PREDICTION OF ATMOSPHERIC $\delta^{13}CO_2$ USING FOSSIL PLANT TISSUES

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Received 1 November 2006; revised 28 April 2007; accepted 31 July 2007; published 11 January 2008.

[1] Reconstruction of the carbon isotope composition of atmospheric CO_2 is critical to the understanding of long-term global carbon cycling. We have suggested that the $\delta^{13}C$ value of land plant carbon ($\delta^{13}C_p$) preserved in the geologic record should reflect the $\delta^{13}CO_2$ at the time during which the plants grew ($\delta^{13}C_a$), based on a meta-analysis of modern plant data. Here we present the results of laboratory experiments designed to quantify the relationship between plant tissue $\delta^{13}C$ and $\delta^{13}CO_2$ values under varying environmental conditions, including differential pCO_2 ranging from 1 to 3 times today's levels. As predicted, plants grown under elevated pCO_2 showed increased average biomass compared to controls grown at the same temperature. Across a very large range in $\delta^{13}C_a$ (≈24‰) and pCO_2 (≈740 ppmv) we observed a consistent correlation between $\delta^{13}C_a$ and $\delta^{13}C_p$ ($p < 0.001$). We show an average isotopic depletion of −25.4‰ for aboveground tissue and −23.2‰ for belowground tissue of *Raphanus sativus* L. relative to the composition of the atmosphere under which it formed. For aboveground and belowground tissue, grown at both ∼23°C and ∼29°C, correlation was strong and significant ($r^2 \geq 0.98$ and $p < 0.001$); variation in pCO_2 level had little or no effect on this relationship. These results validate our initial conclusion that in the absence of environmental stress, plant $\delta^{13}C$ primarily reflects atmospheric $\delta^{13}CO_2$ linearly across pCO_2 levels; the demonstrated excellent correlation in $\delta^{13}C_a$ and $\delta^{13}C_p$ suggests a high level of predictive power across varying environmental conditions.

Citation: Jahren, A. H., N. C. Arens, and S. A. Harbeson (2008), Prediction of atmospheric $\delta^{13}CO_2$ using fossil plant tissues, *Rev. Geophys.*, *46*, RG1002, doi:10.1029/2006RG000219.

1. INTRODUCTION

[2] Modeling the global carbon cycle and associated paleoclimate reconstruction in the geologic past requires an understanding of the size and composition of active carbon reservoirs and the flux among them. Of particular interest are those reservoirs, such as the atmosphere, that vary significantly in both size (pCO_2) and composition ($\delta^{13}CO_2$) through time [*Berner*, 1998; *Strauss and Peters-Kottig*, 2003; *Sundquist*, 1985]. These reservoirs most clearly reflect the dynamic nature of the carbon cycle, and these carbon pools may be important agents of global climate change. The atmospheric carbon reservoir is also of special interest because it links the ocean and biosphere reservoirs over short timescales [*Sundquist*, 1985]. Although this short-term variation may be irrelevant to long-term carbon cycling, the ocean, atmosphere, and biosphere are central players in times of catastrophic perturbation of carbon cycling, such as dramatic climate change and mass extinction [*Arens and Jahren*, 2000; *Bralower et al.*, 1994; *Dickens et al.*, 1997]. The size of the atmospheric carbon reservoir may also influence flux between reservoirs over longer geologic timescales through changes in weathering rates [*Berner*, 1993; *Yapp and Poths*, 1996].

[3] Flux changes among reservoirs can be linked to carbon sources and sinks by their isotopic composition [e.g., *Dickens et al.*, 1997; *Weissert*, 1989]. This is especially true when normal carbon cycling is interrupted [e.g., *D'Hondt et al.*, 1998]. Therefore a quantitative estimate of the isotopic composition of atmospheric CO_2 would greatly facilitate efforts to describe and model carbon cycling through geologic time and the paleoclimatic consequences of carbon cycle change.

[4] Because of its central importance a variety of methods have been developed to assess the size and carbon isotopic composition of the atmospheric carbon pool through geologic time. Some workers have chosen to address the size of the reservoir through modeling [*Berner*, 1994, 2001; *Berner and Kothavala*, 2001]; others have chosen proxy methods that reconstruct pCO_2 through a correlation with the density of stomata on plant leaves observed in modern plants [*Beerling and Royer*, 2002; *Chaloner and McElwain*, 1997; *Greenwood et al.*, 2003;

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8755-1209/08/2006RG000219$15.00
Paper number 2006RG000219

Hesselbo et al., 2003; *McElwain et al.*, 1999; *Retallack*, 2001; *Royer et al.*, 2001a, 2001b] or the carbon isotopic composition of paleosol carbonates [*Bowen and Beerling*, 2004; *Cerling and Hay*, 1986]. Similarly, the isotopic composition of atmospheric CO_2 has been assessed by measuring the carbon isotopic value of various proxies, most notably carbonate carbon and organic matter in marine systems [e.g., *Hayes et al.*, 1989; *Rask and Schoenau*, 1993] and paleosols [e.g., *Bowen and Beerling*, 2004; *Mora et al.*, 1996]. Because land plants sample the atmosphere directly during photosynthesis, we contend that organic carbon derived from plants provides another substrate from which information on the isotopic composition of the atmospheric carbon pool may be sampled [*Arens et al.*, 2000].

[5] The carbon stable isotope value ($\delta^{13}C$ [‰]) assigned to C3 plant tissue (the dominant photosynthetic pathway) during photosynthesis was first conceptualized by G. D. Farquhar and can be described as the following:

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \times (c_i/c_a) \quad (1)$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ represent the carbon stable isotope composition of atmospheric CO_2 and of the resulting plant tissue, respectively; a and b encompass the isotopic discrimination inherent to gaseous diffusion and enzymatic carboxylation, respectively; (c_i/c_a) is a unitless scalar that reflects the partial pressure of CO_2 within the stomatal chamber (c_i) relative to the partial pressure of CO_2 in the external atmosphere (c_a) [*Farquhar et al.*, 1989]. The value a = 4.4‰ was calculated from diffusivity comparisons of $^{13}CO_2$ versus $^{12}CO_2$ in air [*Craig*, 1953, 1954] and is generally accepted. Depending upon the role envisioned for the primary enzyme that facilitates carboxylation, published estimates of b range from 27.0 to 29.0‰ [*Christeller et al.*, 1976; *Farquhar*, 1979; *Schmidt et al.*, 1978; *Wong et al.*, 1979]. Published estimates of (c_i/c_a) range from 0.38 to 0.89 [*Arens et al.*, 2000] and are meant to reflect a time-averaged value resulting from moment-to-moment stomatal regulation of gas exchange.

[6] Because plants regulate stomatal conductance in response to changing environmental conditions, classic studies have showed straightforwardly that $\delta^{13}C_p$ value can be increased by subjecting a plant to water stress [e.g., *Toft et al.*, 1989] or increased salinity [e.g., *Guy et al.*, 1980], both of which force plants to close stomata and result in ^{13}C-enriched tissues. Conversely, the $\delta^{13}C_p$ value was shown to decrease with direct sunlight [e.g., *Madhavan et al.*, 1991] and low environmental temperatures [e.g., *Körner et al.*, 1991]. A wealth of other potential and actual environmental effects on $\delta^{13}C_p$ has been discussed at length elsewhere [i.e., *Dawson et al.*, 2002]. Our 2000 survey of the primary literature revealed a spread of no less than 10‰ in $\delta^{13}C_p$ values all derived from approximately the same atmospheric $\delta^{13}C_a$; this variability reflected a large number of C3 species (n = 176) under an extremely wide range of growth conditions (including pCO_2 level) [*Arens et al.*, 2000]. One striking result of our initial analysis was the strong linear correlation between $\delta^{13}C_p$ and $\delta^{13}C_a$ values (r^2 = 0.91 and 519 measurements on 176 species [*Arens et al.*, 2000, Figure 2]), even across a broad range of environmental conditions and ecological settings. This led us to hypothesize that the $\delta^{13}C_p$ value of fossil plant tissue could be used to reconstruct the $\delta^{13}C_p$ value of the atmosphere, provided that the analytical substrate included the contribution of many species across various states of ecological functioning and could be meaningfully assessed for diagenetic alteration [*Arens and Jahren*, 2000, 2002; *Arens et al.*, 2000; *Jahren*, 2002; *Jahren et al.*, 2001, 2005]. This approach has been questioned at several levels ranging from the possibility of nonlinearity at more extreme values of $\delta^{13}C_p$ and $\delta^{13}C_a$ [*Gröcke*, 2002] to the possibility of variable (c_i/c_a) under changing environmental conditions at both ecological and evolutionary timescales [*Beerling and Royer*, 2002]. In order to address these concerns we designed a set of experiments to answer the question: Do plants grown under conditions of controlled and constant $\delta^{13}C_a$ values and pCO_2 levels show a significant, linear relationship between $\delta^{13}C_a$ and $\delta^{13}C_p$ values?

2. MATERIALS AND METHODS

[7] Two identical growth chambers were built from 1/4-inch Plexiglas (Figure 1b) (91.44 cm wide × 121.92 cm high × 45.72 cm deep). One served as a control with CO_2 concentrations maintained at ambient atmospheric levels (~370 ppmv = RCO_2 = 1×, i.e., equivalent to present levels, here R indicates the carbon dioxide level expressed as a ratio to today's level), while the other chamber was supplied with air enriched with CO_2. In the first experiment the enriched chamber was maintained at pCO_2 of ~780 ppmv = RCO_2 = 2× (i.e., twice present atmospheric pCO_2). During the second experiment the enriched chamber was maintained at ~1090 ppmv = RCO_2 = 3×, and the control chamber was maintained at ambient levels as above. Ambient air was delivered to the chambers through an intake pipe (Figure 1b) via an inline fan at a rate of 49 dm^3 min^{-1}. For the elevated chamber, CO_2 levels were increased to ~780 or ~1090 ppmv by bleeding pure cylinder CO_2 into the intake pipe, where it mixed with the ambient air before reaching the growth chamber. The CO_2 flow rate can be precisely controlled with an inline needle valve (Figure 1b), thus enabling enrichment of air to any desired concentration of CO_2. Air is exhausted through the upper pipe (Figure 1b) and vented to a fume hood. Complete air turnover in both the control and elevated chamber was approximately once every 10.4 min. Adjustable shelving provided support for the samples and grow lights, enabling adjustment of lamp height to maintain a constant distance from plant leaves throughout the growth period (Figure 1b). Four fluorescent grow lamps (33-W GE Brightstik) per shelf provided 600 lumens (approximately 100 microeinsteins m^{-2} s^{-1} photosynthetic photon flux density, the equivalent of full shade at midday [*Halling and Peters*, 1987]) of light intensity as measured at the leaf surface. The lighting cycle was controlled with an electrical timer; lights turned on in the morning at 0700 LT and turned

Figure 1. (a) *Raphanus sativus* L. after 5 weeks of growth within the elevated-CO_2 growth chamber. (b) Plant growth chambers used in all experiments.

off at 1845 LT. Heat generated from the grow lamps provided the daytime temperature difference between the upper and lower sample shelves. The lower shelf provided a "daytime" growing temperature of ~23°C; the upper shelf provided a "daytime" growing temperature of ~30°C. Temperature measurements were taken at each sample shelf (uncertainty = ±1°C). Relative humidity was measured at the lower sample shelf (uncertainty = ±1%), and CO_2 concentrations were measured at the upper sample shelf (uncertainty = ±25 ppmv).

[8] Plants grown were *Raphanus sativus* L. (variant Cherry Belle Radishes, Brassicaceae) ordered from Harris Seeds of Rochester, New York (ID 0071, see www.harris-seeds.com) (Figure 1a). *Raphanus* was selected for the experiment because it grows rapidly and produces a large proportion of its biomass underground, allowing us to separate the significance of leaf versus other tissues. Eight plants were grown on each shelf, with a total of 16 plants per chamber per experiment. Water was delivered to the plants through tubing that entered the chambers from the top (Figure 1b), enabling us to minimize the opening of chambers throughout the growth experiment. Growth was started with soil at "field moist" conditions [*Soil Survey Staff of the Soil Conservation Service*, 1999]. Over the course of the experiment, 690 ± 1 mL of water were added to each plant; two of these water additions contained fertilizer ("Miracle-Gro" at commercially prescribed concentration). Fertilizer additions took place on day 9 and day 21 of the 37-d experiments. Addition of water sometimes resulted in an increase in relative humidity. The response was fleeting: It occurred over 5 h and was about +4% to +10% in magnitude. Experiments were designed to ensure that plant growth was not limited by water, light, or nutrient availability in order to highlight the effects of elevated pCO_2 and minimize the effect of (c_i/c_a) on its carbon isotopic composition.

[9] Upon harvest, aboveground tissue was separated from belowground tissue, dried for 5 d at 100°C, and weighed (uncertainty ±1 mg). All aboveground and belowground tissues were separated from each plant, lyophilized for 24 h, and ground to a fine uniform powder in preparation for stable isotope analysis, thus yielding a bulk or combined tissue value. Plant tissues were analyzed for $\delta^{13}C$ value using a Eurovector automated combustion system in conjunction with an Isoprime stable isotope mass spectrometer at the Johns Hopkins University. All samples were introduced to the combustion system in pure tin capsules; stable isotope values are reported in standard δ notation: $\delta = (R_{sample} - R_{standard}/R_{standard})1000‰$. The reporting standard is Pee Dee Formation limestone with $R = {}^{13}C/{}^{12}C = 0.011237$. Analytical uncertainty associated with each measurement was ±0.05‰. Values presented represented the average of three replicate capsules; the average isotopic variability of replicate capsules was ±0.12‰ for all tissues. Combustion also resulted in a quantification of percent carbon content in each sample. Neither elevated pCO_2 nor elevated growth chamber temperature affected the bulk carbon composition of the plants. Average composition of aboveground tissue was 34.1% C; average for belowground tissue was 35.5% C (all values ±1% C analytical uncertainty; variability was less than this uncertainty).

[10] We sampled CO_2 in air (Tables 1 and 2) using a preevacuated 250-cm^3 glass vessel. We placed the vessel in the environment that we wished to sample (e.g., the control or elevated growth chambers), opened the stopcock, and let it equilibrate with the surrounding air for 15 min. We then isolated the CO_2 from the sampled atmosphere by slowly bleeding the air sample through a vacuum manifold, freezing any water into an ethanol/liquid nitrogen–cooled trap, freezing CO_2 into a series of liquid nitrogen–cooled traps, and pumping away noncondensable gases. Because naturally occurring N_2O will disrupt the isotopic analysis of CO_2 (because of isobaric interference), we further purified the sample by reaction with 0.5 g of reduced copper at 450°C for 2 h. The resulting N_2 was separated from CO_2 cryogenically. Pure CO_2 sample gas was analyzed for $\delta^{13}CO_2$ value through the dual inlet of the mass spectrom-

TABLE 1. Average Stable Isotope Values of CO_2 ($\delta^{13}CO_2$) Presented With the Standard Deviation (σ) Seen in the Number of Days of Measurements (n)[a]

	$\delta^{13}CO_2$, ‰	σ, ‰	n
Control chamber atmosphere ($RCO_2 = 1\times$)	−13.86	0.54	9
Elevated chamber atmosphere ($RCO_2 = 2\times$)	−24.05	0.39	9
Ambient laboratory atmosphere	−14.45	0.44	9
CO_2 cylinder	−42.57[b]	0.10[b]	4

[a]Cylinder measurements took place over one 24-h period.
[b]Stability of these values about a mean is presented in Figure 6.

eter. All statistical analyses were performed in Aabel 1.5.8 (Gigawiz Ltd.) for Macintosh G5 running OSX.4.

3. RESULTS

[11] Environmental conditions were closely monitored in the chambers for an 8-week period prior to the experiments and during the two experiments that followed. Carbon dioxide levels in the control chamber were maintained with less than 8% standard variability throughout preexperiment and experimental periods. Elevated chamber levels were maintained with less than 4% standard variability, with no significant difference in performance between the preexperiment and each of the elevated experiments (Table 2 and Figure 2). Temperatures within the chambers were maintained with great consistency throughout the preexperiment and both experimental periods. Within each chamber the upper growth shelf was maintained at ~29°C; the lower growth shelf was maintained at ~23°C (standard variability was less than 1°C in all circumstances, see Table 2 and Figure 3). Relative humidity (RH) levels (percent) in the chambers did change in response to plant growth (Table 2 and Figure 4). Both chambers exhibited identical RH values during the preexperiment. The introduction of plants during experiment 1 (elevated $RCO_2 = 2\times$) resulted in an average increase in RH of ~10% in both chambers. The increase in the RH of the control chambers from experiments 1 to 2 may also reflect the increasing RH of the greater environment as Maryland's spring turned to summer. In contrast, in experiment 2 (elevated $RCO_2 = 3\times$), RH differed between control and experimental chambers. The enriched pCO_2 chamber experienced at least 10% lower RH than did the control chamber. This may be due to lower stomatal conductance at the highest pCO_2 (Table 2 and Figure 4) [*Hungate et al.*, 2002]. Plants were watered regularly to preclude water limitation [cf. *Beerling and Woodward*, 1995].

[12] Plants grown under elevated pCO_2 showed increased average biomass compared to controls grown at the same temperature. Average aboveground biomass of plants grown under $RCO_2 = 2\times$ at ~23°C was ~36% greater than that grown at ~23°C under $RCO_2 = 1\times$ ($p < 0.001$, see Table 3 and Figure 5a). Average aboveground biomass of plants grown under $RCO_2 = 3\times$ at ~23°C was only ~24% greater than those from the ~23°C control ($p < 0.001$). Belowground biomass showed even greater increase under elevated pCO_2. Average belowground biomass for plants grown under $RCO_2 = 2\times$ at ~23°C was ~190% greater than that grown at ~23°C under $RCO_2 = 1\times$ ($p < 0.001$, see Table 3 and Figure 5c). Similarly, under $RCO_2 = 3\times$ at ~23°C, belowground biomass was ~62% greater than that of the ~23°C control ($p < 0.001$).

[13] Within a given pCO_2 level, mean biomass measurements did show a tendency for elevated temperature to result in decreased biomass. However, this trend was not uniform across pCO_2 levels, nor was it statistically significant. In the first experiment at $RCO_2 = 1\times$, aboveground biomass was significantly lower at higher temperature ($p < 0.001$, see Table 3), but belowground biomass was not ($p = 0.16$). However, this result was not replicated in the second experiment; no statistically significant variation in biomass was observed with temperature (Table 3). At $RCO_2 = 2\times$ and $3\times$ the situation was reversed. Aboveground biomass showed no statistically significant variation with temperature ($p = 0.60$ and 0.36, respectively), while belowground biomass decreased significantly ($p < 0.02$, see Table 3 and Figures 5b and 5d).

TABLE 2. Summary of the Environmental Characteristics of the Control and Elevated Chambers, Separated According to Upper and Lower Growth Shelf, as Measured Throughout the Preexperiment and the $RCO_2 = 2\times$ and $RCO_2 = 3\times$ Experiments[a]

	Preexperiment, 8 Weeks	$RCO_2 = 2\times$, 5 Weeks	$RCO_2 = 3\times$, 7 Weeks
Control chamber pCO_2, ppmv	387 ± 25 (54)	389 ± 30 (36)	348 ± 16 (53)
Control chamber $\delta^{13}CO_2$, ‰	−9.11 ± 0.08 (3)	−9.00 ± 0.10 (3)	−9.04 ± 0.16 (3)
Upper T,°C	29.2 ± 0.8 (42)	29.0 ± 0.6 (30)	28.9 ± 0.7 (51)
Upper RH, %	23.2 ± 3.3 (51)	35.1 ± 7.9 (36)	59.5 ± 4.0 (51)
Lower T,°C	21.8 ± 1.4 (54)	22.5 ± 0.9 (36)	22.9 ± 0.8 (53)
Lower RH, %	21.0 ± 2.8 (51)	31.6 ± 8.3 (36)	52.8 ± 3.8 (51)
Elevated chamber pCO_2, ppmv	764 ± 25 (54)	778 ± 33 (35)	1088 ± 30 (52)
Elevated chamber $\delta^{13}CO_2$, ‰	−27.52 ± 0.13 (3)	−26.73 ± 0.04 (3)	−32.61 ± 0.01 (3)
Upper T,°C	29.5 ± 0.8 (41)	29.4 ± 0.7 (30)	29.3 ± 0.7 (51)
Upper RH, %	19.4 ± 2.8 (51)	27.7 ± 4.7 (36)	39.7 ± 2.1 (51)
Lower T,°C	21.9 ± 1.5 (54)	22.7 ± 1.0 (36)	23.2 ± 0.9 (53)
Lower RH, %	18.9 ± 2.9 (50)	27.3 ± 5.0 (36)	38.0 ± 2.4 (51)

[a]Average values are presented with the standard deviation ($\pm\sigma$) seen in the number of days of measurements (n); these data are presented graphically in Figures 2, 3, and 4. T is temperature; RH is relative humidity.

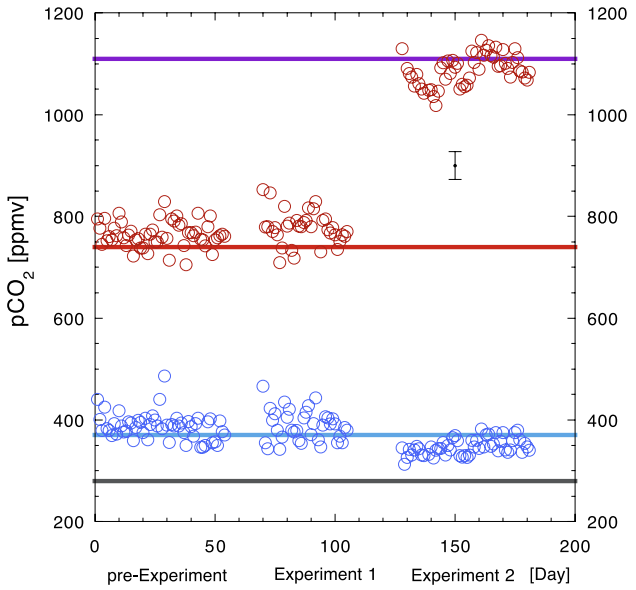

Figure 2. Partial pressure of CO_2 stability in growth chambers before and during each experiment. For reference, the following levels of pCO_2 are included on the graph: Preindustrial (grey line); modern (blue line); $RCO_2 = 2\times$ (red line); and $RCO_2 = 3\times$ (purple line). Data from the control chamber are represented by blue circles, while data from the elevated pCO_2 chamber are represented by red circles. The black bar represents plus/minus the average standard deviation in pCO_2 readings calculated across all experiments.

[14] Measured carbon isotope values for the control and experimental atmospheres ($\delta^{13}C_a$) ranged from −32.6 to −9.0‰ based on the progressive enrichment of laboratory air with isotopically light carbon dioxide from a pressurized CO_2 cylinder (Figure 1 and Table 2). One of the CO_2 cylinders used was characterized to have $\delta^{13}CO_2 = -42.6$‰ and exhibited very low variability over several consecutive measurements (Table 1); however, the values of $\delta^{13}C_a$ against which values of $\delta^{13}C_p$ were ultimately regressed were measured upon atmosphere directly sampled from the chambers (Table 2). In addition to carbon dioxide concentration the carbon isotope composition of carbon dioxide in the chambers was also measured (Figure 6). The standard deviation of $\delta^{13}C_a$ through time is presented as uncertainty when comparing $\delta^{13}C_p$ against $\delta^{13}C_a$ (Figures 7 and 8).

[15] Across a very large range in $\delta^{13}C_a$ (≈24‰) and pCO_2 (≈740 ppmv) we observed a striking and consistent correlation between $\delta^{13}C_a$ and $\delta^{13}C_p$ ($p < 0.001$, Figures 7 and 8). For both aboveground and belowground tissue, grown at both ∼23°C and ∼29°C, correlation was strong and significant ($r^2 \geq 0.98$ and $p < 0.001$). Figures 7 and 8 illustrate an average isotopic depletion of −25.4‰ for aboveground tissue and −23.2‰ for belowground tissue of *Raphanus sativus* L. relative to the composition of the atmosphere under which it formed. Furthermore, variation in temperature and pCO_2 level had little or no effect on this relationship (Figures 7, 8, and 9).

[16] Values of c_i/c_a calculated for the plants in this study ranged from 0.70 to 0.92 (average equals 0.85). Relative to the full range of values reported previously in the literature (0.38 to 0.89 [*Arens et al.*, 2000]), these values are high. At high c_i/c_a values the influence of this ecological term on $\delta^{13}C_p$ is minimal. Moreover, c_i/c_a values did not vary systematically with either temperature ($p = 0.76$), which paralleled relative humidity and thus water potential, or with pCO_2 ($p = 0.17$) level except for the pCO_2 experiment run at 778 ppmv, which had a significantly lower average value ($(c_i/c_a) = 0.76$).

[17] This experiment was not designed to test the influence of c_i/c_a on $\delta^{13}C_p$, which is well defined (see equation (1)). Instead, this experiment sought to consider the relationship between plant and atmosphere under the environmental conditions of low water and nutrient stress necessary when seeking to use the carbon isotope composition of plants to reconstruct the isotopic composition of atmospheric CO_2. High and mostly invariant values of c_i/c_a in this experiment reflect effective control of water and nutrient stress over the various experimental and control groups.

[18] We note that c_i/c_a did not vary at the high and low extremes of pCO_2 evaluated in this study. It has been suggested that c_i/c_a may serve as a proxy for variable

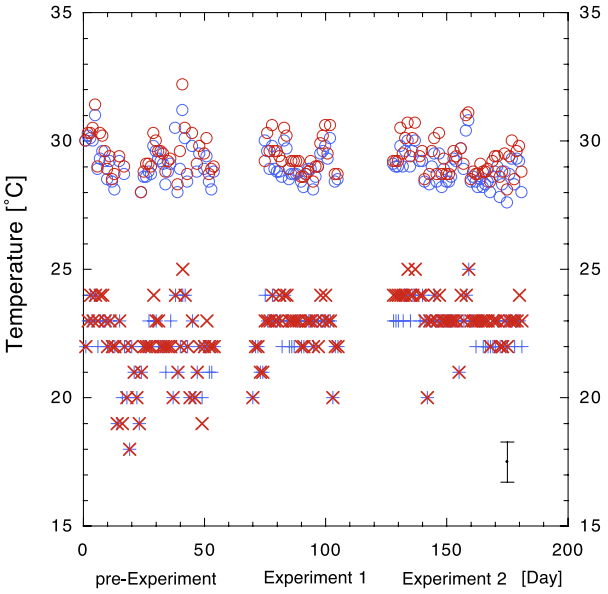

Figure 3. Temperature stability in growth chambers before and during each experiment. Preexperiment and experiment 1 represent control and elevated chambers at $RCO_2 = 1\times$ and $2\times$, respectively. Experiment 2 represents control and elevated chambers at $RCO_2 = 1\times$ and $3\times$, respectively. Data from the lower shelf of the control chamber are represented by plus signs, while data from the lower shelf of the elevated pCO_2 chamber are represented by crosses. Similarly, data from the higher shelf of the control chamber are represented by blue circles, while data from the higher shelf of the elevated pCO_2 chamber are represented by red circles. The black bar represents plus/minus the average standard deviation in temperature readings calculated across all experiments.

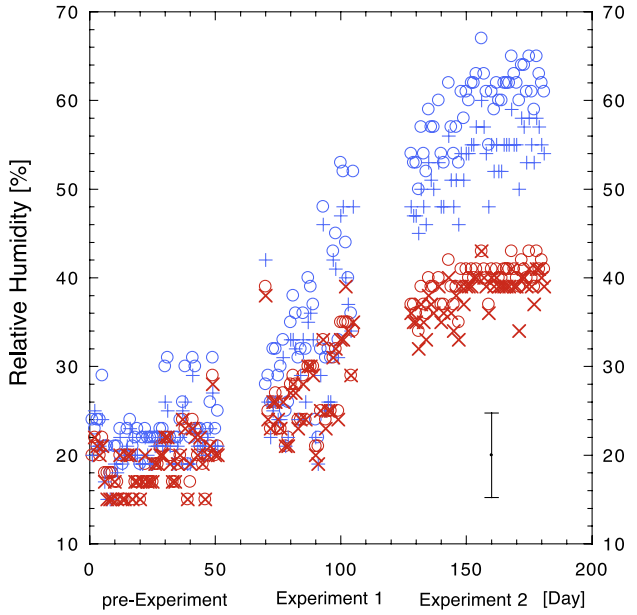

Figure 4. Relative humidity stability in growth chambers before and during each experiment. Preexperiment and experiment 1 represent control and elevated chambers at $RCO_2 = 1\times$ and $2\times$, respectively. Experiment 2 represents control and elevated chambers at $RCO_2 = 1\times$ and $3\times$, respectively. Data from the lower shelf of the control chamber are represented by plus signs, while data from the lower shelf of the elevated pCO_2 chamber are represented by crosses. Similarly, data from the higher shelf of the control chamber are represented by blue circles, while data from the higher shelf of the elevated pCO_2 chamber are represented by red circles. The black bar represents plus/minus the average standard deviation in relative humidity readings calculated across all experiments.

atmospheric mixing ratios in the geologic past. This suggestion is an important basis for the hypothesis that the relationship between plant and atmospheric carbon isotope composition should be nonlinear over a wide range of atmospheric compositions. Our data demonstrate that under conditions of adequate water and nutrients (the only conditions under which we advocate the use of $\delta^{13}C_p$ to reconstruct $\delta^{13}C_a$), c_i/c_a does not covary systematically with changing O_2:CO_2 mixing ratio. Thus the ecological set point was maintained even under conditions where only acclimation, not adaptation, to changing atmospheric composition was possible.

4. DISCUSSION

[19] Elevated atmospheric pCO_2 and its potential and actual effects on photosynthesis has been the subject of no less than 15 years of study and controversy [e.g. *Bazzaz and Garbutt*, 1988; *Schlesinger et al.*, 2006]. An increase of 30% in modern atmospheric CO_2 over preindustrial levels due to human activities has been shown [e.g. *Keeling et al.*, 1984]. Within a geological context, pCO_2 levels may have been as high as $RCO_2 = 10\times$ [*Berner and Kothavala*, 2001] since land plants evolved (~490 Ma). The first comprehensive studies of the effect of CO_2 on plant growth hypothesized that increased pCO_2 should bolster the entire process of plant carbon acquisition, resulting in faster plant growth and greater total biomass [e.g. *Bazzaz*, 1996]. However, many studies have highlighted individualistic responses of plants to elevated atmospheric CO_2 levels [e.g., *Bazzaz and Miao*, 1993; *Bazzaz and Williams*, 1991; *Herrick and Thomas*, 2001; *Körner*, 2000, 2004; *Pataki et al.*, 1998] and rapid acclimation of the photosynthetic mechanism to elevated CO_2 [e.g. *Ainsworth and Long*, 2005; *Isebrands et al.*, 2000; *Vu*, 2005]. These and studies like them call into question any simple model that predicts increased terrestrial productivity from elevated CO_2. Nevertheless, large-scale environmental experiments have generally revealed enhanced production of photosynthetic biomass upon exposure to elevated pCO_2 levels [*Ellsworth et al.*, 2004; *Rogers and Ellsworth*, 2002]. For example, crop plants grown at $RCO_2 = 2\times$ have yielded an average biomass increase of 30% across a variety of environmental conditions [*Mauney et al.*, 1994; *Pinter et al.*, 1996; *Reilly*, 1996]. However, responses in natural systems are likely to be considerably more complex [*Karnosky*, 2003]. For these reasons most discussions of past or future scenarios of $RCO_2 > 1\times$ include a vision of enhanced terrestrial primary productivity.

[20] The "CO_2 fertilization" we documented in *Raphanus sativus* L. is consistent with general descriptions of photosynthetic biomass response to elevated pCO_2 [e.g. *Ward and Strain*, 1999]. Our results are commensurate with a small-scale study performed to specifically compare the effect of elevated pCO_2 on the growth of *Raphanus sativus* L. versus *Daucus carota* L. [*Idso and Kimball*, 1989]. *Idso and Kimball* [1989] documented "significant" though variable increase in dry matter production of *Raphanus sativus* L. under $RCO_2 = \sim 2\times$ when compared to plants grown under $RCO_2 = 1\times$; they also noted that this increase was enhanced when plants grown under elevated temperature (~25°C) were compared. Interestingly, the greatest net biomass increases in our experiment were observed at $RCO_2 = 2\times$, with further increases in pCO_2 yielding biomass increase over control but at levels lower than that of the $RCO_2 = 2\times$ treatment. We also noted lower average relative humidity in the $RCO_2 = 3\times$ treatment. Together, these observations suggest reduced stomatal conductance at $RCO_2 = 3\times$ as part of the plants' short-term acclimation to elevated pCO_2 [*Knapp et al.*, 1996; *Isebrands et al.*, 2000; *Vu*, 2005]. However, the above is speculative: We did not perform the physiological measurements needed to confirm this hypothesis.

[21] One of the strengths of this study, with respect to the relationship between the isotopic composition of atmospheric CO_2 and plant tissue derived from it, is that $\delta^{13}C_a$ was measured directly from within the chambers (Table 2, see section 2 for details); plant growth studies have included determination of $\delta^{13}C_a$ [e.g., *Sternberg and DeAngelis*, 2002] but have not included systematic determinations of resultant tissue $\delta^{13}C_p$. In addition, $\delta^{13}C_a$ variability was

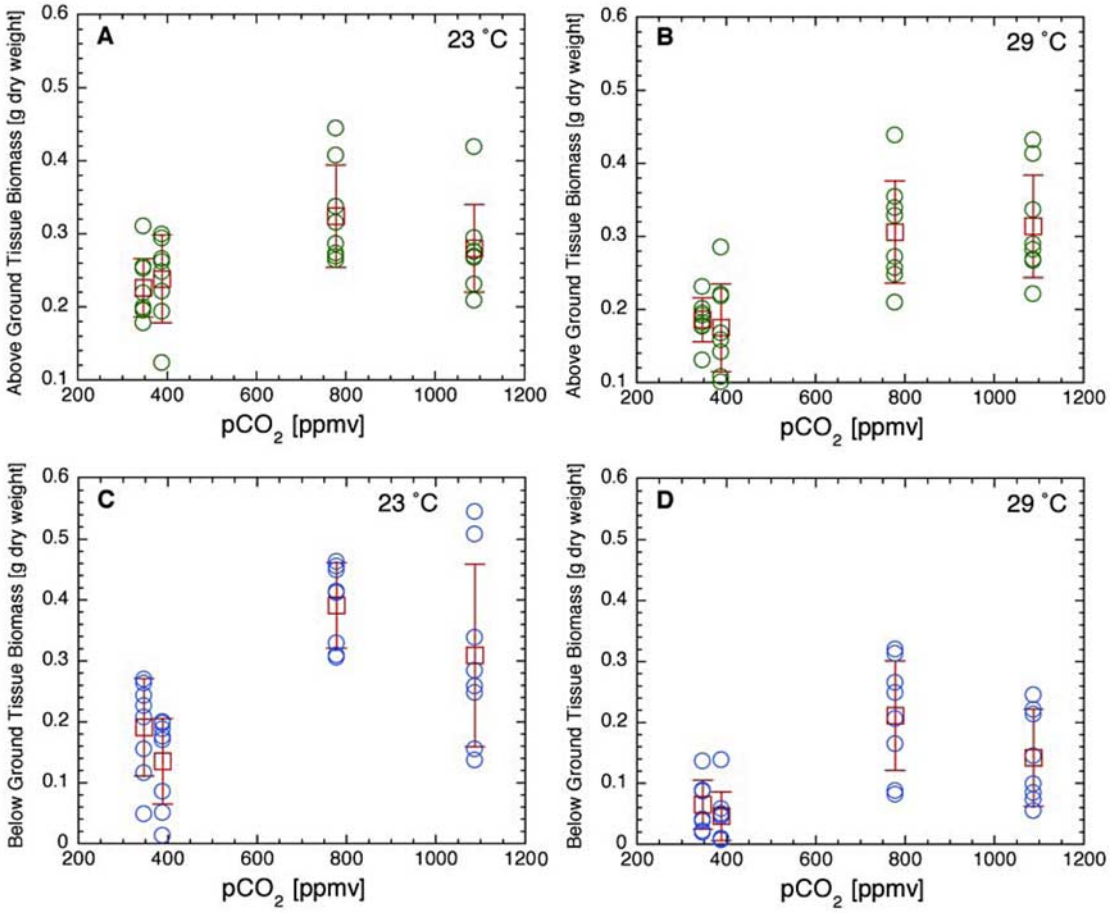

Figure 5. Biomass increases resulting from growth under elevated pCO_2. Significant increases were seen between the mean values (squares) of individual plants (circles) (bars represent the standard deviation of individual measurements) both for (a and b) aboveground and (c and d) belowground tissues grown at all temperatures.

TABLE 3. Summary of the Differences in Biomass Between the Control and Elevated Chambers, Separated According to Upper and Lower Growth Shelf, Resulting From the $RCO_2 = 2\times$ and $RCO_2 = 3\times$ Experiments[a]

	Upper Growth Shelf		
	pCO_2 = 389 ppmv Biomass, g	pCO_2 = 778 ppmv Biomass, g	$RCO_2 = 2\times$ Δ Biomass, %
	Aboveground Biomass		
~22°C	0.24 ± 0.06 (8)[b]	0.32 ± 0.07 (8)	36 ± 55
~29°C	0.18 ± 0.06 (8)[b]	0.31 ± 0.07 (8)	75 ± 74
	Belowground Biomass		
~22°C	0.14 ± 0.07 (8)	0.39 ± 0.07 (8)[b]	190 ± 104
~29°C	0.05 ± 0.04 (8)	0.21 ± 0.09 (8)[b]	359 ± 283
	Lower Growth Shelf		
	pCO_2 = 347 ppmv Biomass, g	pCO_2 = 1088 ppmv Biomass, g	$RCO_2 = 3\times$ Δ Biomass, %
	Aboveground Biomass		
~22°C	0.23 ± 0.04 (8)	0.28 ± 0.06 (8)	24 ± 44
~29°C	0.17 ± 0.03 (8)	0.31 ± 0.07 (8)	69 ± 54
	Belowground Biomass		
~22°C	0.19 ± 0.08 (8)	0.31 ± 0.15 (8)[b]	62± 120
~29°C	0.07 ± 0.04 (8)	0.14 ± 0.08 (8)[b]	118 ± 185

[a]Average biomass values are presented with the standard deviation ($\pm\sigma$) seen in the number of plants (n); change in biomass values are presented plus/minus the uncertainty associated with the propagation of σ; these data are presented graphically in Figure 5.
[b]This value represents a statistically significant difference ($p < 0.02$).

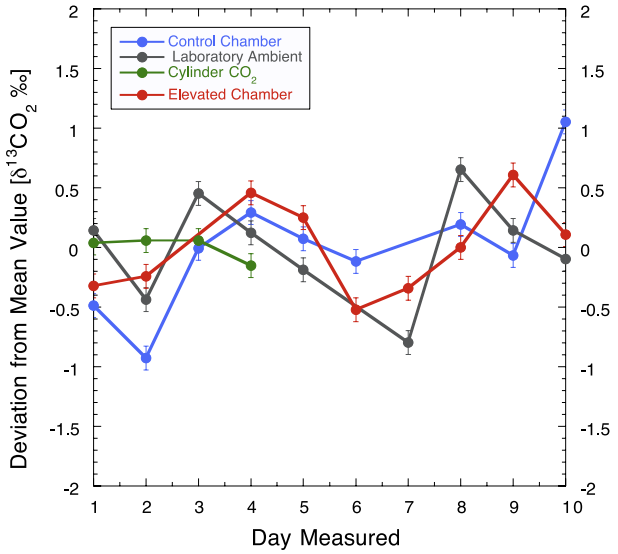

Figure 6. Stability of $\delta^{13}C$ value in the CO_2 of various sources over the course of 10 d of measurements. The data are presented as deviation from the 10-d mean (mean value set at zero).

quantified across 10 d of consecutive measurements during the experimental period (Table 1 and Figure 6). This approach straightforwardly quantifies the carbon isotopic composition of the actual "raw material" from which plants synthesize their tissue. Our method contrasts with many previous studies that estimate $\delta^{13}C_a$ value based on the isotopic composition of the pressurized CO_2 tank used for enrichment [e.g., *Guy and Reid*, 1986] or assume $\delta^{13}C_a$ based on plant tissue composition [e.g., *Beerling and Woodward*, 1995; *Polley et al.*, 1993]. Our work involving previously published values of 176 species [*Arens et al.*, 2000] indicated an average isotopic depletion ≈19‰ in plant tissue relative to the atmosphere under which it formed. We attribute the difference between the isotopic depletion seen in our 2000 study and that recorded here to reflect the individual response of *Raphanus sativus* L. (a species which did not appear in the 2000 study) compared to that of a diverse mix of C3 species used in our 2000 meta-analysis. It has been claimed that single- or few-species isotopic depletion is too variable and represents too poor a correlation to support the idea that $\delta^{13}C_a$ can be effectively predicted from $\delta^{13}C_p$ [*Beerling and Royer*, 2002; *Royer et al.*, 2001a, 2001b]. We have made the same assertion with respect to applying such results to the geologic record [*Arens and Jahren*, 2000; *Arens et al.*, 2000], and the results here seem to bear out that conclusion.

[22] Our experiments further demonstrate an excellent correlation in $\delta^{13}C_a$ and $\delta^{13}C_p$ that suggests a high level of predictive power across a range of pCO_2 levels. As explicitly claimed by *Arens et al.* [2000], prediction of $\delta^{13}C_a$ from $\delta^{13}C_p$ is only legitimately performed upon a substrate that effectively mixes the biomass contributions of many individuals of diverse species, ideally grown under stress-free environmental conditions. *Arens et al.* [2000] asserted that although the $\delta^{13}C_p$ value of individual species would parallel that of changing $\delta^{13}C_a$, individual physiological response would emerge when individual plants of a single species were analyzed. *Raphanus sativus* L. conforms to the general model described by *Arens et al.* [2000], indicating that the relationship is valid and linear across large ranges of pCO_2 [cf. *Gröcke*, 2002]. The lack of dependence of net isotopic depletion on pCO_2 level is worth mention (Figure 9). We demonstrated a similar lack of correlation in meta-analysis of the large data set of *Arens et al.* [2000], and the absence of correlation between fossil plant $\delta^{13}C$ and paleo-pCO_2 has been reported by many authors [e.g., *Bocherens et al.*, 1993; *Ehleringer and Cerling*, 1995; *Raven and Sprent*, 1989; *Tu et al.*, 2004].

5. CONCLUSIONS

[23] Reconstruction of paleo-$\delta^{13}CO_2$ using $\delta^{13}C$ measurements of plant tissues has been a central part of many carbon cycling and paleoclimate reconstructions [e.g., *Arens and Jahren*, 2000, 2002; *Hasegawa et al.*, 2003; *Jahren*, 2002; *Jahren et al.*, 2001, 2005; *Strauss and Peters-Kottig*, 2003]. Perhaps more widely used is the practice of inferring soil $\delta^{13}CO_2$ from soil organic matter $\delta^{13}C$ in order to ultimately solve for atmospheric pCO_2 level, temperature, or other fundamental climate attribute [e.g., *Cerling*, 1991, 1992; *Ekart et al.*, 1999; *Jahren et al.*, 2004a; *Krull and Retallack*, 2000; *Ludvigson et al.*, 1998; *Mora et al.*, 1991, 1996; *Nordt et al.*, 2002; *Tabor et al.*, 2004; *Yapp*, 2004;

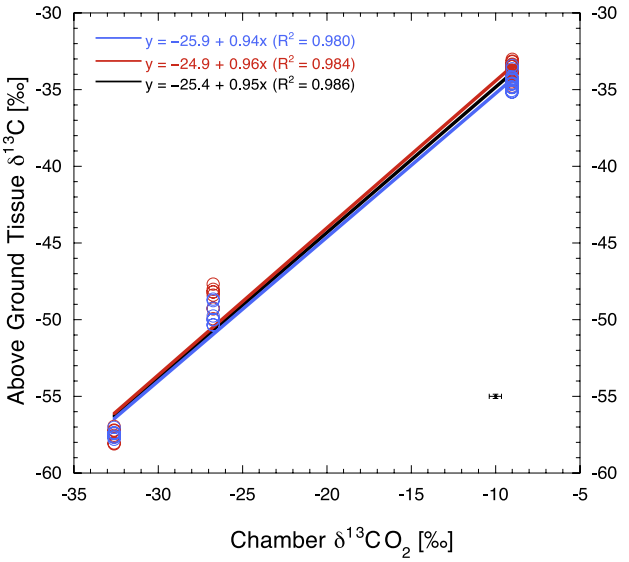

Figure 7. High correlation observed between aboveground tissue $\delta^{13}C$ and growth chamber $\delta^{13}CO_2$ at which the plants grew. R^2 values in all cases are ≥0.98; the linear regression for tissues grown at 23°C (blue circles and line) and those grown at 29°C (red circles and line) are compared to the regression seen for the entire aboveground data set (black line). The black bar represents plus/minus the average standard deviation in measured chamber $\delta^{13}CO_2$ values and the maximum standard deviation seen in $\delta^{13}C$ measured across all tissues.

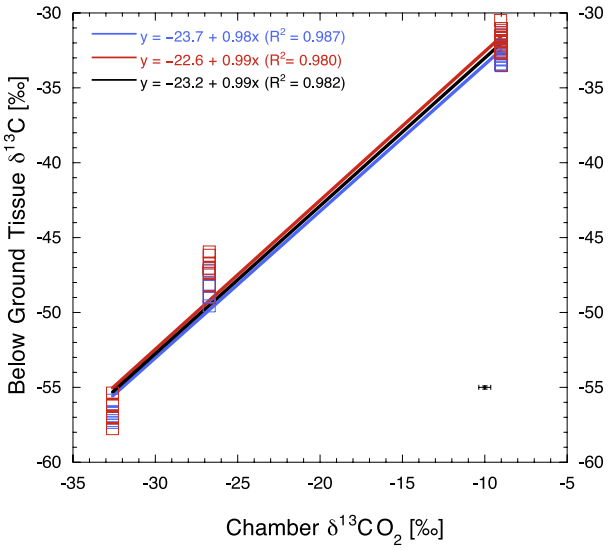

Figure 8. High correlation observed between belowground tissue $\delta^{13}C$ and growth chamber $\delta^{13}CO_2$ at which the plants grew. R^2 values in all cases are $\geq$0.98; the linear regression for tissues grown at 23°C (blue squares and line) and those grown at 29°C (red squares and line) are compared to the regression seen for the entire belowground data set (black line). The black bar represents plus/minus the average standard deviation in measured chamber $\delta^{13}CO_2$ values and the maximum standard deviation seen in $\delta^{13}C$ measured across all tissues.

Yapp and Poths, 1996]. The current study further validates our initial conclusion that in the absence of environmental stress, plant $\delta^{13}C$ primarily reflects atmospheric $\delta^{13}CO_2$ linearly across pCO_2 levels.

[24] It is worth discussing several caveats, both old and new, that arise during the application of our measured isotopic offset to $\delta^{13}C_p$ in order to solve for $\delta^{13}C_a$. First, the results presented here, while reinforcing the idea that such an offset is real and constant in *Raphanus sativus* L., highlight the importance of including many and mixed plant species into the isotopic substrate. The isotopic offset we document for *Raphanus sativus* L. differs from that seen for a mixed-species group [*Arens et al.*, 2000] by no less than 4‰. Similar offsets can be observed in other single-species trials [e.g., *Beerling and Woodward*, 1995; *Gröcke*, 2002; *Guy et al.*, 1989; *Holtum et al.*, 1983]. *Arens et al.* [2000] further reported that single-species variation is systematic, with conifers displaying slightly lower discrimination. We contend that a substrate comprising the contribution of multiple species and many individual plants is available within a few cubic centimeters of many terrestrial rocks and comprises the optimal substrate for $\delta^{13}C_a$ reconstruction. In order to explore substrates that reflect little or no diagenesis, researchers have also explored the isolation and analysis of many types of specific structures and compounds exclusively associated with terrestrial land plants, including wood [*Gröcke et al.*, 1999; *van Bergen and Poole*, 2002], cuticle [*Arens and Jahren*, 2000, 2002; *Jahren*, 2002; *Jahren et al.*, 2001, 2005; *van Bergen et al.*, 1995], pollen [*Jahren*, 2004; *Amundson et al.*, 1997; *Loader and Hemming*, 2000, 2001], *rbcL* gene DNA [*Jahren et al.*, 2004b, 2006], and general "biomarkers" [*Briggs et al.*, 2000; *Pancost and Boot*, 2004]. In each case, discrimination imparted during synthesis of specific plant components must be considered. As with any method of paleoreconstruction, diagenetic alteration must be considered, assessed, and ideally minimized during the selection of isotopic substrate. To facilitate this, the literature contains extensive exploration of the persistence and potential diagenetic alteration of plant tissues and compounds [e.g., *Briggs et al.*, 2000; *Poole et al.*, 2002, 2004; *Tu et al.*, 2004].

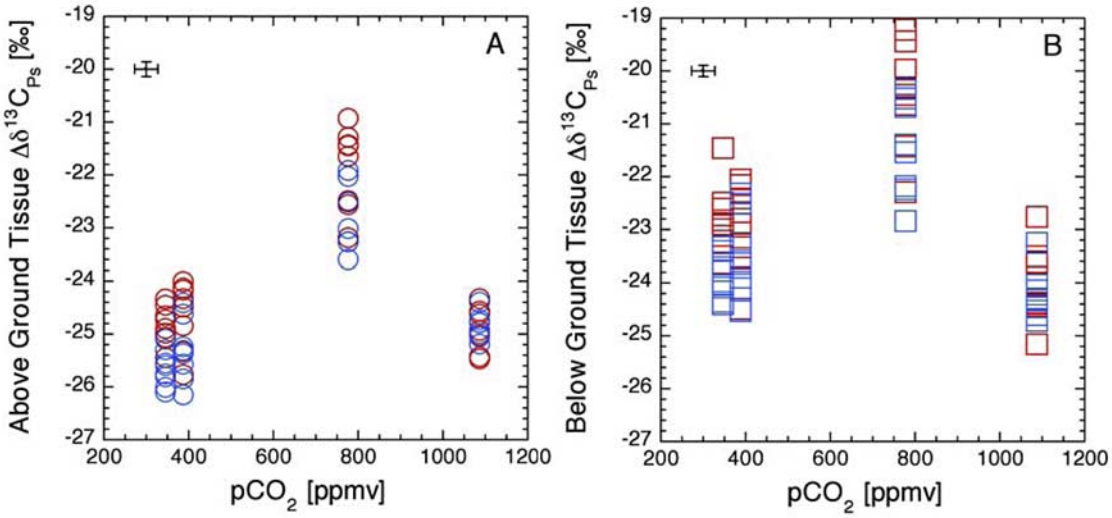

Figure 9. No correlation observed between $\Delta\delta^{13}C_{Ps}$ and the pCO_2 level at which the plants grew either for (a) aboveground or (b) belowground tissue. R^2 values in all cases are $\leq$0.1 with no systematic difference between tissues grown at 23°C (blue symbols) and those grown at 29°C (red symbols). The black bar represents plus/minus the average standard deviation in pCO_2 readings and the maximum standard deviation seen in $\delta^{13}C$ measured across all tissues.

[25] Recent studies have highlighted additional and subtle complications in the measurement of $\delta^{13}C_p$ needed to reconstruct $\delta^{13}C_a$. For example, the fluctuation of pO_2 through geologic time [*Berner*, 2001] may have affected plant metabolic processes [*Beerling et al.*, 2002]. Incubations of isolated plant mitochondria indicate that rates of photorespiration may also affect overall carbon isotope fractionation during photosynthesis more than previously assumed [*Igamberdiev et al.*, 2001]. Both of these issues could be approached using a controlled growth chamber with variable gaseous inputs, such as the experiments described here. The particularity of these results to the single species *Raphanus sativus* L. is counterbalanced by the large ranges of tightly controlled pCO_2 levels and $\delta^{13}CO_2$ values afforded by the experimental design. Additional experiments at several more values of $\delta^{13}C_a$ would strengthen the conclusions presented here and allow for an exhaustive assessment of nonlinearity. Our study illustrates the utility of controlled growth experiments in the investigation of the consistent offset hypothesized between $\delta^{13}C_p$ and $\delta^{13}C_a$ and its application to paleoenvironmental reconstructions.

[26] **ACKNOWLEDGMENTS.** This work benefited from the laboratory expertise of W. M. Hagopian; it was supported by the Howard Hughes Foundation, by NSF/DEB-0107520 and NSF/EAR-0106171, and by DOE-BES:DE-FG02-06ER15831.

[27] The Editor responsible for this paper was Michael Manga. He thanks technical reviewers Isabel Montanez and Gregory J. Retallack and one anonymous cross-disciplinary reviewer.

REFERENCES

Ainsworth, E.-A., and S.-P. Long (2005), What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2, *New Phytol.*, *165*(2), 351–371.

Amundson, R. G., R. R. Evett, A. H. Jahren, and J. Bartolome (1997), Stable carbon isotope composition of Poaceae pollen and its potential in paleovegetational reconstructions, *Rev. Palaeobot. Palynol.*, *99*(1), 17–24.

Arens, N. C., and A. H. Jahren (2000), Carbon isotope excursion in atmospheric CO_2 at the Cretaceous-Tertiary boundary: Evidence from terrestrial sediments, *Palaios*, *15*, 314–322.

Arens, N. C., and A. H. Jahren (2002), Chemostratigraphic correlation of four fossil-bearing sections in southwestern North Dakota, in *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Record of the End of the Cretaceous*, *Spec. Pap. Geol. Soc. Am.*, *361*, 75–93.

Arens, N. C., A. H. Jahren, and R. Amundson (2000), Can C3 plants faithfully record the carbon isotopic composition of atmospheric carbon dioxide?, *Paleobiology*, *26*, 137–164.

Bazzaz, F. A. (1996), *Plants in Changing Environments*, 320 pp., Cambridge Univ. Press, Cambridge, U. K.

Bazzaz, F. A., and K. Garbutt (1988), The responses of annuals in competitive neighbourhoods: Effects of elevated CO_2, *Ecology*, *69*, 937–946.

Bazzaz, F. A., and S. L. Miao (1993), Successional status, seed size, and responses of tree saplings to CO_2, light, and nutrients, *Ecology*, *74*, 104–112.

Bazzaz, F. A., and W. E. Williams (1991), Atmospheric CO_2 concentrations within a mixed forest: Implications for seedling growth, *Ecology*, *72*, 12–16.

Beerling, D. J., and D. L. Royer (2002), Fossil plants as indicators of the Phanerozoic global carbon cycle, *Annu. Rev. Earth Planet. Sci.*, *30*, 527–556.

Beerling, D. J., and F. I. Woodward (1995), Leaf stable carbon isotope composition records increased water-use efficiency of C_3 plants in response to atmospheric CO_2 enrichment, *Funct. Ecol.*, *9*, 394–401.

Beerling, D. J., J. A. Lake, R. A. Berner, L. J. Hickey, D. W. Taylor, and D. L. Royer (2002), Carbon isotope evidence implying high O_2/CO_2 ratios in the Permo-Carboniferous atmosphere, *Geochim. Cosmochim. Acta*, *66*, 3757–3767.

Berner, R. A. (1993), Weathering and its effect on atmospheric CO_2 over Phanerozoic time, *Chem. Geol.*, *107*, 373–374.

Berner, R. A. (1994), GEOCARB II: A revised model of atmospheric CO_2 over Phanerozoic time, *Am. J. Sci.*, *294*, 56–91.

Berner, R. A. (1998), The carbon cycle and CO_2 over Phanerozoic time: The role of land plants, *Philos. Trans. R. Soc. London, Ser. B*, *353*, 75–82.

Berner, R. A. (2001), Modeling atmospheric O_2 over Phanerozoic time, *Geochim. Cosmochim. Acta*, *65*, 685–694.

Berner, R. A., and Z. Kothavala (2001), GEOCARB III: A revised model of atmospheric CO_2 over Phanerozoic time, *Am. J. Sci.*, *301*, 182–204.

Bocherens, H., E. M. Friis, A. Mariotti, and K. R. Pedersen (1993), Carbon isotopic abundances in Mesozoic and Cenozoic fossil plants: Palaeoecological implications, *Lethaia*, *26*, 347–358.

Bowen, G. J., and D. J. Beerling (2004), An integrated model for soil organic carbon and CO_2: Implications for paleosol carbonate pCO_2 paleobarometry, *Global Biogeochem. Cycles*, 18, GB1026, doi:10.1029/2003GB002117.

Bralower, T. J., M. A. Arthur, R. M. Leckie, W. V. Sliter, D. J. Allard, and S. O. Shlanger (1994), Timing and paleoceanography of oceanic dysoxia/anoxia in the Late Barremian to Early Aptian (Early Cretaceous), *Palaios*, *9*, 335–369.

Briggs, D. E. G., R. P. Evershed, and M. J. Lockheart (2000), The biomolecular paleontology of continental fossils, *Paleobiology*, *26*(4), suppl., 169–193.

Cerling, T. E. (1991), Carbon dioxide in the atmosphere; evidence from Cenozoic and Mesozoic paleosols, *Am. J. Sci.*, *291*, 377–400.

Cerling, T. E. (1992), Use of carbon isotopes in paleosols as an indicator of the $P(CO_2)$ of the paleoatmosphere, *Global Biogeochem. Cycles*, *6*(3), 307–314.

Cerling, T. E., and R. L. Hay (1986), An isotopic study of paleosol carbonates from Olduvai Gorge, *Quat. Res.*, *25*(1), 63–78.

Chaloner, W. G., and J. McElwain (1997), The fossil plant record and global climatic change, *Rev. Palaeobot. Palynol.*, *95*(1–4), 73–82.

Christeller, J. T., W. A. Lang, and J. H. Troughton (1976), Isotope discrimination by ribulose 1, 5-diphosphate carboxylase, *Plant Physiol.*, *57*, 580–582.

Craig, H. (1953), The geochemistry of the stable carbon isotopes, *Geochim. Cosmochim. Acta*, *3*, 53–92.

Craig, H. (1954), Carbon-13 in plants and the relationship between carbon-13 and carbon-14 variations in nature, *J. Geol.*, *62*, 115–149.

Dawson, T. E., S. Mambelli, A. H. Plamboeck, P. H. Templer, and K. P. Tu (2002), Stable isotopes in plant ecology, *Annu. Rev. Ecol. Syst.*, *33*, 507–559.

D'Hondt, S., P. Donaghay, J. C. Zachos, D. Luttenberg, and M. Lindinger (1998), Organic carbon fluxes and ecological recovery from the Cretaceous-Tertiary mass extinction, *Science*, *282*, 276–279.

Dickens, G. R., M. M. Castillo, and J. C. G. Walker (1997), A blast of gas in the latest Paleocene: Simulating first-order effects of massive dissociation of oceanic methane hydrate, *Geology*, *25*(3), 259–262.

Ehleringer, J. R., and T. E. Cerling (1995), Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 concentrations in plants, *Tree Physiol.*, *15*, 105–111.

Ekart, D. D., T. E. Cerling, I. P. Montañez, and N. J. Tabor (1999), A 400 million year carbon isotope record of pedogenic carbonate: Implications for paleoatmospheric carbon dioxide, *Am. J. Sci.*, *299*, 805–827.

Ellsworth, D., P. Reich, E. Naumburg, G. Koch, M. Kubiske, and S. Smith (2004), Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert, *Global Change Biol.*, *10*, 2121–2138.

Farquhar, G. D. (1979), Models describing the kinetics of ribulose biphosphate carboxylase-oxygenase, *Arch. Biochem. Biophys.*, *193*, 456–468.

Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick (1989), Carbon isotope discrimination and photosynthesis, *Annu. Rev. Plant. Physiol. Plant Mol. Biol.*, *40*, 503–537.

Greenwood, D. R., M. J. Scarr, and D. C. Christophel (2003), Leaf stomatal frequency in the Australian tropical rainforest tree *Neolitsea dealbata* (Lauraceae) as a proxy measure of atmospheric pCO_2, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *196*, 375–393.

Gröcke, D. R. (2002), The carbon isotope composition of ancient CO_2 based on higher-plant organic matter, *Philos. Trans. R. Soc. London, Ser. A*, *360*, 633–658.

Gröcke, D. R., S. P. Hesselbo, and H. C. Jenkyns (1999), Carbon-isotope composition of Lower Cretaceous fossil wood: Ocean-atmosphere chemistry and relation to sea-level change, *Geology*, *27*(2), 155–158.

Guy, R. D., and D. M. Reid (1986), Photosynthesis and the influence of CO_2-enrichment on $\delta^{13}C$ values in a C_3 halophyte, *Plant Cell Environ.*, *9*, 65–72.

Guy, R. D., D. M. Reid, and H. R. Krouse (1980), Shifts in carbon isotope ratios of two C3 halophytes under natural and artificial conditions, *Oecologia*, *44*, 241–247.

Guy, R. D., P. G. Warne, and D. M. Reid (1989), Stable carbon isotope ratio and an index of water-use efficiency of C_3 halophytes—Possible relationship to strategies of osmotic adjustment, in *Stable Isotopes in Ecological Research*, vol. 68, edited by P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, pp. 55–75, Springer, New York.

Halling, B. P., and G. R. Peters (1987), Influence of chloroplast development on activation of the diphenyl ether herbicide acifluorfen-methyl, *Plant Physiol.*, *84*, 1114–1120.

Hasegawa, T., L. M. Pratt, H. Maeda, Y. Shigeta, T. Okamoto, T. Kase, and K. Uemura (2003), Upper Cretaceous stable carbon isotope stratigraphy of terrestrial organic matter from Sakhalin, Russian Far East: A proxy for the isotopic composition of paleoatmospheric CO_2, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *189*, 97–115.

Hayes, J. M., B. N. Popp, R. Takigiku, and M. W. Johnson (1989), An isotopic study of biogeochemical relationships between carbonates and organic carbon in the Greenhorn Formation, *Geochim. Cosmochim. Acta*, *53*, 2961–2972.

Herrick, J. D., and R. B. Thomas (2001), No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO_2 enrichment at the Duke Forest FACE experiment, *Plant Cell Environ.*, *24*, 53–64.

Hesselbo, S. P., B. H. S. Morgans, J. C. McElwain, P. M. Rees, S. A. Robinson, and C. E. Ross (2003), Carbon-cycle perturbation in the Middle Jurassic and accompanying changes in the terrestrial paleoenvironment, *J. Geol.*, *111*(3), 259–276.

Holtum, J. A. M., M. H. O'Leary, and C. B. Osmond (1983), Effect of varying CO_2 partial pressure on photosynthesis on the carbon isotope composition of carbon-4 of malate from the Crassulean acid metabolism plant *Kalanchoë daigremontiana* Hamet et Perr, *Plant Physiol.*, *71*, 602–609.

Hungate, B. A., M. Reichstein, P. Dijkstra, D. Johnson, G. Hymus, J. D. Tenhunen, R. Hinkle, and B. G. Drakes (2002), Evapotranspiration and soil water content in a scrub-oak woodland under carbon dioxide enrichment, *Global Change Biol.*, *8*, 1–10.

Idso, S. B., and B. A. Kimball (1989), Growth response of carrot and radish to atmospheric CO_2 enrichment, *Environ. Explor. Bot.*, *29*(2), 135–139.

Igamberdiev, A. U., A. A. Ivlev, N. V. Bykova, C. N. Threlkeld, P. J. Lea, and P. Gardeström (2001), Decarboxylation of glycine contributes to carbon isotope fractionation in photosynthetic organisms, *Photosynth. Res.*, *67*, 177–184.

Isebrands, J. G., R. E. Dickson, J. Rebbeck, and D. F. Karnosky (2000), Interacting effects of multiple stresses on growth and physiological processes in northern forest trees, in *Responses of Northern U. S. Forests to Environmental Change*, edited by R. A. Mickler, R. A. Birdsey, and J. Hom, pp. 149–180, Springer, New York.

Jahren, A. H. (2002), The biogeochemical consequences of the mid-Cretaceous superplume, *J. Geodyn.*, *34*(2), 177–191.

Jahren, A. H. (2004), The carbon stable isotope composition of pollen, *Rev. Palaeobot. Palynol.*, *132*(3–4), 291–313.

Jahren, A. H., N. C. Arens, G. Sarmiento, J. Guerrero, and R. Amundson (2001), Terrestrial record of methane hydrate dissociation in the Early Cretaceous, *Geology*, *29*(2), 159–162.

Jahren, A. H., B. A. LePage, and S. P. Werts (2004a), Methanogenesis in Eocene Arctic soils inferred from $\delta^{13}C$ of tree fossil carbonates, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *214*, 347–358.

Jahren, A. H., G. Petersen, and O. Seberg (2004b), Plant DNA: A new substrate for carbon stable isotope analysis and a potential paleoenvironmental indicator, *Geology*, *32*(3), 241–244.

Jahren, A. H., C. P. Conrad, N. C. Arens, G. Mora, and C. R. Lithgow-Bertelloni (2005), A plate tectonic mechanism for methane hydrate release along subduction zones, *Earth Planet. Sci. Lett.*, *236*(3–4), 691–704.

Jahren, A. H., K. B. Kelm, B. Wendland, G. Petersen, and O. Seberg (2006), Carbon stable isotope composition of DNA isolated from an incipient paleosol, *Geology*, *34*(5), 381–384.

Karnosky, D. F. (2003), Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: Knowledge gaps, *Environ. Int.*, *29*, 161–169.

Keeling, C. D., A. Carter, and W. G. Mook (1984), Seasonal, latitudinal, and secular variations in the abundance and isotopic ratios of atmospheric CO_2: 2. Results from oceanographic cruises in the tropical Pacific Ocean, *J. Geophys. Res.*, *89*(3), 4615–4628.

Knapp, A. K., E. P. Hamerlynck, J. M. Ham, and C. E. Owensby (1996), Responses in stomatal conductance to elevated CO_2 in 12 grassland species that differ in growth form, *Plant Ecol.*, *125*(1), 31–41.

Körner, C. (2000), Biosphere responses to CO_2 enrichment, *Ecol. Appl.*, *10*, 1590–1619.

Körner, C. (2004), Through enhanced tree dynamics carbon dioxide enrichment may cause tropical forests to lose carbon, *Philos. Trans. R. Soc. London, Ser. B*, *369*, 493–498.

Körner, C., G. D. Farquhar, and C. S. Wong (1991), Carbon isotope discrimination by plants follows latitudinal and altitudinal trends, *Oecologia*, *88*, 30–40.

Krull, E. S., and G. J. Retallack (2000), $\delta^{13}C$ depth profiles from paleosols across the Permian-Triassic boundary: Evidence for methane release, *Geol. Soc. Am. Bull.*, *112*(9), 1459–1472.

Loader, N. J., and D. L. Hemming (2000), Preparation of pollen for stable carbon isotope analyses, *Chem. Geol.*, *165*, 339–344.

Loader, N. J., and D. L. Hemming (2001), Spatial variation in pollen $\delta^{13}C$ correlates with temperature and seasonal development timing, *Holocene*, *11*(5), 587–592.

Ludvigson, G. A., L. A. González, R. A. Metzger, B. J. Witzke, B. L. Brenner, A. P. Murillo, and T. S. White (1998), Meteoric sphaerosiderite lines and their use for paleohydrology and paleoclimatology, *Geology*, *26*(11), 1039–1042.

Madhavan, S., I. Treichel, and M. H. O'Leary (1991), Effects of relative humidity on carbon isotope fractionation in plants, *Bot. Acta*, *104*, 292–294.

Mauney, J. R., B. A. Kimball, P. J. Pinter, R. L. Lamorte, K. F. Lewin, J. Nagy, and G. R. Hendrey (1994), Growth and yield of cotton in response to a free-air carbon dioxide enrichment (FACE) environment, *Agric. For. Meteorol.*, *70*, 49–67.

McElwain, J. C., D. J. Beerling, and F. I. Woodward (1999), Fossil plants and global warming at the Triassic-Jurassic boundary, *Science*, *285*, 1386–1390.

Mora, C. I., S. G. Driese, and P. G. Seager (1991), Carbon dioxide in the Paleozoic atmosphere: Evidence from carbon-isotope compositions of pedogenic carbonate, *Geology*, *19*(10), 1017–1020.

Mora, C. I., S. G. Driese, and L. A. Colarusso (1996), Middle to late Paleozoic atmospheric CO_2 levels from soil carbonate and organic matter, *Science*, *271*, 1105–1107.

Nordt, L., S. Atchley, and S. I. Dworkin (2002), Paleosol barometer indicates extreme fluctuations in atmospheric CO_2 across the Cretaceous-Tertiary boundary, *Geology*, *30*(8), 703–706.

Pancost, R. D., and C. S. Boot (2004), The paleoclimatic utility of terrestrial biomarkers in marine sediments, *Mar. Chem.*, *92*, 239–261.

Pataki, D. E., R. Oren, and D. T. Tissue (1998), Elevated carbon dioxide does not affect stomatal conductance of *Pinus taeda* L., *Oecologia*, *117*, 47–52.

Pinter, P. J., B. A. Kimball, R. L. Garcia, G. W. Wall, D. J. Hunsaker, and R. L. Lamorte (1996), Free-air CO_2 enrichment: Responses of cotton and wheat crops, in *Carbon Dioxide and Terrestrial Ecosystems*, edited by G. W. Koch and H. A. Mooney, pp. 215–249, Academic, San Diego, Calif.

Polley, H. W., H. B. Johnson, B. D. Marino, and H. S. Mayeux (1993), Increase in C3 plant water-use efficiency and biomass over glacial to present CO_2 concentrations, *Nature*, *361*, 61–64.

Poole, I., F. Braadbaart, J. J. Boon, and P. F. van Bergen (2002), Stable carbon isotope changes during artificial charring of propagules, *Org. Geochem.*, *33*, 1675–1681.

Poole, I., P. F. van Bergen, J. Kool, S. Schouten, and D. J. Cantrill (2004), Molecular isotopic heterogeneity of fossil organic matter: Implications for $\delta^{13}C_{biomass}$ and $\delta^{13}C_{palaeoatmosphere}$ proxies, *Org. Geochem.*, *35*, 1261–1274.

Rask, H. M., and J. J. Schoenau (1993), ^{13}C natural abundance variations in carbonates and organic carbon from boreal forest wetlands, *Biogeochemistry*, *22*, 23–35.

Raven, J. A., and J. J. Sprent (1989), Phototrophy, diazotrophy and palaeoatmospheres: Biological catalysts and H, C, N and O biological cycles, *J. Geol. Soc. London*, *146*, 161–170.

Reilly, J. (1996), Agriculture in a changing climate: Impacts and adaptation, in *Climate Change 1995: Impacts, Adaptations, and Mitigation of Climate Change*, edited by R. T. Watson, M. C. Zinyowera, and R. H. Moss, pp. 429–467, Cambridge Univ. Press, Cambridge, U. K.

Retallack, G. (2001), A 300-million year old record of atmospheric carbon dioxide from fossil plant cuticles, *Nature*, *411*, 287–290.

Rogers, A., and D. Ellsworth (2002), Photosynthetic acclimation of *Pinus taeda* (loblolly pine to long-term growth in elevated pCO_2 (FACE), *Plant Cell Environ.*, *25*, 851–858.

Royer, D. L., R. A. Berner, and D. J. Beerling (2001a), Phanerozoic atmospheric CO_2 change: Evaluating geochemical and paleobiological approaches, *Earth Sci. Rev.*, *54*(4), 349–392.

Royer, D. L., S. L. Wing, D. J. Beerling, D. W. Jolley, P. L. Koch, L. J. Hickey, and R. A. Berner (2001b), Paleobotanical evidence for near present-day levels of atmospheric CO_2 during part of the Tertiary, *Science*, *292*, 2310–2313.

Schlesinger, W. H., et al. (2006), The Duke Forest FACE experiment: CO_2 enrichment of a loblolly pine forest, in *Managed Ecosystems and CO_2 Case Studies, Processes and Perspectives*, vol. 187, edited by J. Nösberger et al., Springer, Berlin.

Schmidt, H. L., F. J. Winkler, E. Latsko, and E. Wirth (1978), ^{13}C-kinetic isotope effects in photosynthetic carboxylation reactions and $\delta^{13}C$ values of plant material, *Isr. J. Chem.*, *17*, 223–224.

Soil Survey Staff of the Soil Conservation Service (1999), *Keys to Soil Taxonomy*, Pocahontas, Inc., Blacksburg, Va.

Sternberg, L. S. L., and D. L. DeAngelis (2002), The carbon isotope composition of ambient CO_2 and recycling: A matrix simulation model, *Ecol. Model.*, *154*, 179–192.

Strauss, H., and W. Peters-Kottig (2003), The Paleozoic to Mesozoic carbon cycle revisited: The carbon isotopic composition of terrestrial organic matter, *Geochem. Geophys. Geosyst.*, *4*(10), 1083, doi:10.1029/2003GC000555.

Sundquist, E. T. (1985), Geological perspective on CO_2 and the C cycle, in *The Carbon Cycle and Atmospheric CO_2: Natural Variations Archaean to Present*, *Geophys. Monogr. Ser.*, vol. 32, edited by E. T. Sundquist and W. S. Broecker, pp. 5–59, AGU, Washington, D. C.

Tabor, N. J., C. J. Yapp, and I. P. Montañez (2004), Goethite, calcite, and organic matter from Permian and Triassic soils: Carbon isotopes and CO_2 concentrations, *Geochim. Cosmochim. Acta*, *68*, 1503–1517.

Toft, N. L., J. E. Anderson, and R. S. Nowak (1989), Water use efficiency and carbon isotope composition of plants in a cold desert environment, *Oecologia*, *80*, 11–18.

Tu, T. T. N., W. M. Kürschner, S. Schouten, and P. F. Van Bergen (2004), Leaf carbon isotope composition of fossil and extant oaks grown under differing atmospheric CO_2 levels, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *212*, 199–213.

van Bergen, P. F., and I. Poole (2002), Stable carbon isotopes of wood: A clue to palaeoclimate?, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *182*, 31–45.

van Bergen, P. F., M. E. Collinson, D. E. G. Briggs, J. W. de Leeuw, A. C. Scott, R. P. Evershed, and P. Finch (1995), Resistant biomacromolecules in the fossil record, *Acta Bot. Neerl.*, *44*, 319–342.

Vu, J.-C.-V. (2005), Acclimation of peanut (*Arachis hypogaea* L.) leaf photosynthesis to elevated growth CO_2 and temperature, *Environ. Exp. Bot.*, *53*(1), 85–95.

Ward, J. K., and B. R. Strain (1999), Elevated CO_2 studies: Past, present and future, *Tree Physiol.*, *19*, 211–220.

Weissert, H. (1989), C-isotope stratigraphy, a monitor of paleoenvironmental change: A case study from the Early Cretaceous, *Surv. Geophys.*, *10*, 1–61.

Wong, W. W., C. R. Benedict, and R. J. Kohel (1979), Enzymic fractionation of the stable carbon isotopes of carbon dioxide by ribulose 1, 5-diphosphate carboxylase, *Plant Physiol.*, *63*, 852–856.

Yapp, C. J. (2004), Fe (CO_3)OH in goethite from a mid-latitude North American Oxisol: Estimate of atmospheric CO_2 concentration in the Early Eocene "climatic optimum," *Geochim. Cosmochim. Acta*, *68*, 935–947.

Yapp, C. J., and H. Poths (1996), Carbon isotopes in continental weathering environments and variations in ancient atmospheric CO_2 pressure, *Earth Planet. Sci. Lett.*, *137*(1–4), 71–82.

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