range of species growing in diverse conditions according to the following equation (Schubert and Jähren, 2012) (Fig. 1):

$$S = (A^2)(B) / [A + (B)(p\text{CO}_2 + C)]^2 \quad (1)$$

where A, B and C are curve fitting parameters, discussed below. The integration of Eq. 1 yields a generalized hyperbolic relationship between $\Delta^{13}\text{C}$ and $p\text{CO}_2$ (Eq. 2):

$$\Delta^{13}\text{C} = [(A)(B)(p\text{CO}_2 + C)] / [A + (B)(p\text{CO}_2 + C)] \quad (2)$$

The classic equation modeling carbon isotope fractionation between plants and the atmosphere (Farquhar et al., 1989) states that:

$$\Delta^{13}\text{C} = a + (b - a)(c_i/c_a) \quad (3)$$

where a and b are constants representing the isotopic fractionation due to diffusion through the plant's stomata and subsequent catalysis by Ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO), respectively; and c_i/c_a is the ratio of intercellular and ambient partial pressures of CO_2 . The value for a is set to 4.4‰ (Craig, 1953; Cerling et al., 1991) and values for b range in the literature from 26 to 30‰ (Christeller et al., 1976; Wong et al., 1979; Farquhar et al., 1982; Roeske and O'Leary, 1984; Guy et al., 1993; Lloyd and Farquhar, 1994; Suits et al., 2005) and Schubert and Jahren (2012, 2013, and 2015) used a value of 28.26‰ based on data from their elevated $p\text{CO}_2$ experiments.

Because c_i/c_a must be between 0 and 1, Eq. 3 constrains the maximum and minimum values for $\Delta^{13}\text{C}$ where: 1) $\Delta^{13}\text{C} = a$ at $c_i/c_a = 0$ (i.e., $\Delta^{13}\text{C} = 4.4\text{‰}$ at $p\text{CO}_2 = 0$ ppmv) and 2) $\Delta^{13}\text{C} = b$ when $c_i/c_a = 1$ (i.e., $\Delta^{13}\text{C}$ cannot exceed b). We note that this maximum fractionation value, b , in Eq. 3 is equivalent to the asymptote, A , in Eq. 2. Values for B and C were determined iteratively by minimizing the root mean square error (RMSE) between the observed data and the fitted curve (Eq. 1) across the full range of reported values for b (i.e., $A = 26$ to 30‰ , subject to these two constraints, using the “Solver” add-in within Excel (Table 1).

In order to assess the uncertainty in the curve fitting (Fig. 1) and calculate a statistically robust confidence interval, we performed a bootstrap on the residual between the curve fitting and the data following the methods described in Efron and Tibshirani (1994). We calculated the residual, i.e. the differences between the 40 observed data points and the best-fit curve, using three A values ($A=26$, 28.26, and 30), which span the

full range of reported values. We then performed a random sampling of the residuals (n=40) with replacement for each A value, assuming equal probability of each residual. The newly sampled residual was then added back to the original fitted curve to create a pseudo dataset. A new curve fitting with the best-fit B and C values was determined using the pseudo dataset by minimizing the RMSE while maintaining the two constraints described above. The minimization of RMSE for the non-linear curve with constraints was realized in R (<http://www.r-project.org>) using function “solnp” in the “Rsolnp” package (Ye, 1987). This process was repeated 10,000 times to generate 10,000 values of B and the corresponding C values. The confidence interval of B was constructed by specifying the 16th and 84th percentile of all the bootstrapped B values. The R code, which includes instructions on how to use it for the bootstrap analysis, is provided in the supplementary material.

2.2 Quantifying paleo- $p\text{CO}_2$

Because the $\Delta^{13}\text{C}$ value of modern C_3 plant tissue shows high variability (e.g., Diefendorf et al., 2010; Matson et al., 2012), even among plants growing under the same environmental conditions (e.g. Flanagan et al., 1997; Leavitt and Newberry, 1992; Bonal et al., 2000), and within highly managed arboretums and botanical gardens (Jahren, 2004), Eq. 2 cannot be used directly to calculate $p\text{CO}_2$ (Schubert and Jahren, 2012). Instead, the terrestrial C_3 plant organic-carbon isotope $p\text{CO}_2$ proxy works by examining relative changes in the $\delta^{13}\text{C}$ value between the time of interest ($\delta^{13}\text{C}_{(t)}$) and the $\delta^{13}\text{C}$ value at a chosen initial time ($\delta^{13}\text{C}_{(t=0)}$), designated within Schubert and Jahren (2015) as “ $\Delta(\delta^{13}\text{C})$ ” where:

$$\Delta(\Delta^{13}\text{C}) = \Delta^{13}\text{C}_{(t)} - \Delta^{13}\text{C}_{(t=0)} \quad (4)$$

which can be expanded as:

$$\Delta(\Delta^{13}\text{C}) = (\delta^{13}\text{C}_{\text{CO}_2(t)} - \delta^{13}\text{C}_{\text{org}(t)}) / (1 + \delta^{13}\text{C}_{\text{org}(t)} / 1000) - (\delta^{13}\text{C}_{\text{CO}_2(t=0)} - \delta^{13}\text{C}_{\text{org}(t=0)}) / (1 + \delta^{13}\text{C}_{\text{org}(t=0)} / 1000) \quad (5)$$

We can also rewrite Eq. 2 in terms of a relative change in $\Delta^{13}\text{C}$ value [i.e., $\Delta(\Delta^{13}\text{C})$]

resulting from a change in $p\text{CO}_2$:

$$\Delta(\Delta^{13}\text{C}) = [(A)(B)(p\text{CO}_{2(t)} + C)] / [(A + (B)(p\text{CO}_{2(t)} + C))] - [(A)(B)(p\text{CO}_{2(t=0)} + C)] / [(A + (B)(p\text{CO}_{2(t=0)} + C))] \quad (6)$$

Then, by rearrange Eq. 6, one can solve for $p\text{CO}_2$ at any time t ($p\text{CO}_{2(t)}$):

$$p\text{CO}_{2(t)} = \frac{\Delta(\Delta^{13}\text{C}) \times A^2 + \Delta(\Delta^{13}\text{C}) \times A \times B \times p\text{CO}_{2(t=0)} + 2 \times \Delta(\Delta^{13}\text{C}) \times A \times B \times C + \Delta(\Delta^{13}\text{C}) \times B^2 \times C \times p\text{CO}_{2(t=0)} + \Delta(\Delta^{13}\text{C}) \times B^2 \times C^2 + A^2 \times B \times p\text{CO}_{2(t=0)}}{A^2 \times B - \Delta(\Delta^{13}\text{C}) \times A \times B - \Delta(\Delta^{13}\text{C}) \times B^2 \times p\text{CO}_{2(t=0)} - \Delta(\Delta^{13}\text{C}) \times B^2 \times C} \quad (7)$$

Within Eq. 7, values for A, B and C are the curve fitting parameters, $p\text{CO}_{2(t=0)}$ is the initial $p\text{CO}_2$ level (known), and $\Delta(\Delta^{13}\text{C})$ is calculated using Eq. 5.

2.3 Monte Carlo Error Assessment

We used R to perform Monte Carlo error propagation through Eq. 7 for $p\text{CO}_{2(t)} = \sim 110 - \sim 2000$ ppmv. Error related to the curve fitting equation (A, B, and C within Eqs. 1 and 2) and all the input values ($\delta^{13}\text{C}_{\text{CO}_2(t)}$, $\delta^{13}\text{C}_{\text{org}(t)}$, $\delta^{13}\text{C}_{\text{CO}_2(t=0)}$, $\delta^{13}\text{C}_{\text{org}(t=0)}$, $p\text{CO}_{2(t=0)}$) required for Eq. 7 were considered in the Monte Carlo error assessment. The precision for each input is reported as $\pm 1\sigma$. We note that depending on the time period of interest, values for $\delta^{13}\text{C}_{\text{CO}_2(t)}$ and $\delta^{13}\text{C}_{\text{CO}_2(t=0)}$ can be determined from air bubbles trapped in ice (e.g., [Schmitt et al., 2012](#)) or reconstructions based on marine carbonate $\delta^{13}\text{C}$ values (e.g. [Passey et al., 2002](#); [Strauss and Peters-Kottig, 2003](#); [Tippie et al., 2010](#)).

The errors of the input variables were assumed to be Gaussian distributed. Values for each input were randomly drawn from normal distributions defined by the pre-determined means and standard deviations. 10,000 $p\text{CO}_{2(t)}$ values were calculated using 10,000 randomly generated sets of input values. The $p\text{CO}_{2(t)}$ value we report is the median of the 10,000 iterations. The 16th and 84th percentiles of the 10,000 iterations of $p\text{CO}_{2(t)}$ values were determined to construct the 68% confidence interval, similar to that in [Breecker \(2013\)](#), [Frank et al., \(2014\)](#), and [Royer et al., \(2014\)](#). The positive error of the reconstructed $p\text{CO}_{2(t)}$ represents the difference between the 84th percentile value and the median, and the negative error represents the difference between the 16th percentile value and the median.

3. Results

3.1 Uncertainties in curve fitting parameters A, B and C

Differences among the hyperbolic and S curves for $A = 26, 28.26$, and 30 are shown in Fig. 1. Across the entire range in possible A values, the absolute $\Delta^{13}\text{C}$ value for a given $p\text{CO}_2$ level differs for each of the hyperbolic curves (Fig. 1 inset), but the relative changes in $\Delta^{13}\text{C}$ for a given change in $p\text{CO}_2$ (i.e., the slopes of each hyperbolic curve; S , ‰/ppmv) are nearly identical (Fig. 1). Thus, regardless of the value for A chosen, the calculated $p\text{CO}_{2(t)}$ will be similar at low to moderate $p\text{CO}_{2(t)}$ (Supplementary Fig. 1), consistent with the result shown for subambient $p\text{CO}_{2(t=0)}$ conditions ([Schubert and Jahren, 2015](#)). The $\Delta^{13}\text{C}$ value approaches an asymptote of 26 ($A = 26$), 28.26 ($A = 28.26$), and 30‰ ($A = 30$) for the three choices of A , and all the curves cross the y-axis at 4.4‰ (i.e., $\Delta^{13}\text{C} = 4.4\text{‰}$ when $p\text{CO}_2 = 0$ ppmv) (Fig. 1 inset). The 68% confidence

intervals for the three S curves determined from the bootstrap analysis overlap, further suggesting that the choice of A plays a minor role in the reconstructed $p\text{CO}_{2(t)}$ value, especially at low $p\text{CO}_{2(t)}$. The lowest RMSE was determined to occur with $A = 30$, but the RMSE value was only marginally lower than with $A = 28.26$ (RMSE = 0.002591 *versus* 0.002625) (Table 1). Because the choice of A value within this range ($A = 26$ -30) is somewhat subjective, we here maintain the $A = 28.26$ value used by Schubert and Jahren (2012, 2013, and 2015). This choice of A value could potentially improve the accuracy in the reconstructed $p\text{CO}_{2(t)}$, especially at very high $p\text{CO}_{2(t)}$, as it falls near the midpoint of reported values (Supplementary Fig. 1). The uncertainty on the reconstructed $p\text{CO}_{2(t)}$, however, will be greater with $A = 28.26$ than with $A = 30$ as a result of the higher RMSE (and greater error in B) (Table 1). The 68% confidence intervals on the hyperbolic functions for the three choices of A values (Fig. 1 inset) show the asymmetric feature of the bootstrap-determined B value, with B value slightly skewed to the left for $A = 30$ ($B = 0.28 + 0.022/-0.024$) and $A = 26$ ($B = 0.17 + 0.018/-0.024$), and skewed to the right for $A = 28.26$ ($B = 0.22 + 0.028/-0.014$). Although these curve-fitting errors were not normally distributed, we assumed a Gaussian distribution when conducting the Monte Carlo error analysis. Therefore, we conservatively applied the highest error on B for each A value (i.e., for $A = 28.26$, we used $B = 0.22 \pm 0.028$).

Fig. 2 shows the Monte Carlo determined errors in $p\text{CO}_{2(t)}$ caused by the errors in the curve fitting only, while keeping the errors for all other input terms fixed at zero. The errors increase with increasing $p\text{CO}_{2(t)}$, with the positive error increasing at a greater rate than the negative error, especially at $p\text{CO}_{2(t)} > \sim 1500$ ppmv. Despite the model relationship being well constrained (Fig. 1), the saturating nature of our proxy augments

the small errors in the curve fitting, resulting in increasing error, especially for the positive error, with increasing $p\text{CO}_{2(t)}$. However, we note that the positive error across the full range of $p\text{CO}_{2(t)}$ (i.e., up to ~2000 ppmv) remains < 50%, with the negative error remaining < 24% (Fig. 2 bottom). For $p\text{CO}_{2(t)}$ levels common to much of the Cenozoic (i.e., < 1000 ppmv), the errors are less than 15% (Fig. 2 bottom).

3.2 Full error propagation

We conducted a full Monte Carlo error propagation using the uncertainties listed in Table 2. The errors in the input parameters represent the precision associated with a scenario in which Holocene data are used for the reference period ($t = 0$) and Cenozoic data are used for time t . Fig. 3 shows the result of this full error propagation using the curve fitting uncertainty (section 3.1) and the uncertainties associated with the other inputs to Eq. 7 (Table 2; see discussion below) across a wide range of reconstructed $p\text{CO}_{2(t)}$ (~ 110 to ~ 2000 ppmv). As expected, the error in $p\text{CO}_{2(t)}$ increases with increasing $p\text{CO}_{2(t)}$ and the positive error is higher than negative error (Fig. 3). The positive error reaches ~200% (~3730 ppmv) at $p\text{CO}_{2(t)} = \sim 2000$ ppmv, but the negative error remains < ~50% (~930 ppmv) across the entire range of $p\text{CO}_{2(t)}$ tested (Fig. 3). The calculated uncertainty (+122/-40%) when the reconstructed $p\text{CO}_{2(t)}$ is below 1000 ppmv suggests that the C_3 plant proxy can be widely used across most of the Cenozoic, which is characterized by $p\text{CO}_2 \leq \sim 1000$ ppmv (e.g., [Breecker et al., 2010](#); [Beerling and Royer, 2011](#); [Pearson et al., 2009](#); [Zhang et al., 2013](#); [Franks et al., 2014](#)). During brief carbon isotope excursion events when $p\text{CO}_2$ may exceed 2000 ppmv (e.g. [Cui et al., 2011](#); [Kiehl](#)

and Shields, 2013; Schubert and Jahren, 2013; Meissner et al., 2014), the relatively small negative error may be used to produce a minimum bound on the $p\text{CO}_2$ level reached.

Figure 3 (bottom) shows that the 10,000 Monte Carlo resampling process generated a limited number of invalid $p\text{CO}_{2(t)}$ values (i.e., $p\text{CO}_{2(t)} < 0$ or $> 10^6$ ppmv). The percentage of invalid $p\text{CO}_{2(t)}$ values reached a maximum of 28% at $p\text{CO}_{2(t)}$ close to 2000 ppmv, but accounted for $< \sim 5\%$ of all values for $p\text{CO}_{2(t)} < \sim 1000$ ppmv, and $< 1\%$ of values were invalid for $p\text{CO}_{2(t)} < 400$ ppmv. These invalid numbers are a result of the hyperbolic relationship used, such that if $\Delta(\Delta^{13}\text{C})$ exceeded 8.6‰ (assuming $p\text{CO}_{2(t=0)} = 270$ ppmv), calculated $p\text{CO}_{2(t)}$ will jump from infinitely high values (i.e., $>> 10^6$ ppmv) to the symmetrical branch of the hyperbola, and thus yield infinitely low $p\text{CO}_{2(t)}$ (i.e., $<< 0$ ppmv) (Supplementary Fig. 2). Because these negative $p\text{CO}_{2(t)}$ values resulted from exceedingly high $\Delta(\Delta^{13}\text{C})$, and therefore do not indicate very low $p\text{CO}_{2(t)}$, these invalid $p\text{CO}_{2(t)}$ values (which can approach negative infinity) were removed. This is in contrast to an error analysis for the paleosol carbonate CO_2 paleobarometer, which replaces negative values with zeros (Breecker, 2013); for these paleosols, negative $p\text{CO}_2$ resulted from estimates that were slightly too low, rather than being exceedingly high.

Because of the nonlinear nature of the curve fitting function (Fig. 1), we expected the errors we quantified here to cause the median $p\text{CO}_{2(t)}$ determined *via* Monte Carlo resampling to be higher than if $p\text{CO}_{2(t)}$ were calculated simply using Eq. 7 (i.e., with no errors). However, for $p\text{CO}_{2(t)} > \sim 1000$ ppmv, removal of the increasing number of invalid values (i.e., $p\text{CO}_{2(t)} < 0$ or $> 10^6$) generated during the Monte Carlo resampling caused a decrease in the median $p\text{CO}_{2(t)}$ value. Although $p\text{CO}_{2(t)} < 0$ or $> 10^6$ can be calculated as a result of the hyperbolic function used (Supplementary Fig. 2), we conclude that the effect

of these invalid values on reconstructed $p\text{CO}_{2(t)}$ is small at $p\text{CO}_{2(t)} < \sim 1000$ ppmv, and well within the errors quantified here.

3.3 Application to the Paleogene

We reconstruct atmospheric $p\text{CO}_2$ from 63 to 53 Ma using uncertainties listed in Table 2 and the $\delta^{13}\text{C}_{\text{org}}$ dataset presented in Diefendorf et al., (2015) (Fig. 4). The reconstructed Paleogene CO_2 levels based on 75 data points average $470 +288/-147$ ppmv, and are consistent with other proxy estimates for this time period (Table 3). The calculated $p\text{CO}_{2(t)}$ estimates assume $\delta^{13}\text{C}_{\text{org}(t=0)} = -25.1\text{‰}$ at 270 ppmv (Holocene average $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{org}}$) (Table 2). Uncertainty in $\delta^{13}\text{C}_{\text{org}(t=0)}$ is estimated as $\pm 1.6\text{‰}$, which represents the standard deviation of 479 $\delta^{13}\text{C}$ measurements made on a wide diversity of modern leaves collected across $\sim 55^\circ\text{S}$ to 70°N latitude, and includes sites with MAP = ~ 1 to 3700 mm/yr (Kohn, 2010). We acknowledge, however, that inclusion of independent data on MAP could be used to better constrain the value for $\delta^{13}\text{C}_{\text{org}(t=0)}$ within this range (i.e., 1σ range = -26.7 to -23.5‰), with wetter and drier sites approximating values near the lower and higher ends of this range, respectively. However, by using the global uncertainty value of $\pm 1.6\text{‰}$ observed in leaves today, our analysis makes no attempt to include climate information in order to constrain the value for $\delta^{13}\text{C}_{\text{org}(t=0)}$ further. As a result, the errors in $p\text{CO}_2$ calculated here reflect conservative estimates, and provide opportunity for decreasing the errors in $p\text{CO}_{2(t)}$ provided that the paleoenvironment in which the plants grew can be constrained.

4. Sensitivity analysis of input errors

Because this proxy analyzes the relative change in $\Delta^{13}\text{C}$ in response to a change in $p\text{CO}_2$, quantifying $p\text{CO}_{2(t)}$ requires knowledge of $\delta^{13}\text{C}_{\text{CO}_2}$, $\delta^{13}\text{C}_{\text{org}}$, and $p\text{CO}_2$ for an initial time period ($t = 0$), as well as data on $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{CO}_2}$ at the time period of interest (t). Here we describe how uncertainty in these five input parameters, each in isolation, affects the error in $p\text{CO}_{2(t)}$ across $p\text{CO}_{2(t)} = \sim 110$ to ~ 2000 ppmv.

4.1 Sensitivity analysis of $p\text{CO}_{2(t=0)}$

The errors in $p\text{CO}_{2(t=0)}$ depend on the reference time period used. Modern measurements of $p\text{CO}_2$ have extremely low error from direct measurement of air (Keeling et al., 2009), but these are limited to only the most recent ~ 50 years; ice core records extend back 800,000 years while maintaining low error (e.g., Holocene error = ± 7 ppmv, Kawamura et al., 2007); and various proxies can extend the record back millions of years, but have significantly higher error (e.g., Neogene error is as low as ~ 35 ppmv for the alkenone and boron proxies; Royer, 2014). Because the error in $p\text{CO}_2$ is negligible when measured directly from today's atmosphere, we focus on comparison of the error in $p\text{CO}_{2(t)}$ that results when using $p\text{CO}_{2(t=0)}$ data from ice cores (± 7 ppmv) *versus* Neogene proxy data (± 35 ppmv), as our low and high uncertainty scenarios (Fig. 5). For both scenarios we set $p\text{CO}_{2(t=0)} = 270$ ppmv (pre-industrial value), but note that this is also similar to the median Neogene value determined from other proxies (i.e., ~ 314 ppmv; Royer, 2014). Because the error in $p\text{CO}_{2(t)}$ is a function of $p\text{CO}_{2(t=0)}$, our use of a low $p\text{CO}_2$ period for $t = 0$ results in greater error in $p\text{CO}_{2(t)}$ than if a reference period with higher $p\text{CO}_2$ were chosen, especially for when investigating periods with high $p\text{CO}_{2(t)}$.

As expected, the high $p\text{CO}_{2(t=0)}$ error scenario (i.e., ± 35 ppmv) yielded more error on $p\text{CO}_{2(t)}$ than the low $p\text{CO}_{2(t=0)}$ error scenario (i.e., ± 7 ppmv) across all $p\text{CO}_{2(t)}$ values (Fig. 5). Errors in $p\text{CO}_{2(t)}$ increased with increasing $p\text{CO}_{2(t)}$; however, the error in $p\text{CO}_{2(t)}$ increased at a faster rate for the ± 35 ppmv scenario than the ± 7 ppmv scenario, especially for the positive error (Fig. 5). For $p\text{CO}_{2(t)} > 270$ ppmv, the positive error is greater than the negative error, consistent with expectations based on the curved shape of proxy relationship, but this offset is minimal (i.e., $< 3\%$) for the ± 7 ppmv error scenario. The error in $p\text{CO}_{2(t)}$ associated with the curve fitting (Fig. 2) is in general higher than the error in $p\text{CO}_{2(t)}$ determined for the ± 7 ppmv scenario, but lower than that determined using the ± 35 ppmv scenario (Fig. 5). This result demonstrates the importance of minimizing the error in $p\text{CO}_{2(t=0)}$, especially if $p\text{CO}_{2(t)}$ is expected to be high.

4.2 Sensitivity analysis of $\delta^{13}\text{C}_{\text{CO}_2}$

Here, we assessed how the uncertainty in the $\delta^{13}\text{C}_{\text{CO}_2}$ value used to calculate $\Delta(\delta^{13}\text{C})$ (Eq. 5) affected the error in $p\text{CO}_{2(t)}$ for two scenarios: $\pm 0.1\text{‰}$ and $\pm 0.5\text{‰}$. An uncertainty of $\pm 0.1\text{‰}$ corresponds with the uncertainty in $\delta^{13}\text{C}_{\text{CO}_2}$ value from ice core records for the Holocene (Schmitt et al., 2012); an uncertainty of $\pm 0.5\text{‰}$ corresponds to the uncertainty reported for the Cenozoic from a reconstruction based on benthic foraminifera (Tippie et al., 2010). Error in $\delta^{13}\text{C}_{\text{CO}_2}$ of $\pm 0.2\text{‰}$ assumed by Breecker and Retallack (2014) for the paleosol carbonate $p\text{CO}_2$ proxy falls within this range. We show the error analysis for $\delta^{13}\text{C}_{\text{CO}_2(t)}$ specifically, but note that the error in $p\text{CO}_{2(t)}$ is nearly identical ($< 0.4\%$ difference across all $p\text{CO}_{2(t)}$) whether these errors are applied to $\delta^{13}\text{C}_{\text{CO}_2(t=0)}$ or $\delta^{13}\text{C}_{\text{CO}_2(t)}$. For both scenarios, error increases with increasing $p\text{CO}_{2(t)}$, both

in terms of absolute values (i.e., ppmv) and percent error (Fig. 6), similar to what we observed with the error in curve fitting (Fig. 2) and error in $p\text{CO}_{2(t=0)}$ (Fig. 5). The positive and negative errors in $p\text{CO}_{2(t)}$ are similar to each other when using an uncertainty of $\pm 0.1\text{‰}$, but for uncertainty of $\pm 0.5\text{‰}$ the positive error is significantly higher than the negative error, especially at high $p\text{CO}_{2(t)}$ (Fig. 6). The $\pm 0.1\text{‰}$ scenario yields smaller $p\text{CO}_{2(t)}$ error than the $p\text{CO}_{2(t=0)}$ uncertainty of ± 7 ppmv scenario, while the $\pm 0.5\text{‰}$ scenario yields slightly smaller error than the ± 35 ppmv scenario.

4.3 Sensitivity analysis of $\delta^{13}\text{C}_{\text{org}}$

A vast amount of $\delta^{13}\text{C}_{\text{org}}$ measurements have been produced from terrestrial plant tissue including bulk organic matter (e.g., [Matson et al., 2012](#)), wood (e.g., [Treydte et al., 2007](#); [Young et al., 2012](#)), leaf (e.g. [Graham et al., 2014](#)), and leaf waxes (e.g., *n*-alkanes) (e.g. [Collister et al., 1994](#); [Rieley et al., 1991](#)). Measurement error for $\delta^{13}\text{C}_{\text{org}}$ is almost always better than 0.2‰ and commonly better than 0.1‰ (e.g., [Werner et al., 1999](#); [King et al., 2012](#)) and an error of $\pm 0.12\text{‰}$ for soil $\delta^{13}\text{C}_{\text{org}}$ was used in a recent analysis of the uncertainty in the paleosol carbonate $p\text{CO}_2$ proxy ([Breecker, 2013](#)). We note that significant changes in the measured $\delta^{13}\text{C}_{\text{org}}$ value can also result from changes in precipitation (e.g., [Stewart et al., 1995](#); [Kohn, 2010](#)), plant communities (e.g., [O’Leary, 1988](#); [Flanagan et al., 1997](#)), $p\text{O}_2$ (e.g., [Bernier et al., 2000](#); [Beerling et al., 2002](#); [Tappert et al., 2013](#)), and source inputs (e.g., [Walsh et al., 2008](#); [Rebolledo et al., 2015](#)), and thus can affect the accuracy of the reconstructed $p\text{CO}_2$ value. Similar effects are commonly resolved in other $p\text{CO}_2$ proxies by producing multiple reconstructed $p\text{CO}_2$ values for a given time period, with each reconstructed $p\text{CO}_2$ value having its own uncertainty (e.g.,

Royer et al., 2014; Whiteside et al., 2015; Steinthorsdottir et al., 2011; Cleveland et al., 2008). By producing a large number of values, consensus in the $p\text{CO}_2$ value may be achieved (Beerling and Royer, 2011), but the accuracy of such a proxy value is impossible to verify for deep time applications. Instead, workers often assess the accuracy of the proxies by comparison with available ice core or modern $p\text{CO}_2$ data (e.g., Tripathi et al., 2009; Franks et al., 2014; Schubert and Jahren, 2015).

Leaves from modern C_3 plants growing in equilibrium with a well-mixed atmosphere show a large range of $\delta^{13}\text{C}_{\text{org}}$ values (-31.2‰ to -22.8‰, $n = 479$, Kohn, 2010). The standard deviation of this global dataset is $\pm 1.6\text{‰}$ and represents a good estimate of the $\pm 1\sigma$ uncertainty of $\delta^{13}\text{C}_{\text{org}(t=0)}$. A similar approach was used in a recent error quantification study on hydrogen isotopes by Polissar and D'Andrea (2014). Use of this uncertainty value assumes that the Holocene experienced similar variability in $\delta^{13}\text{C}_{\text{org}}$ as modern plants and no information on plant type or climate is known for the site. Here we present a sensitivity analysis in $\delta^{13}\text{C}_{\text{org}(t=0)}$ by comparing two end-member scenarios for the uncertainty in $p\text{CO}_{2(t)}$: natural, environmental variability across diverse environments and plant types (i.e., $\pm 1.6\text{‰}$) and uncertainty only associated with measurement error (i.e., $\pm 0.1\text{‰}$). For both scenarios, error increases with increasing $p\text{CO}_{2(t)}$, both in terms of absolute values (i.e., ppmv) and percent error, but the higher uncertainty scenario (i.e., $\pm 1.6\text{‰}$) results in a much faster increase in uncertainty of $p\text{CO}_{2(t)}$ (Fig. 7). The positive and negative errors in $p\text{CO}_{2(t)}$ are similar to each other when using an uncertainty of $\pm 0.1\text{‰}$, but for uncertainty of $\pm 1.6\text{‰}$ the positive error is >3 times higher than the negative error, especially at high $p\text{CO}_{2(t)}$ (Fig. 7). This illustrates that without independent knowledge of environmental conditions at the site, uncertainty

in $\delta^{13}\text{C}_{\text{org}(t=0)}$ produces the largest error in $p\text{CO}_{2(t)}$ of any of the input parameters. This suggests that improvement in the estimate of $\delta^{13}\text{C}_{\text{org}(t=0)}$ is a useful target for reducing the uncertainty in this proxy.

5. Comparison with other proxies

We compare the error of our C_3 land plant $p\text{CO}_2$ proxy to the errors of other proxies (e.g. fossil leaf stomatal indices, boron isotopes, paleosol carbonates, liverworts, alkenones, and stomatal ratio) across a wide range of $p\text{CO}_2$ (Fig. 8). The positive error of the C_3 plant proxy increases at a somewhat faster rate with increasing $p\text{CO}_2$ than most other proxies at very high $p\text{CO}_2$, and is comparable to the liverworts-based proxy at $p\text{CO}_2 = \sim 1000$ ppmv). The negative error of the C_3 plant proxy increases more slowly with increasing $p\text{CO}_2$ and follows general trends observed in the other $p\text{CO}_2$ proxies. If one can independently constrain the plant functional type or the climate, the error on $p\text{CO}_{2(t)}$ can be reduced significantly (Fig. 8).

The right-skewed error of the C_3 plant proxy is similar to that observed for liverworts (Fletcher et al., 2008), stomata (Beerling et al., 2009) and paleosols (Royer 2014). Unlike the paleosol carbonate $p\text{CO}_2$ proxy, for which the right skewed error can be minimized by choosing evenly balanced soil CO_2 mixtures (Breecker, 2013), the right skew to the error in $p\text{CO}_2$ is inherent to the C_3 plant $p\text{CO}_2$ proxy because of the nonlinear curve fitting function.

Typically, high-resolution $p\text{CO}_2$ reconstructions are limited to marine substrates. However, recent application of the C_3 land plant proxy towards reconstructing $p\text{CO}_2$ across the last 30 kyr (Schubert and Jahren, 2015) demonstrated the potential for

generating nearly continuous records of $p\text{CO}_2$ from terrestrial environments. The relatively low error determined here for this proxy (e.g., Fig. 4), especially at low to moderate $p\text{CO}_2$, combined with the large abundance of terrestrial organic matter throughout the fossil record, suggests great potential for generating similar high-resolution $p\text{CO}_2$ records across millions of years of Earth history. Comparison of high-resolution terrestrial and marine $p\text{CO}_2$ records would help to validate these reconstructions and provide necessary data for comparing to available high-resolution temperature records (e.g., high-resolution oxygen isotope records, [Zachos et al., 2001](#)). The application of statistically robust uncertainty analyses to these reconstructions can improve our understanding of uncertainty when evaluating Earth system climate sensitivity (e.g. [Royer et al., 2007](#)).

6. Implications and Conclusion

This study provides a rigorous statistical analysis of the error in the C_3 land plant $p\text{CO}_2$ proxy using a Monte Carlo resampling method. The errors in $p\text{CO}_{2(t)}$ increase with increasing $p\text{CO}_{2(t)}$ and the positive errors are generally at least as high as the negative errors for all scenarios (Fig. 3), similar to several other $p\text{CO}_2$ proxies (Fig. 8). The percent error we estimate for $p\text{CO}_{2(t)}$ during the Paleogene (+61%/-31%) are within the range of errors determined from the other $p\text{CO}_2$ proxies (+20% to +82% and -14% to -44%) (Table 3). For time periods with large changes in $p\text{O}_2$ (e.g., the late Paleozoic, [Berner, 2006](#)), we acknowledge the need to better quantify the effect of $p\text{O}_2$ on $\Delta^{13}\text{C}$ in plants grown from seed to maturity across multiple levels of $p\text{O}_2$ spanning this range. In addition, we point to better determination of the paleoenvironment of a site as an

opportunity to reduce the errors in $p\text{CO}_{2(t)}$ presented here. Last, although calculated $p\text{CO}_{2(t)}$ values show little variability within each time interval examined here (Fig. 4), we acknowledge that use of large $\delta^{13}\text{C}_{\text{org}(t)}$ datasets is desirable. For example, a recent analysis determined that by averaging 50 randomly selected paleosols within each time slice, one could significantly reduce the uncertainty in paleosols-based $p\text{CO}_2$ estimates (Breecker, 2013). The increasing availability of terrestrial $\delta^{13}\text{C}$ measurements from fossil organic matter, leaves, and specific compounds (e.g., leaf waxes, cellulose) and the relatively low errors for this proxy across a wide range of $p\text{CO}_2$ suggests great potential for applying this proxy throughout much of the fossil record of C_3 land plants, when $p\text{CO}_2$ may have remained < 1000 ppmv (Franks et al., 2014).

Acknowledgements

This research was supported by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy under award DE-FG02-13ER16412 to B.A.S.

REFERENCES

- Beerling, D. J., Fox, A. and Anderson, C. W. (2009) Quantitative uncertainty analyses of ancient atmospheric CO₂ estimates from fossil leaves, *American Journal of Science*, **309**(9), 775-787.
- Beerling, D. J., Lake, J. A., Berner, R. A., Hickey, L. J., Taylor, D. W., and Royer, D. L., (2002) Carbon isotope evidence implying high O₂/CO₂ ratios in the Permo-Carboniferous atmosphere: *Geochimica et Cosmochimica Acta*, **66**, 3757–3767.
- Beerling, D. J. and D. L. Royer (2011) Convergent Cenozoic CO₂ history, *Nature Geoscience*, **4**(7), 418-420.
- Berner, R. A., Petsch, S., Lake, J., Beerling, D., Popp, B., Lane, R., Laws, E., Westley, M., Cassar, N. And Woodward, F. (2000), Isotope fractionation and atmospheric oxygen: Implications for Phanerozoic O₂ evolution: *Science*, **287**, 1630–1633.
- Berner, R. A., 2006, GEOCARBSULF: A combined model for Phanerozoic atmospheric O₂ and CO₂: *Geochimica et Cosmochimica Acta*, **70**, 5653-5664.
- Bonal, D., Sabatier, D., Montpied, P., Tremeaux, D., Guehl, J., 2000. Interspecific variability of $\delta^{13}\text{C}$ among trees in rainforests of French Guiana: functional groups and canopy integration. *Oecologia* **124**, 454-468.
- Breecker, D. O., Sharp, Z. D., and McFadden, L. D. (2010) Atmospheric CO₂ concentration during ancient greenhouse climates were similar to those predicted for A.D. 2100: *Proceedings of the National Academy of Sciences USA*, **107**(2), 576-580.
- Breecker, D. (2013) Quantifying and understanding the uncertainty of atmospheric CO₂ concentrations determined from calcic paleosols, *Geochemistry, Geophysics, Geosystems*, **14**(8), 3210-3220.
- Breecker, D. O. and Retallack, G. J. (2014) Refining the pedogenic carbonate atmospheric CO₂ proxy and application to Miocene CO₂: *Palaeogeography, Palaeoclimatology, Palaeoecology*, **406**, 1-8.
- Cerling, T. (1991) Carbon dioxide in the atmosphere: evidence from Cenozoic and Mesozoic paleosols, *American Journal of Science*, **291**(4), 377-400.
- Cerling, T. E., Solomon, D. K., Quade, J. and Bowman, J. R. (1991) On the isotopic composition of carbon in soil carbon dioxide, *Geochimica et Cosmochimica Acta*, **55**(11), 3403-3405.
- Christeller, J. T., Laing, W. A. and Troughton, J. H. (1976), Isotope Discrimination by Ribulose 1, 5-Diphosphate Carboxylase: No Effect of Temperature or HCO₃⁻ Concentration, *Plant Physiology*, **57**(4), 580-582.
- Cleveland, D. M., Nordt, L. C., Dworkin, S. I. and Atchley, S. C. (2008) Pedogenic carbonate isotopes as evidence for extreme climatic events preceding the Triassic-Jurassic boundary: Implications for the biotic crisis?: *Geological Society of America Bulletin*, **120**(11-12), 1408-1415.
- Collister, J. W., Rieley, G., Stern, B., Eglinton, G. and Fry, B. (1994) Compound-specific $\delta^{13}\text{C}$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms, *Organic Geochemistry*, **21**(6-7), 619-627.
- Craig, H. (1953) The geochemistry of the stable carbon isotopes, *Geochimica et Cosmochimica Acta*, **3**(2), 53-92.
- Cui, Y., Kump, L. R., Ridgwell, A. J., Charles, A. J., Junium, C. K., Diefendorf, A. F., Freeman, K. H., Urban, N. M. and Harding, I. C. (2011) Slow release of fossil

carbon during the Palaeocene-Eocene Thermal Maximum, *Nature Geosci*, **4**(7), 481-485.

Demicco, R. V., Lowenstein, T. K., and Hardie, L. A. (2003) Atmospheric $p\text{CO}_2$ since 60 Ma from records of seawater pH, calcium, and primary carbonate mineralogy: *Geology*, **31**(9), 793-796.

Diefendorf, A. F., Mueller, K. E., Wing, S. L., Koch, P. L. and Freeman, K. H. (2010) Global patterns in leaf ^{13}C discrimination and implications for studies of past and future climate, *Proceedings of the National Academy of Sciences*, **107**(13), 5738-5743.

Efron, B. and Tibshirani, R. J. (1994) *An introduction to the bootstrap*, CRC press.

Ekart, D. D., Cerling, T. E., Montanez, I. P. and Tabor, N. J. (1999) A 400 million year carbon isotope record of pedogenic carbonate: implications for paleoatmospheric carbon dioxide, *American Journal of Science*, **299**(10), 805-827.

Farquhar, G. D., O'Leary, M. H. and Berry, J. A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, **9**, 121-137.

Flanagan, L. B., Brooks, J. R. and Ehleringer, J. R. (1997) Photosynthesis and carbon isotope discrimination in boreal forest ecosystems: A comparison of functional characteristics in plants from three mature forest types, *Journal of Geophysical Research: Atmospheres* (1984-2012), **102**(D24), 28861-28869.

Fletcher, B. J., Beerling, D. J., Brentnall, S. J. and D. L. Royer (2005) Fossil bryophytes as recorders of ancient CO_2 levels: Experimental evidence and a Cretaceous case study, *Global Biogeochemical Cycles*, **19**(3).

Fletcher, B. J., Brentnall, S. J., Quick, W. P. and Beerling, D. J. (2006) BRYOCARB: A process-based model of thallose liverwort carbon isotope fractionation in response to CO_2 , O_2 , light and temperature, *Geochimica et Cosmochimica Acta*, **70**(23), 5676-5691.

Fletcher, B. J., Brentnall, S. J., Anderson, C. W., Berner, R. A. and Beerling, D. J. (2008) Atmospheric carbon dioxide linked with Mesozoic and early Cenozoic climate change, *Nature Geoscience*, **1**(1), 43-48.

Franks, P. J., Royer, D. L., Beerling, D. J., Van de Water, P. K., Cantrill, D. J., Barbour, M. M., and Berry, J. A. (2014) New constraints on atmospheric CO_2 concentration for the Phanerozoic: *Geophysical Research Letters*, 2014GL060457.

Freeman, K. H., and Pagani, M. (2005) Alkenone-Based Estimates of Past CO_2 Levels: A Consideration of Their Utility Based on an Analysis of Uncertainties, in *A History of Atmospheric CO_2 and Its Effects on Plants, Animals, and Ecosystems*, edited by J. Ehleringer, T. Cerling and D. Dearing, pp. 55-78, American Geophysical Union.

Graham, H.V., Patzkowsky, M.E., Wing, S.L., Parker, G.G., Fogel, M.L., Freeman, K.H., 2014. Isotopic characteristics of canopies in simulated leaf assemblages, *Geochimica et Cosmochimica Acta*, **144**, 82-95.

Guy, R. D., Fogel, M. L., and Berry, J. A. (1993) Photosynthetic fractionation of the stable isotopes of oxygen and carbon, *Plant Physiology*, **101**, 37-47.

Hoins, M., Van de Waal, D.B., Eberlein, T., Reichart, G.-J., Rost, B., Sluijs, A. (2015) Stable carbon isotope fractionation of organic cyst-forming dinoflagellates:

- Evaluating the potential for a CO₂ proxy. *Geochimica et Cosmochimica Acta*,
160, 267-276.
- Jahren, A. H. (2004) The carbon stable isotope composition of pollen: Review of
Palaeobotany and Palynology, **132**, 291-313.
- Kanzaki, Y. and Murakami, T. (2015) Estimates of atmospheric CO₂ in the Neoproterozoic–
Paleoproterozoic from paleosols: *Geochimica et Cosmochimica Acta*, **159**, 190-
219.
- Kawamura, K., Nakazawa, T., Aoki, S., Sugawara, S., Fujii, Y. and Watanabe, O. (2007)
Dome Fuji Ice Core 338KYr Wet Extraction CO₂ Data, in *World Data Center for
Paleoclimatology, Boulder and NOAA Paleoclimatology Program*.
- Keeling, R. F., Piper, S. C., Bollenbacher, A. F. and Walker, J. S. (2009) Atmospheric
CO₂ records from sites in the SIO air sampling network. In Trends: A
Compendium of Data on Global Change. Carbon Dioxide Information Analysis
Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge,
Tenn., U.S.A. doi: 10.3334/CDIAC/atg.035
- Kiehl, J. T., and Shields, C. A. (2013) Sensitivity of the Palaeocene–Eocene Thermal
Maximum climate to cloud properties, *Philosophical Transactions of the Royal
Society A: Mathematical, Physical and Engineering Sciences*, **371**(2001),
20130093.
- King, D. C., Schubert, B. A. and Jahren, A. H. (2012) Practical considerations for the use
of pollen $\delta^{13}\text{C}$ value as a paleoclimate indicator: *Rapid Communications in Mass
Spectrometry*, **26**, 2165-2172.
- Kohn, M. J. (2010) Carbon isotope compositions of terrestrial C3 plants as indicators of
(paleo)ecology and (paleo)climate, *Proceedings of the National Academy of
Sciences*, **107**(46), 19691-19695.
- Konrad W, Roth-Nebelsick A, Grein M. 2008. Modelling of stomatal density response to
atmospheric CO₂. *Journal of Theoretical Biology*, **253**: 638-58.
- Leavitt, S. and Newberry, T. (1992) Systematics of stable-carbon isotopic differences
between gymnosperm and angiosperm trees, *Plant Physiology*, **11**, 257–262.
- Lloyd, J. and Farquhar, G. D. (1994) ¹³C discrimination during CO₂ assimilation by the
terrestrial biosphere, *Oecologia*, **99**(3-4), 201-215.
- Lowenstein, T. K. and Demicco, R. V. (2006) Elevated Eocene atmospheric CO₂ and its
subsequent decline: *Science*, **313**(5795), 1928.
- Martínez-Botí MA, Foster GL, Chalk TB, Rohling EJ, Sexton PF, et al. 2015. Plio-
Pleistocene climate sensitivity evaluated using high-resolution CO₂ records.
Nature, **518**, 49-54.
- Matson, S. D., Rook, L., Oms, O. and Fox, D. L. (2012) Carbon isotopic record of
terrestrial ecosystems spanning the Late Miocene extinction of *Oreopithecus
bambolii*, Baccinello Basin (Tuscany, Italy): *Journal of Human Evolution*, **63**(1),
127-139.
- Meissner, K., Bralower, T., Alexander, K., Jones, T. D., Sijp, W. and Ward, M. (2014)
The Paleocene-Eocene Thermal Maximum: How much carbon is enough?,
Paleoceanography, **29**(10), 946-963.
- O'Leary, M. H. (1988) Carbon isotopes in photosynthesis: *BioScience*, **38**, 328-336.
- Pagani, M., Arthur, M. A. and Freeman, K. H. (1999) Miocene evolution of atmospheric
carbon dioxide, *Paleoceanography*, **14**(3), 273-292.

- Passey, B. H., Cerling, T. E., Perkins, M. E., Voorhies, M. R., Harris, J. M. and Tucker, S. T. (2002) Environmental change in the Great Plains: an isotopic record from fossil horses, *The Journal of Geology*, **110**(2), 123-140.
- Pearson, P. N. and Palmer, M. R. (2000) Atmospheric carbon dioxide concentrations over the past 60 million years: *Nature*, **406**, 695-699.
- Pearson, P. N., Foster, G. L. and Wade, B. S. (2009) Atmospheric carbon dioxide through the Eocene-Oligocene climate transition, *Nature*, **461**, 1110-1113.
- Polissar, P.J., D'Andrea, W.J., 2014. Uncertainty in paleohydrologic reconstructions from molecular δD values, *Geochimica et Cosmochimica Acta*, **129**, 146-156.
- R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Rebolledo, L., Lange, C. B., Bertrand, S., Muñoz, P., Salamanca, M., Lazo, P., Iriarte, J. L., Vargas, G., Pantoja, S. and Dezileau, L. (2015) Late Holocene precipitation variability recorded in the sediments of Reloncaví Fjord (41°S, 72°W), Chile: *Quaternary Research*, doi: 10.1016/j.yqres.2015.05.006.
- Rieley, G., Collier, R. J., Jones, D. M., Eglinton, G., Eakin, P. A. and Fallick, A. E. (1991) Sources of sedimentary lipids deduced from stable carbon-isotope analyses of individual compounds, *Nature*, **352**, 425-427.
- Roeske, C. and O'Leary, M. H. (1984) Carbon isotope effects on enzyme-catalyzed carboxylation of ribulose biphosphate, *Biochemistry*, **23**(25), 6275-6284.
- Royer, D. L., Berner, R. A. and Beerling, D. J. (2001) Phanerozoic atmospheric CO₂ change: evaluating geochemical and paleobiological approaches, *Earth-Science Reviews*, **54**(4), 349-392.
- Royer, D. L., Berner, R. A. and Park, J. (2007) Climate sensitivity constrained by CO₂ concentrations over the past 420 million years, *Nature*, **446**(7135), 530-532.
- Royer, D. (2014) Atmospheric CO₂ and O₂ during the Phanerozoic: Tools, patterns, and impacts, *Treatise on Geochemistry*, **6**, 251-267.
- Royer, D. L., Donnadieu, Y., Park, J., Kowalczyk, J. and Goddérís, Y. (2014) Error analysis of CO₂ and O₂ estimates from the long-term geochemical model GEOCARBSULF, *American Journal of Science*, **314**(9), 1259-1283.
- Schmitt, J., Schneider, R., Elsig, J., Leuenberger, D., Laurantou, A., Chappellaz, J., Köhler, P., Joos, F., Stocker, T. F. and Leuenberger, M. (2012) Carbon isotope constraints on the deglacial CO₂ rise from ice cores, *Science*, **336**(6082), 711-714.
- Schubert, B. A. and Jahren, A. H. (2012) The effect of atmospheric CO₂ concentration on carbon isotope fractionation in C₃ land plants, *Geochimica et Cosmochimica Acta*, **96**, 29-43.
- Schubert, B. A. and Jahren, A. H. (2013) Reconciliation of marine and terrestrial carbon isotope excursions based on changing atmospheric CO₂ levels, *Nature Communications*, **4**, 1653, doi: 10.1038/ncomms2659.
- Schubert, B. A. and Jahren, A. H. (2015) Global increase in plant carbon isotope fractionation following the Last Glacial Maximum caused by increase in atmospheric pCO₂, *Geology*, **43**(5), 435-438.
- Steinthorsdottir, M., Jeram, A. J. and McElwain, J. C. (2011) Extremely elevated CO₂ concentrations at the Triassic/Jurassic boundary: *Palaeogeography, Palaeoclimatology, Palaeoecology*, **308**, 418-432.

- Stewart, G. R., Turnbull, M. H., Schmidt, S. and Erskine, P. D. (1995) ^{13}C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability: *Australian Journal of Plant Physiology*, **22**, 51-55.
- Strauss, H. and Peters-Kottig, W. (2003) The Paleozoic to Mesozoic carbon cycle revisited: The carbon isotopic composition of terrestrial organic matter, *Geochemistry, Geophysics, Geosystems*, **4**(10).
- Suits, N. S., Denning, A. S., Berry, J. A., Still, C. J., Kaduk, J., Miller, J. B. and Baker, I. T. (2005) Simulation of carbon isotope discrimination of the terrestrial biosphere, *Global Biogeochem. Cycles*, **19**, GB1017, doi:10.1029/2003GB002141.
- Tappert, R., McKellar, R. C., Wolfe, A. P., Tappert, M. C., Ortega-Blanco, J. and Muehlenbachs, K. (2013) Stable carbon isotopes of C3 plant resins and ambers record changes in atmospheric oxygen since the Triassic, *Geochimica et Cosmochimica Acta*, **121**, 240-262.
- Tipple, B. J., Meyers, S. R. and Pagani, M. (2010) Carbon isotope ratio of Cenozoic CO_2 : A comparative evaluation of available geochemical proxies, *Paleoceanography*, **25**(3).
- Treydte et al., (2007) Signal strength and climate calibration of a European tree-ring isotope network: *Geophysical Research Letters*, **34**, L24302.
- Tripati, A. K., Roberts, C. D., and Eagle, R. A. (2009) Coupling of CO_2 and ice sheet stability over major climate transitions of the last 20 million years: *Science*, **326**(5958), 1394-1397.
- van de Wal, R. S. W., de Boer, B., Lourens, L. J., Köhler, P., and Bintanja, R. (2011) Reconstruction of a continuous high-resolution CO_2 record over the past 20 million years: *Clim. Past*, **7**, 1459-1469.
- Walsh, E. M., Ingalls, A. E. and Keil, R. G. (2008) Sources and transport of terrestrial organic matter in Vancouver Island fjords and the Vancouver-Washington Margin: A multiproxy approach using $\delta^{13}\text{C}_{\text{org}}$, lignin phenols, and the ether lipid BIT index, *Limnology and Oceanography*, **53**(3), 1054-1063.
- Werner, R. A., Bruch, B. A. and Brand, W. A. (1999) ConFlo III-An Interface for High Precision $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Analysis with an Extended Dynamic Range, *Rapid Communications in Mass Spectrometry*, **13**(13), 1237-1241.
- Whiteside, J. H., Lindström, S., Irmis, R. B., Glasspool, I. J., Schaller, M. F., Dunlavey, M., Nesbitt, S. J., Smith, N. D. and Turner, A. H. (2015) Extreme ecosystem instability suppressed tropical dinosaur dominance for 30 million years: *Proceedings of the National Academy of Sciences*, **112**(26), 7909-7913.
- Wong, W. W., Benedict, C. R. and Kohel, R. J. (1979) Enzymic fractionation of the stable carbon isotopes of carbon dioxide by ribulose-1, 5-bisphosphate carboxylase, *Plant physiology*, **63**(5), 852-856.
- Ye, Y. (1987) Interior algorithms for linear, quadratic, and linearly constrained non linear programming, *PhD Dissertation*, Stanford University.
- Young, G., McCarroll, D., Loader, N., Gagen, M., Kirchhefer, A., and Demmler, J., (2012) Changes in atmospheric circulation and the Arctic Oscillation preserved within a millennial length reconstruction of summer cloud cover from northern Fennoscandia: *Climate Dynamics*, **39**(1), 495-507.
- Zachos, J. C., Pagani, M., Sloan, L., Thomas, E., Billups, K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 686-693.

658 Zhang, Y. G., Pagani, M., Liu, Z., Bohaty, S. M. and DeConto, R. (2013) A 40-million-
659 year history of atmospheric CO₂, *Philosophical Transactions of the Royal Society*
660 *A: Mathematical, Physical and Engineering Sciences*, **371**(2001), 20130096.

Figure Captions

Figure 1. The effect of $p\text{CO}_2$ on C_3 land plant carbon isotope fractionation based on field and chamber experiments on a wide range of C_3 land plant species. The amount of carbon isotope fractionation per change in $p\text{CO}_2$ (S , ‰/ppmv) as a function of $p\text{CO}_2$ (Eq. 1) is plotted for three choices of A ($A=26, 28.26$, and 30 ; calculated values for B and C , for each A , are provided in Table 2). Note the similarity among the three S-curves. Circles are plotted as the midpoint of the range of $p\text{CO}_2$ tested for each study. All $p\text{CO}_2$ data and references are provided in Table DR2 within Schubert and Jahren (2015). Inset: $\Delta^{13}\text{C}$ value plotted as a function of $p\text{CO}_2$ (Eq. 2) with the same values for A , B , and C (Table 2). The shaded regions represent the 68% confidence interval based on 10,000 bootstrap iterations.

Figure 2. The effect of uncertainty in the curve fitting (Fig. 1) on the error in $p\text{CO}_{2(t)}$ (top, ppmv; bottom, % error); all other input uncertainties were kept at 0. Error is shown for $A = 28.26$ with $B = 0.223 \pm 0.028$ ($\pm 1\sigma$). For each randomly chosen B value, corresponding values for C were calculated by solving Eq. 2 with $p\text{CO}_2 = 0$ and $\Delta^{13}\text{C} = 4.4$, such that $C = [4.4 \times (A)] / [(A - 4.4) \times (B)]$.

Figure 3. Full error propagation showing the effect of all the uncertainties for the inputs listed in Table 2 on the error in $p\text{CO}_{2(t)}$. Top: the positive and negative $p\text{CO}_{2(t)}$ error in ppmv; middle: the positive and negative $p\text{CO}_{2(t)}$ error in percentage; bottom: the percent of invalid $p\text{CO}_{2(t)}$ values (i.e., $0 > p\text{CO}_{2(t)} > 10^6$ ppmv) generated during the 10,000 Monte Carlo resampling.

684

685 Figure 4. Reconstruction of $p\text{CO}_{2(t)}$ (red circles with black error bars) for the Paleogene
686 (52-63 Ma) using the C_3 land plant proxy and $\delta^{13}\text{C}_{\text{org}(t)}$ data from Diefendorf et al.,
687 (2015). All inputs and their uncertainties for calculation of $p\text{CO}_{2(t)}$ are listed in Table 2.
688 The error bars on each calculated $p\text{CO}_{2(t)}$ value assume a lack of knowledge of the
689 paleoenvironment of the site (i.e., use 1σ uncertainty of 1.6‰ based on a global dataset
690 of modern leaf $\delta^{13}\text{C}_{\text{org}}$ values; Kohn, 2010). Note that the variability in reconstructed
691 $p\text{CO}_{2(t)}$ for each time interval is small relative to the error bars, consistent with little
692 variability in $\delta^{13}\text{C}_{\text{org}(t)}$ within each time interval. $p\text{CO}_2$ reconstructions based on the data
693 compilation of Royer (2014) for three other proxies (gray symbols and error bars:
694 squares, stomata; diamonds, paleosols; triangles, liverworts) are shown for comparison.
695 $p\text{CO}_{2(t)}$ calculated using the C_3 land plant proxy indicates levels above the pre-industrial
696 value of 270 ppmv (dashed line). Average $p\text{CO}_{2(t)}$ data for each proxy within this time
697 interval are presented in Table 3.

698

699 Figure 5. Sensitivity analysis showing the effect of uncertainty in $p\text{CO}_{2(t=0)}$ on the errors
700 in $p\text{CO}_{2(t)}$. Errors in $p\text{CO}_{2(t)}$ are shown for $p\text{CO}_{2(t=0)} = 270 \pm 7$ ppmv ($t = 0$, Holocene,
701 shaded triangles) and $p\text{CO}_{2(t=0)} = 270 \pm 35$ ppmv ($t = 0$, Neogene, open triangles); all
702 other input uncertainties are kept at 0. Top: $p\text{CO}_{2(t)}$ error in ppmv; bottom: $p\text{CO}_{2(t)}$ error
703 in percentage.

704

705 Figure 6. Sensitivity analysis showing the effect of uncertainty in $\delta^{13}\text{C}_{\text{CO}_2}$ on the errors in
706 $p\text{CO}_{2(t)}$. Errors in $p\text{CO}_{2(t)}$ are shown for $\delta^{13}\text{C}_{\text{CO}_2(t)} = \pm 0.1\text{‰}$ (shaded triangles) and

707 $\delta^{13}\text{C}_{\text{CO}_2(t)} = \pm 0.5\text{‰}$ (open triangles); all other input uncertainty is kept at 0. The error in
708 $p\text{CO}_{2(t)}$ is applied here to the $\delta^{13}\text{C}_{\text{CO}_2(t)}$ term within Eq. 7, but is nearly identical ($< \sim 0.4\%$
709 difference across all $p\text{CO}_{2(t)}$) whether this error is applied to $\delta^{13}\text{C}_{\text{CO}_2(t=0)}$ or $\delta^{13}\text{C}_{\text{CO}_2(t)}$.
710 Top: $p\text{CO}_{2(t)}$ error in ppmv; bottom: $p\text{CO}_{2(t)}$ error in percentage.

711

712 Figure 7. Sensitivity analysis showing the effect of uncertainty in $\delta^{13}\text{C}_{\text{org}}$ on the errors in
713 $p\text{CO}_{2(t)}$. Errors in $p\text{CO}_{2(t)}$ are shown for $\delta^{13}\text{C}_{\text{org}(t=0)} = \pm 0.1\text{‰}$ (shaded triangles) and
714 $\delta^{13}\text{C}_{\text{org}(t=0)} = \pm 1.6\text{‰}$ (open triangles); all other input uncertainty is kept at 0. The error in
715 $p\text{CO}_{2(t)}$ is applied here to the $\delta^{13}\text{C}_{\text{org}(t=0)}$ term within Eq. 7, but is nearly identical ($<$
716 $\sim 0.4\%$ difference across all $p\text{CO}_{2(t)}$) whether this error is applied to $\delta^{13}\text{C}_{\text{org}(t=0)}$ or
717 $\delta^{13}\text{C}_{\text{org}(t)}$. Top: $p\text{CO}_{2(t)}$ error in ppmv; bottom: $p\text{CO}_{2(t)}$ error in percentage.

718

719 Figure 8. Positive and negative errors of $p\text{CO}_2$ proxies redrawn from Figure 3 within
720 Royer (2014) plotted with the errors of the C_3 land plant proxy from this study. Errors in
721 $p\text{CO}_{2(t)}$ are calculated using the uncertainties shown in Table 2, but with error for $\delta^{13}\text{C}_{\text{org}(t=0)}$
722 ranging from $\pm 0.1\text{‰}$ (measurement error) to $\pm 1.6\text{‰}$ (environmental error) (shaded
723 region). Bold black line represents a 2nd order polynomial fit through the error data if the
724 $\delta^{13}\text{C}_{\text{org}(t=0)}$ error is reduced to $\pm 0.7\text{‰}$.

725

726

727 Table 1. B and C values calculated across the full range of potential A values.

A	B	C	RMSE
26	0.16	32.88	0.002732
27	0.19	28.40	0.002672
28	0.21	24.70	0.002633
28.26*	0.22	23.85	0.002625
29	0.24	21.68	0.002607
30	0.27	19.20	0.002591

728 *A = 28.26 is the value reported within Schubert and Jahren (2012) and used in their
729 previous $p\text{CO}_2$ reconstructions (Schubert and Jahren 2013, 2015). The lowest RMSE
730 results from A = 30.

731

Table 2. The uncertainty of each input variable used to calculate the propagated error of $p\text{CO}_{2(t)}$ where the reference period ($t = 0$) is the Holocene ($p\text{CO}_{2(t=0)} = 270$ ppmv).

Input Variable (Eq. 7)	Input Values	Source	Uncertainty ($\pm 1\sigma$)	Source
$p\text{CO}_{2(t=0)}$	270 ppmv	Kawamura et al., (2007)	7 ppmv	Kawamura et al., (2007)
$\delta^{13}\text{C}_{\text{CO}_2(t=0)}$	-6.4‰	Schubert and Jahren (2015)	0.1‰	Schmitt et al., (2012)
$\delta^{13}\text{C}_{\text{CO}_2(t)}$	variable [#]	Tipple et al., (2010) [#]	0.5‰	Tipple et al., (2010)
$\delta^{13}\text{C}_{\text{org}(t=0)}$	-25.1‰	Schubert and Jahren (2015)	1.6‰	Kohn (2010)
$\delta^{13}\text{C}_{\text{org}(t)}$	variable [#]	Diefendorf et al., (2015) [#]	~0.1‰	Diefendorf et al., (2015) [#]
A	28.26*	Schubert and Jahren (2015)	0*	Schubert and Jahren (2012, 2013, 2015)
B	0.22	This study	0.028	Bootstrapping (this study)
C	Median value determined in Monte Carlo analysis [†]	This study	Determined in Monte Carlo analysis [†]	This study

[#]Dataset used for calculation of $p\text{CO}_{2(t)}$ within Figure 4 is provided in the Appendix.

*The value for A was fixed at 28.26.

[†]Calculated by solving Eq. 2 with $p\text{CO}_2 = 0$ and $\Delta^{13}\text{C} = 4.4$, such that $C = [4.4 \times (A)] / [(A - 4.4) \times (B)]$.

739 Table 3. $p\text{CO}_2$ estimates and their reported errors (Royer, 2014) for the Paleogene (52-63
740 Ma) compared with calculated values for the C_3 plant proxy.

Paleogene					
Proxy	Age range (Ma)	Mean $p\text{CO}_2$ (ppmv)	Mean negative error in ppmv (%)	Mean positive error in ppmv (%)	No. of $p\text{CO}_2$ estimates
C_3 plant proxy (this study)	52-63	470	-147 (31%) [†]	288 (61%) [†]	75
Liverworts	58.8	583	-182 (31%)	235 (40%)	1
Paleosols	52-63	397	-173 (44%)	327 (82%)	16
Stomata	52-63	400	-54 (14%)	79 (20%)	22

741 [†]Determined from the Monte Carlo resampling (Fig. 4).

Figure1

Figure2

Figure3

Figure4

Figure5

Figure 6

Figure7

Figure8