



## On the use of phloem sap $\delta^{13}\text{C}$ to estimate canopy carbon discrimination

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Although the carbon stable isotope composition ( $\delta^{13}\text{C}$ ) of bulk leaf material is a good integrative parameter of photosynthetic discrimination and can be used as a reliable ecological index of plant functioning; it is not a good tracer of short-term changes in photosynthetic discrimination. In contrast,  $\delta^{13}\text{C}$  of phloem sap is potentially useful as an indicator of short-term changes in canopy photosynthetic discrimination. However, recent research indicates that  $\delta^{13}\text{C}$  signatures may be substantially altered by metabolic processes downstream of initial leaf-level carbon fixation (e.g. post-photosynthetic fractionation). Accordingly, before phloem sap  $\delta^{13}\text{C}$  can be used as a proxy for canopy level carbon discrimination an understanding of factors influencing the degree and magnitude of post-photosynthetic fractionation and how these vary between species is of paramount importance.

In this study, we measured the  $\delta^{13}\text{C}$  signature along the basipetal transport pathway in two co-occurring tree species in the field – an understory invasive exotic legume, *Acacia longifolia*, and a native pine, *Pinus pinaster*. We measured  $\delta^{13}\text{C}$  of bulk leaf and leaf water soluble organic matter (WSOM), phloem sap sampled at two points along the plant axis and leaf and root dark respiration. In general, species differences in photosynthetic discrimination resulted in more enriched  $\delta^{13}\text{C}$  values in the water-conserving *P. pinaster* relative to the water-spending *A. longifolia*. Post-photosynthetic fractionation led to differences in  $\delta^{13}\text{C}$  of carbon pools along the plant axis with progressively more depleted  $\delta^{13}\text{C}$  from the canopy to the trunk ( $\sim 6.5$  per mil depletion in *A. longifolia* and  $\sim 0.8$  per mil depletion in *P. pinaster*). Leaf and root respiration,  $\delta^{13}\text{C}$ , were consistently enriched relative to putative substrates. We hypothesize that the pronounced enrichment of leaf respiration  $\text{CO}_2$  relative to leaf WSOM may have left behind relatively depleted carbon to be loaded into the phloem resulting in  $\delta^{13}\text{C}$  depletion along the canopy to trunk continuum. We further hypothesize that pronounced depletion along the basipetal transport pathway in *A. longifolia* (more than 6 per mil from leaf water soluble organic matter to trunk phloem sap) may be due to high stem photosynthesis rates in this green-barked legume.

Regardless of these fractionation effects, phloem sap  $\delta^{13}\text{C}$  correlated well with environmental parameters driving photosynthesis (photosynthetic photon flux density, soil moisture, vapor pressure deficit) for both species indicating that phloem sap  $\delta^{13}\text{C}$  is a good integrative tracer of changes in canopy-level carbon discrimination once species-specific differences in post-photosynthetic fractionation are accounted for. Furthermore, we illustrate that combining sap flow estimated canopy stomatal conductance ( $g_s$ ) with measurements of phloem sap  $\delta^{13}\text{C}$  (adjusted for post-photosynthetic fractionation) has significant potential as a relatively non-intensive method for estimating canopy-level carbon assimilation rates in field studies.