



## Commentary

# Where and when does stem cellulose $\delta^{18}\text{O}$ reflect a leaf water enrichment signal?

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Early studies of oxygen isotope composition ( $\delta^{18}\text{O}$ ) of cellulose noted strong relationships with mean annual temperature and humidity (Libby et al. 1976, Burk and Stuiver 1981). Since then the  $\delta^{18}\text{O}$  signals in plant materials have become increasingly important for the reconstruction of paleoclimates and plant physiological responses to environmental variation. Many researchers have contributed to advancing how we understand the primary controls on the  $\delta^{18}\text{O}$  composition of plant materials, but arguably the most influential of these were experimental and modeling studies (Sternberg et al. 1986, Yakir and DeNiro 1990, Roden et al. 2000). Thereafter, many studies have clarified various controls over leaf water enrichment and the subsequent incorporation of these  $\delta^{18}\text{O}$  signals in cellulose (Barbour et al. 2004, Kahmen et al. 2011, Voelker et al. 2014, Cernusak et al. 2016).

In parallel to these advancements was the wider application of  $\delta^{18}\text{O}$  for analyses of tree-rings. For example, tree-ring  $\delta^{18}\text{O}$  has been used to reconstruct precipitation  $\delta^{18}\text{O}$  in relatively humid locations (Miller et al. 2006, Brien et al. 2012, Boysen et al. 2014), whereas in other locations tree-ring  $\delta^{18}\text{O}$  often reflects evaporative demand—some combination of vapor pressure deficit, relative humidity and sunshine (Roden and Ehleringer 2007, Labuhn et al. 2014, Xu et al. 2011, Hartl-Meier et al. 2015). Where tree-ring  $\delta^{18}\text{O}$  clearly reflects more than one driving variable, it can often still be used to improve paleoclimatic signals within a multi-proxy framework (Loader et al. 2008, Etien et al. 2009, Berkelhammer and Stott 2012, Johnstone et al. 2013, Schollaen et al. 2013, Voelker et al. 2014, 2015). Finally, tree-ring cellulose  $\delta^{18}\text{O}$  used within a mechanistic modeling framework has elucidated patterns in leaf temperature (Helliker and

Richter 2008, Song et al. 2011), and when combined with carbon isotope variation of the same plant materials, has been used to infer how environmental variation has impacted stomatal conductance versus photosynthetic assimilation rates (Scheidegger et al. 2000, Brooks and Coulombe 2009, Barnard et al. 2012, Guerrieri et al. 2016, Jennings et al. 2016).

The original Roden et al. (2000) mechanistic model of cellulose  $\delta^{18}\text{O}$  lacks some complexities that could potentially modify our conventional interpretations of climatic signals imprinted upon tree-ring  $\delta^{18}\text{O}$  (Sternberg and Ellsworth 2011, Gessler et al. 2014, Song et al. 2014). In this issue of *Tree Physiology*, Cheesman and Cernusak (2017) reveal yet another layer of complexity that is vexing to the conventional knowledge of controls on tree-ring  $\delta^{18}\text{O}$ . In their study, stem water and leaf and branch cellulose of five *Eucalyptus* and three *Corymbia* species were sampled across 11 sites representing a strong aridity gradient across northeastern Australia. The difference between leaf cellulose  $\delta^{18}\text{O}$  and source water  $\delta^{18}\text{O}$  could largely be explained using a slightly modified version of the conventional steady state models for leaf water and cellulose  $\delta^{18}\text{O}$  (i.e., Roden et al. 2000, Barbour et al. 2004). However, the difference between stem cellulose  $\delta^{18}\text{O}$  and source water  $\delta^{18}\text{O}$  was not affected by aridity as expected. In essence, their data, when combined with those of two other studies (Cooper and Solis 2003, Kahmen et al. 2011), suggest that the leaf water enrichment signal can be almost completely obscured by a post-photosynthetic process that is strongly correlated in space with relative humidity (Figure 1). If this were a generalizable phenomenon it would be contrary to many studies using stem cellulose  $\delta^{18}\text{O}$  as a record of evaporative enrichment within the now commonly applied

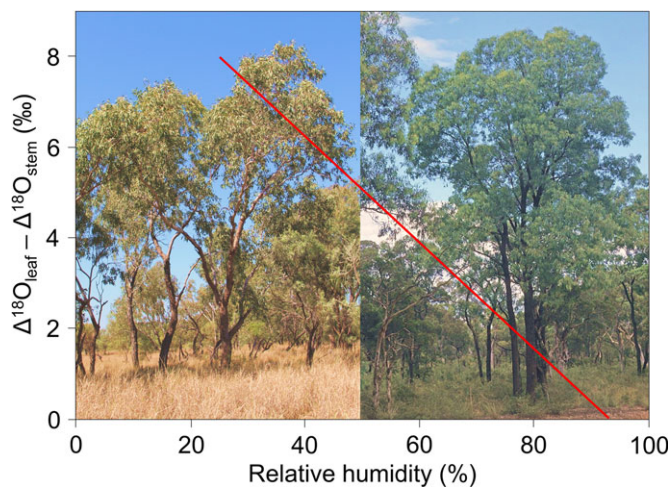


Figure 1. The red line (after Cheesman and Cernusak 2017) is a regression relationship indicating that the difference between leaf cellulose and stem water  $\delta^{18}\text{O}$  ( $\Delta^{18}\text{O}_{\text{leaf}}$ ) is much greater than the difference between branch wood cellulose and stem water  $\delta^{18}\text{O}$  ( $\Delta^{18}\text{O}_{\text{stem}}$ ) at sites with lower daytime relative humidity and associated increases in aridity. Two of the species sampled, *Eucalyptus coolabah* (from drier sites 7, 8, 10 and 11, on left) and *Eucalyptus crebra* (from wetter sites 1 and 5, on right), are shown in the background.

dual isotope frameworks for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  (Scheidegger et al. 2000, Brooks and Coulombe 2009, Barnard et al. 2012, Guerrieri et al. 2016, Jennings et al. 2016) as well as  $\delta\text{D}$  and  $\delta^{18}\text{O}$  (Voelker et al. 2014, 2015). It is clear from the literature noted above that an inter-annual evaporative signal is often captured by tree-ring cellulose  $\delta^{18}\text{O}$ , suggesting that in most cases there is not complete exchange between oxygen atoms in sucrose transported in the phloem and cytosolic water at the sites of cellulose synthesis in the cambium. Nonetheless, in situations where a near complete exchange was well documented over time, the resulting record of xylem source water  $\delta^{18}\text{O}$  could represent a very valuable response to past changes in temperature and/or atmospheric circulation and the associated origins of storm tracks.

The hypothesized mechanisms put forward by Cheesman and Cernusak (2017) to explain how the leaf evaporative enrichment signal is increasingly diluted at more arid sites are twofold and non-exclusive. The first hypothesis is that longer turnover times for sucrose between the phloem and the cambial sites of incorporation into cellulose molecules creates greater opportunities for hexose phosphates to undergo futile triose phosphate cycling, thereby allowing oxygen atoms to undergo greater exchange with local cytosolic water that is ostensibly unenriched in  $\delta^{18}\text{O}$  within suberized tissues (Hill et al. 1995, Barbour and Farquhar 2000, Song et al. 2014). The same process could also be invoked when non-structural carbohydrates (NSCs) are transported out of the phloem and stored (i.e., starch or lipids) and later remobilized. In ring-porous oak species, the formation of earlywood before leaves have expanded necessitates the use

of stored NSCs (Hill et al. 1995, Voelker et al. 2012). However, we lack detailed knowledge regarding the extent to which remobilized NSCs are used for early versus late-season stem growth in most tree species. Moreover, we know even less about how remobilization of stored NSCs for growth may depend on how drought stress reduces sink strength in the cambium relative to stomatal limitations on photosynthetic carbon gain. More research is needed, particularly experimental testing using tracers (e.g.,  $\delta^{13}\text{C}$ ,  $^{14}\text{C}$  and even highly enriched  $\delta^{18}\text{O}$ ) to document when and where stored NSCs show up in the stem cellulose trees grown at differing levels of drought stress. Observational studies may also contribute to our knowledge. For example, the  $\delta^{18}\text{O}$  signal in tree-rings from relatively dry locations should be less responsive to variation in relative humidity during periods of long-term drought compared with pluvial periods. Together, these types of studies will help discern whether inter-annual variability in aridity can modify the ability of stem cellulose  $\delta^{18}\text{O}$  to record leaf water enrichment to the same extent that Cheesman and Cernusak (2017) have documented across sites differing in aridity (Figure 1).

Part of the difference between how  $\delta^{18}\text{O}$  is recorded in cellulose across gradients in space (Figure 1) versus time (i.e., correlations between tree-ring  $\delta^{18}\text{O}$  and relative humidity or vapor pressure deficit) may derive from the second hypothesized mechanism: that stem cortical photosynthesis contributes a significant proportion of carbohydrates to the formation of stem wood (Cernusak and Hutley 2011), and that proportion may increase with greater drought stress (Cernusak and Cheesman 2015). In the current study by Cheesman and Cernusak (2017) the stem cellulose sampled came from small branches where cortical photosynthesis may have been a more important contributor to growth locally, particularly during short wetting events. In contrast, these newly fixed NSCs may not greatly impact phloem supply of NSCs near the stem base before drying once again reduces total photosynthesis and total NSC supply. A re-sampling of cellulose  $\delta^{18}\text{O}$  near stem bases and different heights in the canopy from each site studied by Cheesman and Cernusak could provide additional insights into mechanisms driving within-tree variation in cellulose  $\delta^{18}\text{O}$ .

Overall, the work by Cheesman and Cernusak (2017) underscores that oxygen isotopic exchanges occurring in the phloem, cambium and xylem may impact the extent to which leaf water enrichment is ultimately fixed within stem cellulose of plants across aridity gradients (Figure 1). Moreover, these new data raise questions for the dendrochronological community about whether leaf water  $\delta^{18}\text{O}$  enrichment recorded in cellulose may differ depending on how drought stress differentially impacts cambial activity versus stomatal conductance for any given year. Until these questions are better resolved, they deserve further study and the application of innovative techniques to better inform our basic understanding of  $\delta^{18}\text{O}$  signals in plant tissues.

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