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Steady state or non-steady state? Identifying driving mechanisms of oxygen isotope signatures of leaf transpiration in functionally distinct plant species

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Isotope techniques are widely applied in ecosystem studies. For example, isoflux models are used to separate soil evaporation from transpiration in ecosystems. These models often assume that plant transpiration occurs at isotopic steady state, i.e. that the transpired water shows the same isotopic signature as the source water. Yet, several studies found that transpiration did not occur at isotopic steady state, under both controlled and field conditions. Here we focused on identifying the internal and external factors which drive the isotopic signature of leaf transpiration. Using cavity ring-down spectroscopy (CRDS), the effect of both environmental variables and leaf physiological traits on $\delta^{18}O_T$ was investigated under controlled conditions. Six plant species with distinct leaf physiological traits were exposed to step changes in relative air humidity (RH), their response in $\delta^{18}O_T$ and gas exchange parameters and their leaf physiological traits were assessed. Moreover, two functionally distinct plant types (tree, i.e. *Quercus suber*, and grassland) of a semi-arid Mediterranean oak-woodland were observed under natural conditions throughout an entire growth period in the field.

The species differed substantially in their leaf physiological traits and their turn-over times of leaf water. They could be grouped in species with fast (<60 min.), intermediate (ca. 120 min.) and slow (>240 min.) turn-over times, mostly due to differences in stomatal conductance, leaf water content or a combination of both. Changes in RH caused an immediate response in $\delta^{18}O_T$, which were similarly strong in all species, while leaf physiological traits affected the subsequent response in $\delta^{18}O_T$. The turn-over time of leaf water determined the speed of return to the isotopic steady or a stable $\delta^{18}O_T$ value (Dubbert & Kübert et al., in prep.). Under natural conditions, changes in environmental conditions over the diurnal cycle had a huge impact on the diurnal development of $\delta^{18}O_T$ in both observed plant functional types. However, in accordance with our findings in the lab, species specific differences in the leaf water turn over time, significantly influenced the amount of time plants transpired at non-steady state during the day (Dubbert et al., 2013, 2014).

Our results emphasize the significance of considering isotopic non-steady state of transpiration and specifically to account for the specific differences of plant species resulting from distinct physiological traits of their leaves when applying isoflux models in ecosystem studies.

Dubbert, M; Cuntz, M; Piayda, A; Maguas, C; Werner, C: Partitioning evapotranspiration - Testing the Craig and Gordon model with field measurements of oxygen isotope ratios of evaporative fluxes. *J Hydrol* (2013)

Dubbert, M; Piayda, A; Cuntz, M; Correia, AC; Costa e Silva, F; Pereira, JS; Werner, C: Stable oxygen isotope and flux partitioning demonstrates understory of an oak savanna contributes up to half of ecosystem carbon and water exchange, *Frontiers in Plant Science* (2014a)