Is the dual-isotope conceptual model fully operational?

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The stable carbon isotopic composition (δ¹³C) of organic matter is a valuable measure of plant physiological processes. Unfortunately, the impacts of carbon assimilation (A, demand for CO₂) and stomatal conductance (gₛ, supply of CO₂) on Rubisco discrimination (through Cᵢ/Cₐ) are difficult to separate. Thus, a conceptual model (Scheidegger et al. 2000) was developed to constrain the interpretation of δ¹³C variation by measuring oxygen isotopic composition (δ¹⁸O) on the same material (the ‘dual-isotope’ approach). The idea is that δ¹⁸O, as a proxy for evaporative flux, will be modified by gₛ but not A. Therefore, if changes in environmental conditions cause a long-term change in A and/or gₛ, this should be reflected in the carbon and oxygen isotope ratios of organic matter, respectively. This model is conceptually sound and a number of papers (Sidorova et al. 2009, Brooks and Mitchell 2011) have used this model for interpreting δ¹³C variation in tree rings.

In the current issue of Tree Physiology, Barnard et al. have utilized the dual-isotope model to infer physiological responses of mature Pseudotsuga menziesii (Mirb) Franco trees to environmental variation associated with site differences and canopy position. They developed a novel approach to eliminate confounding factors by plotting relative changes (from the grand mean) in tree ring δ¹³C and δ¹⁸O values. They used the conceptual model to infer temporal changes in gₛ, spatial variation in A associated with leaf nitrogen content and different levels of physiological responsiveness to environmental forcing for trees from different canopy positions. This was an ambitious use of the dual-isotope model due to complex environmental variation associated with sites (slope and aspect differences), canopy position (three crown classes), seasonal separation (earlywood and latewood) and inter-annual climate (8 years). The dual-isotope model has not been tested under such complex conditions, and the highly variable results of Barnard et al. (2012) make model interpretation challenging.

Our ability to take advantage of this conceptual model is limited by a number of model assumptions and constraints that are often overlooked. Barnard et al. (2012) recognized many of these assumptions and tried to quantify or limit confounding variation; however, they did not test whether model predictions regarding A and gₛ were realized in their system, which would have been a valuable confirmation of model applicability. They assumed that this conceptual model was operational for interpreting tree ring isotope variation in complex forest ecosystems. The purpose of this commentary is to highlight areas of caution that must be recognized before the dual-isotope model can be routinely utilized and encourage research that could help reduce model uncertainties.

1. The dual-isotope model interprets changes in δ¹⁸O as primarily influenced by gₛ. This requires that environmental influences on evaporative enrichment (Craig and Gordon 1965) including source water δ¹⁸O, atmospheric vapor δ¹⁸O, ambient humidity and leaf temperature must either be constant over time (for tree rings) or between treatments (common garden) or that all relevant fractionation events also influence gₛ (e.g., vapor pressure deficit [D] differences modify gₛ through a well-described relationship; thus, increasing D leads to enhanced evaporative enrichment in ¹⁸O, but it can also reduce gₛ leading to a concomitant increase in leaf water δ¹⁸O, amplifying the effect of D on organic matter δ¹⁸O). Documented inter-annual variation in the δ¹⁸O of precipitation, soil evaporative enrichment profiles and the fact that trees can tap different sources of water may reduce one’s confidence that constant source water δ¹⁸O is an appropriate assumption (Sarris et al. 2013).
makes it difficult to assess this assumption. We recommend measuring stem water δ18O and other parameters to ensure model applicability and strengthen model interpretation.

2. Differences in humidity (and D) have strong effects on 18O enrichment, and thus variation between plots, within complex canopies or between years (for historical studies using tree rings) could make interpretation of results problematic. The assumption that any humidity differences would directly translate to changes in gs needs better justification. Studies have developed algorithms to describe gs as a function of D for some species, but species differ in their sensitivity to D and the relationship may not be uniform across the full range of D (Oren et al. 1999). In addition, other factors (hydraulic conductivity, soil moisture, light levels, [CO2], genetic provenance, etc.) can modify the D/gs relationship. Roden and Farquhar (2012) tested the dual-isotope model in controlled environments and found a poor relationship between humidity and gs. Prior to utilizing the dual-isotope model for a particular species the D/gs relationship should be evaluated in order to ensure that potential humidity variation will likely translate to reasonable changes in gs.

3. The dual-isotope model was tested primarily for leaf organic matter (Scheidegger et al. 2000, Grams et al. 2007) and not tree rings (although see Roden and Farquhar 2012). Compared with leaf cellulose, tree rings have a dampened signal in δ18O due to isotope exchange with medium water (xylem water in the bole, Roden et al. 2000). δ13C and δ18O inputs from the leaf may also be modified by dilution from stored carbon reserves, but these two dampening processes (xylem water exchange and dilution by stored carbohydrates) are independent and thus the relationship between these two isotopes could change. The model uses a plot of δ13C vs. δ18O and the directional change (using arrows) between two or more treatments is diagnostic for predicting treatment-caused changes in A or gs. Thus, in tree ring studies, if one isotope has more dampening than the other, the slope of the arrow could be modified as compared with a plot using leaf organic matter.

4. A sensitivity analysis using standard mechanistic models that predict δ18O variation in organic matter (Roden et al. 2000, Barbour et al. 2004) demonstrates that, at high humidity, subtle changes in gs would be undetectable even for leaf cellulose (within the measurement error for δ18O, Figure 1). For tree ring cellulose only large changes in gs would be detectable due to reduced signal strength (dampening; see above). Prior to utilizing the dual-isotope model, one should determine if the humidity environment will allow sufficient sensitivity for viable interpretation. A site with humidity values consistently >80% is a poor choice if one wishes to assess subtle changes in gs. This analysis also shows that small errors in humidity estimation (10%) substantially influence predicted δ18O values potentially obscuring the influence of gs. Thus, assumptions of similar

humidity/D environments within canopies, between sites or years must be carefully assessed.

5. Some studies use regression analysis for model interpretation (Sidorova et al. 2009, Barnard et al. 2012). This can provide some level of confidence with variable data sets, but one must be aware that non-significant regressions (vertical or horizontal slopes) are also interpretable in the dual-isotope conceptual model. The model tries to highlight directional change in the δ18O vs. δ13C graph often by plotting an arrow between treatments. The directional change in the arrow is then interpreted as a directional change in the A vs. gs plot. Isotope data that plot vertically or horizontally have meaningful interpretations that a regression analysis may not highlight or lead to false conclusions. These are not plots of dependent and independent variables. The plot is simply trying to assess how these two isotopes co-vary. Barnard et al. (2012) seem to confuse the issue by presenting only four possible A/gs interpretations in the four quadrants in Figures 4 to 7. The scatter in their data indicates that arrows could proceed from the center to each observation in all directions (Figure 4). Does this indicate that each tree has a different physiological response or simply that there is immense variation in their complex system? They attempt to deal with this variation through a normalization procedure and statistical analysis. Results from dual-isotope plots must always be interpreted within the context of plant function. Statistical significance, though extremely important, may indicate differences in physiological response that turn out to be trivial or even not viable.

6. Both the Scheidegger et al. (2000) and Grams et al. (2007) papers interpret changes in assimilation using AMAX, but this is problematic as AMAX can be defined in a number of ways. AMAX is commonly a measure of the maximal photosynthetic capacity; however, the demand side of the equation that
sets the C\textsubscript{i}/C\textsubscript{a} ratio (more correctly, C\textsubscript{i}/C\textsubscript{a} is the driving force for discrimination, i.e., chloroplast rather than intercellular [CO\textsubscript{2}], Farquhar et al. 1989) is more dependent on integrated or average A than on maximum photosynthetic potential under optimal conditions. As such, when comparing the effects of treatments using the conceptual model one must be sure that only the parameters of interest are variable. For example, variation in light environments can have a strong effect on integrated A without necessarily changing A\textsubscript{MAX}. Variation in temperature and plant water status can also affect A without changing A\textsubscript{MAX}. Thus, we would recommend dropping A\textsubscript{MAX} from these models and instead interpreting results of dual-isotope analysis as indicative of changes in mean or integrated A (as in Barnard et al. 2012).

7. A minor scaling issue comes up when testing these models using A and g\textsubscript{s} data. Because both δ\textsuperscript{18}O and δ\textsuperscript{13}C generally vary by similar amounts, a plot where 1‰ on the δ\textsuperscript{18}O axis is equal in distance to 1‰ on the δ\textsuperscript{13}C axis would produce an unbiased analysis. However, A\textsubscript{MAX} (or A, see above) and g\textsubscript{s} do not vary on similar scales and depending on the choice of axis range, changes in either A or g\textsubscript{s} could appear more or less significant than they really are. In other words, by manipulating the axis scale a response arrow could have a variety of apparent slopes. We would recommend the development of an objective scaling protocol for A vs. g\textsubscript{s} plots.

8. Mechanistic models that predict δ\textsuperscript{18}O values in organic matter (Barbour et al. 2004) use g\textsubscript{s} inputs in two ways. Firstly, g\textsubscript{s} along with boundary layer conductance are used to calculate the kinetic fractionation factor (ε\textsubscript{k}) in the leaf water enrichment model. Secondly, g\textsubscript{s} will influence transpiration (E, either modeled or measured) and thus modify leaf water enrichment via the Péclet effect (Farquhar and Lloyd 1993). The influence of g\textsubscript{s} on E can be considerably stronger than its effect on kinetic fractionation, particularly under low ambient humidity when E is high. One aspect that is not considered, however, is the effect of g\textsubscript{s} on isotopic exchange with atmospheric vapor. This might significantly influence leaf water enrichment under conditions where atmospheric vapor δ\textsuperscript{18}O is not in equilibrium with source water (Christina Reynolds, personal communication). More research is needed to understand how g\textsubscript{s} influences leaf water δ\textsuperscript{18}O through bi-directional water vapor flux.

9. Some studies (e.g., Saurer et al. 1995) have shown that air pollutants (O\textsubscript{3}, SO\textsubscript{2}, etc.) can directly impact stomatal and photosynthetic physiology altering the relationship between g\textsubscript{s} and C\textsubscript{i}/C\textsubscript{a}. Thus, we advise caution when applying the dual-isotope model in urban environments or trees subject to chronic inputs of strong oxidizing agents. On the other hand, Siegwolf et al. (2001) found the dual-isotope approach instrumental for the interpretation of long-term effects of air pollutants (e.g., NO\textsubscript{3}) on plant physiological processes. Thus, we would like to see more research into this question of air pollution and the applicability of the standard model for 13C discrimination.

10. Another concern with the use of this conceptual model with tree ring studies is that it may not detect the effects of severe drought. In some instances, a severe lack of moisture will inhibit assimilate transport to the vascular cambium essentially shutting down lateral growth (Sarris et al. 2013). As such, the isotopic composition of tree ring cellulose may only reflect less stressful time periods of the growing season. Leaf organic matter (especially non-structural carbohydrates) may retain a drought signal that is potentially interpretable. Similar periods of limited cambial activity during the growing season may occur with unusually cold periods and anaerobic soils during flooding.

In conclusion, the dual-isotope conceptual model has clear benefits for constraining our interpretation of δ\textsuperscript{13}C variation, but researchers planning to utilize this model must design their experiments carefully in order to meet the assumptions of the model and avoid various pitfalls of over-interpretation. Finally, situations where the dual-isotope model yields implausible results might be valuable for diagnosing model limitations and point to areas that require further research.

References


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