Supplemental File 5 – Method for estimation of $\Delta^{13}C$ from fossil wood $\delta^{13}C$ values

Overview

Photosynthetic isotope discrimination ($\Delta^{13}C$) is usually presented in the following simplified form (after Farquhar et al., 1989):

$$\Delta^{13}C = a + (b - a)\frac{C_i}{C_a} \quad (1)$$

where a is the kinetic isotope fractionation from diffusion of CO_2 in air (+4.4‰), b is the isotope fractionation due to the enzymatic fixation of CO_2 by Rubisco (we choose +27.5‰ after Lloyd and Farquhar (1994)), and C_i/C_a is the ratio of intercellular CO_2 to atmospheric CO_2. The term $\Delta^{13}C$ is simply the difference between the $\delta^{13}C$ value of atmospheric CO_2 and the $\delta^{13}C$ value of plant tissue ($\delta^{13}C_a - \delta^{13}C_p$). Furthermore, the term b actually reflects the enzymatic fixation of CO_2 by Rubisco and PEP-c and in the expanded form has the following relationship:

$$b = b_3 - \beta(b_3 - b_4) \quad (2)$$

where b_3 is the fractionation due solely to Rubisco, β is the ratio of PEP-c to Rubisco carboxylation, and b_4 is the isotope fractionation due to PEP-c and includes equilibrium isotope fractionations due to hydration of CO_2 (Mook et al., 1974) and is thus temperature dependent.

Application to fossils

In order to estimate $\Delta^{13}C$ values for our isotopic data from fossil wood we need to reconstruct reasonable estimates of the C_i/C_a ratio and the $\delta^{13}C$ value of atmospheric CO_2 during the Late Permian.

We selected C_i/C_a ratios based on the relationship of C_i/C_a for leaf-to-air vapor mole fraction difference (which directly impacts stomatal response) for conifers (i.e., gymnospermous wood that has similar characteristics to the studied fossils than wood of angiosperms) growing in a cool/cold climate (Lloyd and Farquhar, 1994). We selected 4 values of C_i/C_a to use in our calculations: 1) a mean C_i/C_a value of 0.66, 2) a moderate leaf-to-air vapor mole fraction C_i/C_a mean of 0.62 that we think is representative of spring-like conditions, 3) a low leaf-to-air vapor mole fraction C_i/C_a mean of 0.81, which we think is representative of peak summer conditions, and 4) a high leaf-to-air vapor mole fraction C_i/C_a mean value of 0.51 that we think is representative of fall-like conditions.

We estimated the $\delta^{13}C$ value of Late Permian atmospheric CO_2 using two methods: 1) the approach of Arens et al. (2000) where $\delta^{13}C_a = \frac{\delta^{13}C_p + 18.67}{1.1}$ (3), and 2) equilibrium carbon isotope fractionation between atmospheric CO_2 and carbonate in the surface ocean. For the second approach we use the fractionation effect of 8‰ after Ekart et al. (1999), which they inferred by comparing $\delta^{13}C$ values of CO_2 trapped in glacial ice to $\delta^{13}C$ values of surface ocean carbonate and arguing that the ocean-atmosphere is in [isotopic] equilibrium with respect to carbon in CO_2 and has maintained this condition on geologic timescales. The approach of Arens et al. (2000) applied to our carbon isotope data from fossil wood yield $\delta^{13}C_a$ values that range from -3.6‰ for Mt. Achernar, -3.9‰

for Wahl Glacier, and -5.9‰ for Graphite Peak, and these values were calculated from the mean $\delta^{13}C$ values of wood at each locality. When we correct for a 1‰ ^{13}C-enrichment during charcoalification of wood (Deines, 1980; Jones and Chaloner, 1991; Gröcke, 2002) these values are -4.5‰, -4.8‰, and -6.8‰, respectively. The equilibrium isotope effect between atmospheric CO_2 and carbonate of the surface ocean is estimated at 8‰, surface ocean carbonate during the Late Permian was variable, but a mean value of +3‰ reported in Ekart et al. (1999) yields a $\delta^{13}C_a$ value of -5‰. The Late Permian $\delta^{13}C_a$ estimates are in agreement only after an isotope enrichment of the fossil wood is taken into account, and only for the Mt. Achernar and Wahl Glacier localities. Moreover, the approach of Arens et al. (2000) for nearly time equivalent fossil plants can yield different $\delta^{13}C_a$ values, which is confusing since differences in the $\delta^{13}C$ value of plant material are not solely reflective of differences in $\delta^{13}C_a$, but can be the result of varying Ci/Ca ratios that can be impacted by light availability, temperature, water stress; and the additive effects of the fractionation factors that affects the carbon isotope composition of source CO_2. These confounding results are likely due to the fact that the regression equation used does not permit Ci/Ca to vary, as it is known to do over a range of timescales (Beerling and Royer, 2002). Thus, it is unclear how meaningful the different $\delta^{13}C_a$ results are between the different fossil forest localities. If these localities are not exactly time equivalent, then it is possible that $\delta^{13}C_a$ varied, but a variation of ~2‰ is excessive when compared to the chemostratigraphic results of Krull and Retallack (2000) over the same stratigraphic interval, which suggest a variation of <0.9‰ in $\delta^{13}C_a$, whereas the 0.3‰ difference in $\delta^{13}C_a$ between Wahl Glacier and Mt. Achernar could reflect real variation in atmospheric CO_2 $\delta^{13}C$ values. Therefore, we select a $\delta^{13}C_a$ estimate of -5‰ for the Late Permian to use in our calculations because of the partial agreement between the method of assuming isotope equilibrium between surface ocean carbonate and atmospheric CO_2 and the method of Arens et al. (2000), but we use caution in drawing additional inferences from the method of Arens et al. (2000).

Calculating $\Delta^{13}C$ for a range of Ci/Ca for a $\delta^{13}C_a$ of -5‰

We calculate $\Delta^{13}C$ using two established relationships: 1) equation 1, and 2) $\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p}$ (4). We allowed equation 1 to vary by the Ci/Ca ratios quoted above, and we used the mean $\delta^{13}C$ values of fossil wood from each locality for $\delta^{13}C_p$. Our $\Delta^{13}C$ results from equation 1 range from 19.6, 18.7, 23.1, 16.8 for the mean, moderate, high, and low Ci/Ca values, respectively. The results of applying equation 4 yield $\Delta^{13}C$ values of 18.0 for Mt. Achernar, 18.3 for Wahl Glacier, and 20.7 for Graphite Peak. Therefore, while $\Delta^{13}C$ did likely vary between each locality, long-term Ci/Ca values between 0.59 and 0.66 result in $\Delta^{13}C$ values that bracket the $\Delta^{13}C$ estimated from the carbon isotope data, and describes the possible range of Ci/Ca variation between these localities.

For an additional test we employed the $\Delta^{13}C$ modeling approach of Lloyd and Farquhar (1994) to estimate $\Delta^{13}C$ values on the basis of CO_2 concentration, the seasonal variation of CO_2 over a 4-month growing period (similar to polar regions), the expected seasonal variation in the Ci/Ca ratio, the CO_2 photocompensation point, and seasonal

temperature change over a 4-month growing period. These parameters are related in the following way (Lloyd and Farquhar, 1994):

$$\Delta^{13}C = a\left(1 - \frac{C_i}{C_a} + 0.025\right) + 0.075(e_s + a_s) + b\left(\frac{C_i}{C_a} - 0.1\right) - \frac{\frac{eR_d}{k} + f\Gamma^*}{C_a} \quad (5)$$
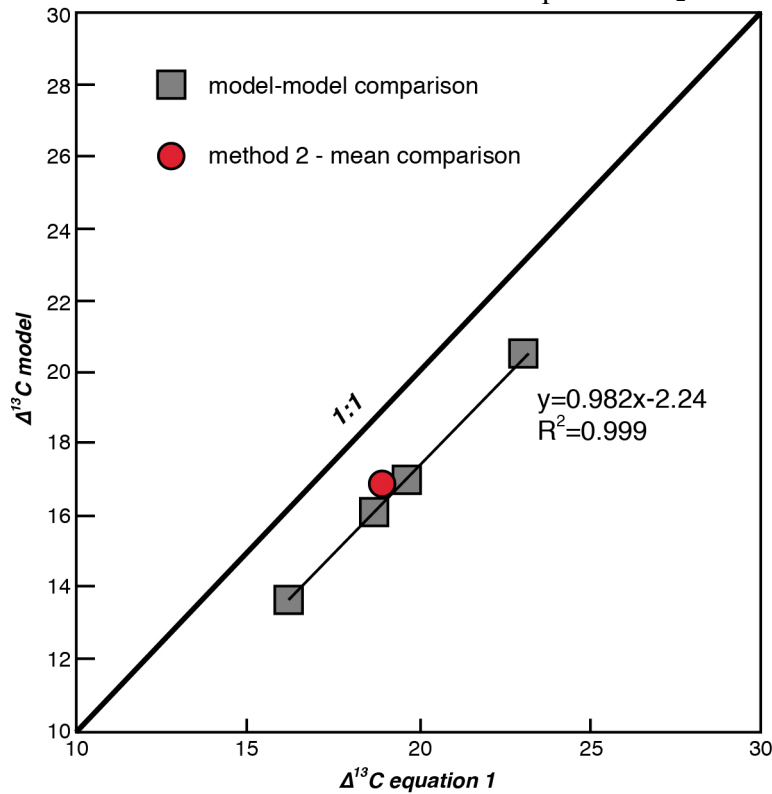
where a, b, and C_i/C_a are the same as equation 1, e_s is the equilibrium fractionation for CO_2 going into solution (1.1‰, Mook et al., 1974), a_s is the kinetic fractionation of CO_2 diffusion in water (0.7‰, O'Leary, 1984), e is the fractionation from respiration (0‰), R_d is the rate of foliar respiration in light, k is the carboxylation efficiency, f is the fractionation from photorespiration (8‰), and Γ^* is the CO_2 photocompensation point. Γ^* is modeled based on the temperature dependence to "leaf temperature" (T_L), where $\Gamma^*=1.54T_L$, and $T_L=1.05(T_a+2.5)$, where T_a is the air temperature in ºC. We report the values for these variables in Table S5-1.

Table S5-1: Explanation of model parameters for $\Delta^{13}C$ calculation

Variable	definition	Value/relationship
a	Diffusion of CO_2 in air	4.4‰
b	Enzymatic fixation of CO_2 by PEP-c and Rubisco	27.5‰
a_s	Diffusion of CO_2 in water	0.7‰
e_s	CO_2 into solution	1.1‰
f	photorespiration	8‰
Γ^*	CO_2 photocompensation point	$1.54T_L$
T_L	Leaf temperature	$1.05(T_a+2.5)$
T_a	Air temperature	N/A
C_i	Intercellular CO_2 concentration	N/A
C_a	Atmospheric CO_2 concentration	1500 µmol mol^{-1}
ΔCO_2 (@85ºN) May-Oct.	Seasonal change in atmospheric CO_2 concentration	60 µmol mol^{-1}

For temperature estimates we referred to paleoclimate reconstructions of the Late Permian from Roscher et al. (2011) and Kiehl and Shields (2005). For 85ºS paleolatitude the DJF, austral summer, mean temperatures are 10ºC in both models. For the JJA, austral winter, mean temperatures are -45ºC in Roscher et al. (2011) and ~-18ºC in Kiehl and Shields (2005). We compared these model results to a modern analogue to try to constrain the seasonal variation in temperature. For Yakutsk, Russia the mean maximum spring temperature is 17ºC, the mean maximum temperature in the peak summer is 25ºC,

and the mean maximum temperature in the fall is -4ºC (World Meteorological Organization). For the temperatures used in the model calculations we subtracted the DJF mean temperature for the Late Permian from the mean maximum temperatures at Yakutsk to yield a seasonal temperature fluctuation over the growing season. For Ca we used the estimate of global atmospheric CO_2 concentrations during the Late Permian (Berner, 2006) of 1500 μmol mol^{-1} for the peak summer value, a value of 1530 μmol mol^{-1} for the spring, and a value of 1470 μmol mol^{-1} for the fall. The seasonal variation in CO_2 concentration is determined from NOAA compilations of atmospheric CO_2 concentrations for 85ºN latitude (GLOBALVIEW-CO2, 2013) to account for the greater landmass and vegetative cover of the modern northern polar latitudes as compared to the southern polar latitudes during the Late Permian. We selected Ci/Ca as before, and our model results yield $\Delta^{13}C$ values for the spring of 16.1, 20.5 for the summer, and 14.0 for the fall. These model results are significantly lower than the previous estimates of $\Delta^{13}C$ using equation 1, indicating that we likely underestimated the temperatures for growth and/or the seasonal variation in atmospheric CO_2 concentration when we used the model equation of Lloyd and Farquhar (1994). The differences, however, are systematically offset by ~2.2‰ (Fig. S5-1), and when we compare the mean $\delta^{13}C$ values of the fossil wood from all of the localities (equation 4) to mean annual conditions in the model (equation 5) we find that they fall onto the trendline, suggesting that our estimates of $\Delta^{13}C$ based on fossil plant carbon isotopes and estimates of atmospheric CO_2 (equation 4) have good correspondence with the method using equation 1, which gives greater confidence in our estimates of $\Delta^{13}C$.

Figure S5-1: Model comparison of $\Delta^{13}C$ estimates. Y-axis represents model results from equation 5 where $\Delta^{13}C$ was estimated from reconstructed temperature variations, CO_2 concentrations, and Ci/Ca ratios. X-axis represents model results from equation 1 which reflects estimates using the reconstructed $\delta^{13}C_a$, $\delta^{13}C_p$, and assumed Ci/Ca ratios. Square symbols represent model–model comparison. The red circle represents the model results of equation 5 using mean annual values for temperature, CO_2, and Ci/Ca versus the mean $\Delta^{13}C$ estimated from the mean $\delta^{13}C$ value of the entire fossil wood data set using equation 4.

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