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Comment

# Comment on "Oxygen isotope ratios ($^{18}O/^{16}O$) of hemicellulose-derived sugar biomarkers in plants, soils and sediments as paleoclimate proxy I: Insight from a climate chamber experiment" by Zech et al. (2014)

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**Abstract**

Zech et al. (2014) reported oxygen isotope ratios of stem hemicellulose for three species of plants grown at different temperatures and humidity. The authors did not consider temperature effects on biochemical fractionation during hemicellulose synthesis as an important determinant of the oxygen isotope ratio of hemicellulose. However, a closer examination of their data shows that, indeed, temperature has a significant effect on the oxygen isotope biochemical fractionation. Lower temperature has no effect on the proportion of oxygen isotope exchange with cell water, but it increases the biochemical fractionation of the exchange reaction. These results are consistent with previous observations.


## 1. INTRODUCTION

Zech et al. (2014) described a method by which hemicellulose derived sugar biomarkers (arabinose and xylose) can be extracted from plant biomass and their oxygen isotope ratios determined by gas chromatography–pyrolysis isotope ratio mass spectrometry. They cultured three species of plants (*Eucalyptus globulus*, *Vicia fava* and *Brassica olearacea*) under different temperatures and relative humidities to test the use of the oxygen isotope ratios of these biomarkers as paleoclimate proxies. They also compared their results with modeled stem cellulose oxygen isotope ratio values ($\delta^{18}O_{cell}$) and showed a good agreement indicating that their analysis offers a promising isotopic proxy of ancient climates. Using the average of the oxygen isotope ratios of their extracted arabinose and xylose to represent the oxygen isotope ratio of stem hemicellulose ($\delta^{18}O_{hemicell}$), they concluded that the main factors influencing the $\delta^{18}O$ values of hemicellulose are: (1) the oxygen isotope ratio of source water which depends on the oxygen isotope ratio of precipitation and (2) the isotopic enrichment of leaf water due to transpiration which is principally affected by ambient relative humidity. Although the authors admit that temperature can have indirect effects on the isotopic enrichment of leaf water that propagates on to the isotopic signatures of carbohydrates; they reject any possibility that temperature can have a direct impact on biochemical processes responsible for the $^{18}O$ labeling of carbohydrates by plant water. The rejection of temperature effects on biochemical isotopic fractionations is puzzling, particularly because their data strongly supports such an effect. Here, I demonstrate with their data (presented in Table 1 of their manuscript) that temperature does affect biochemical isotope fractionations. Such an effect must be taken into account when using oxygen isotope ratios of cellulose and hemicellulose as a paleoclimate proxy.

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## 2. THEORETICAL FRAMEWORK

In order to show temperature effects on the biochemical fractionation, we require a brief background on the theory of how cellulose or any other stem carbohydrate becomes isotopically labeled by the $^{18}O$ content of the cell water. Sugars synthesized in the leaf acquire the oxygen isotopic signature of the leaf water mostly by exchange reactions between carbonyl oxygen of carbohydrates and water (Sternberg, 2009). As these simple sugars are exported out of the leaf and into the stem they exchange their oxygen with water in the stem, which is less enriched than that of the leaves (Sternberg, 2009; Gessler et al., 2013). Stem carbohydrates including cellulose or hemicellulose, therefore, have a mixed isotopic signal of the oxygen isotope ratios of the leaf and stem water. The equation describing the cellulose or hemicellulose oxygen isotopic enrichment relative to stem water is given by the following equation (Barbour et al., 2001):

$$\Delta_{carbohydrate} = \Delta_{lw}(1 - p_{ex}) + \epsilon_{bio} \quad (1)$$

where $\Delta_{carbohydrate}$ is the oxygen isotopic enrichment of cellulose ($\Delta_{cell}$) or hemicellulose ($\Delta_{hemicell}$) above the oxygen isotope ratio of stem water ($\delta^{18}O_{sw}$), $\Delta_{lw}$ is the oxygen isotope enrichment of leaf water above that of the stem water, $p_{ex}$ is the proportional amount of carbohydrate oxygen that exchange with stem water during the respective carbohydrate synthesis (cellulose or hemicellulose) and $\varepsilon_{bio}$ is the biochemical fractionation of this exchange which can differ between cellulose and hemicellulose. Hence, there are physiological and biochemical components that dictates the isotopic enrichment of cellulose relative to stem water (Sternberg, 2009). Physiological components determine the isotopic enrichment of the leaf water relative to stem water ($\Delta_{lw}$). I will not go into detail how leaf water becomes isotopically enriched as it is well covered in the literature (Barbour and Farquhar, 2003; Farquhar and Cernusak, 2005). For Eq. (1) above, $\Delta_{lw}$ will not be altered regardless of whether we are calculating the oxygen isotope ratio of cellulose or hemicellulose. On the other hand, the biochemical components of the above equation ($p_{ex}$ and $\varepsilon_{bio}$) could be different for the two different carbohydrates. Zech et al (2014) claim that the only factor in Eq. (1) affected by temperature is the physiological component ($\Delta_{lw}$). Temperature can alter the vapor pressure gradient across the leaf, which would then affect the oxygen isotope ratio of leaf water. Our previous study (Sternberg and Ellsworth, 2011), however, showed that temperature affects the biochemical component $\varepsilon_{bio}$ but not $p_{ex}$ in Eq. (1).

## 3. TEMPERATURE EFFECTS ON $\delta^{18}O_{HEMICELL}$

The first indication from their data that temperature has an effect on the biochemical fractionation during hemicellulose synthesis is the observation that the discrepancy between the average oxygen isotope ratios of hemicellulose and the modeled oxygen isotope ratio of cellulose ($\delta^{18}O_{hemicell} - \delta^{18}O_{cell}$) is related to temperature (Fig. 1A). Their cellulose model takes into account any factor which might alter the leaf water isotopic enrichment, including temperature. Their model, however, assumes no temperature effect on the biochemical component of the isotopic labeling process ($\varepsilon_{bio}$ and $p_{ex}$). We would, therefore, expect that if temperature does not affect the biochemical fractionation during hemicellulose synthesis, the $\delta^{18}O_{hemicell}$ values should be offset by a constant amount relative to those of the cellulose model. However, it can be seen that there is a general trend of an increasing difference as temperatures decreases (Fig. 1A); i.e., hemicellulose becomes progressively isotopically enriched relative to that predicted by the cellulose model with lower temperatures. This progressive discrepancy between $\delta^{18}O_{hemicell}$ and $\delta^{18}O_{cell}$ with

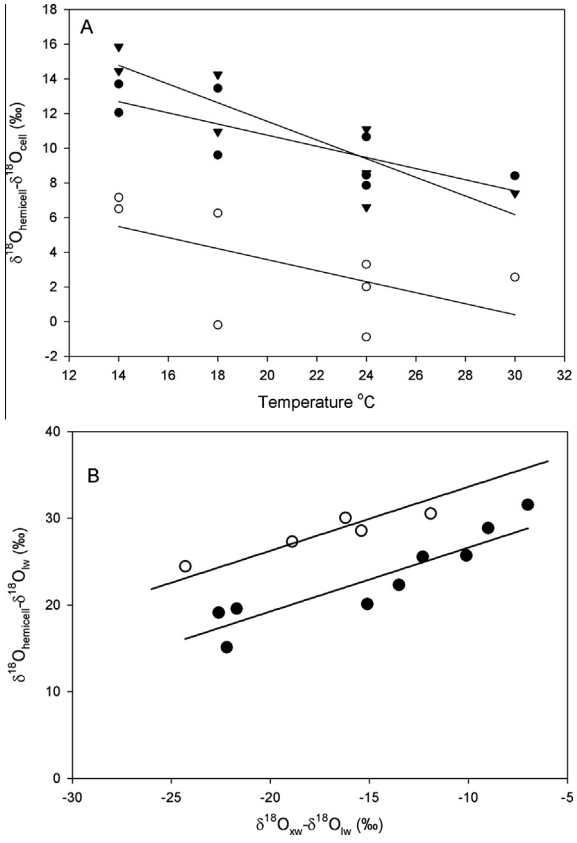


Fig. 1. (A) Discrepancy between the $\delta^{18}O_{hemicell}$ and modeled $\delta^{18}O_{cellulose}$ as a function of temperature. Symbols are (●) for *E. globulus*, (○) for *V. fava* and (▼) for *B. olearacea*. Regressions were significant for *E. globulus* (slope = −0.32, intercept = 17.2, $r = 0.78$, $p < 0.05$) and *B. olearacea* (slope = −0.54, intercept = 22.29, $r = 0.87$, $p < 0.01$) but not for *V. fava* (slope = −0.32, intercept = 9.9, $r = 0.58$, $p > 0.05$). (B) Relationship between ($\delta^{18}O_{hemicell} - \delta^{18}O_{lw}$) and ($\delta^{18}O_{sw} - \delta^{18}O_{lw}$) for two growth temperatures 24 °C (●, slope = 0.81, intercept = 35.1, $r = 0.92$, $p < 0.01$) and 14 °C (○, slope = 0.50, intercept = 36.9, $r = 0.95$, $p < 0.05$). Parallel lines are plotted with the common slope (0.74) with the distinctive intercepts for the two temperatures.

temperature cannot be caused by differences in leaf water isotopic enrichment as the cellulose model takes into account these differences. We therefore conclude that temperature is affecting biochemical processes that determine the oxygen isotope ratio of hemicellulose. The above analysis, however, does not allow us to determine whether temperature is affecting $\varepsilon_{bio}$ or $p_{ex}$.

Eq. (1) above can be rearranged to:

$$\delta^{18}O_{carbohydrate} - \delta^{18}O_{lw} = p_{ex}(\delta^{18}O_{sw} - \delta^{18}O_{lw}) + \epsilon_{bio}, \quad (2)$$

which states that a plot of the difference between the oxygen isotope ratio of the carbohydrate in question and that of the leaf water *versus* the difference between the oxygen isotope ratio of stem water and leaf water will yield a linear relationship having a slope equivalent to $p_{ex}$ and an intercept equivalent to $\varepsilon_{bio}$. There is a significant linear relationship for plants grown by Zech et al (2014) at 24 and 14 °C ($r = 0.92$, $p < 0.01$ at 24 °C and $r = 0.95$, $p < 0.05$ at 14 °C) when their respective isotopic values are plotted according to Eq. (2) (Fig. 1B). We selected data from these two temperatures as they are the extreme of temperatures with enough points and variation to derive a linear regression. These two different regressions had different slopes (0.81 at 24 °C and 0.50 at 14 °C) and different intercepts (35.1 and 36.9 at 24 °C and 14 °C, respectively). However, a statistical comparison between the two regression lines (Armitage et al., 2008) indicate no significant differences in the slopes of the two lines ($p = 0.21$), but a significant difference ($p < 0.01$) in the adjusted means of 22.4 for plants grown at 24 °C and 29.4 for plants grown at 14 °C. Since there are no significant differences between the slopes (i.e., $p_{ex}$) of the two regressions, we can conclude that the rate of oxygen isotope exchange between carbohydrates and water during hemicellulose synthesis is not altered by temperature. Therefore, we show the line having the common slope (0.74) for the two regressions (Fig. 1B). On the other hand, because there are significant differences between the adjusted means for the two temperatures, we conclude that there are differences in $\varepsilon_{bio}$. The biochemical fractionation ($\varepsilon_{bio}$) for hemicellulose at these temperatures can be calculated by using the common slope, the adjusted mean of the values from horizontal and vertical axis to yield the following values: at 24 °C, $\varepsilon_{bio} = 34.0$ and at 14 °C, $\varepsilon_{bio} = 41.0$.

## 4. CONCLUSION

The temperature effects observed in Zech et al.'s (2014) data are entirely consistent with our previous findings (Sternberg and Ellsworth, 2011): (1) temperature affects $\varepsilon_{bio}$ but not $p_{ex}$ and (2) $\varepsilon_{bio}$ increases with lower temperatures. Nevertheless, there is much room for experimentation to more precisely determine the exact effect of temperature on the synthesis of stem cellulose for specific trees used in paleoclimate studies.

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