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Supplementary Information for

Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water

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Supplementary Information Text

Growth Conditions for Mangroves

Pots containing mangrove plants were placed in plastic containers (height: 18 cm, top diameter: 25 cm, bottom diameter: 23 cm) holding 20 ppt saline water, which was produced by adding commercial sea salts into a reference water of known isotope ratios. The water level within the container was kept close to the soil surface within pots, so to ensure the soil remained constantly saturated. We took the following two measures to maintain stability in salinity and isotope ratios of the water inside the container throughout the growth period: 1) the exposed parts of the water surface (i.e, surface not occupied with pots) were all covered with tinfoil so as to minimize evaporation; 2) the saline water within each container was changed twice every week.

Transpiration Isotope Measurement System

As described in Fig. S1, the main components of the measurement system included three whole-plant through-flow chambers coupled to a water vapor isotope ratio infrared spectrometer (IWA-45-EP, Los Gatos Research, San Jose, CA, USA), a relative humidity (RH) stabilizer, a self-regenerative desiccant drying unit, and an air compressor.

Each of the whole-plant chambers has a cubic shape with edge length of 40 cm. The chamber was made of transparent acrylic, with its base consisting of two halves that are readily removable. The chamber body can be elevated from the ground by 20 to 120 cm with the aid of four adjustable, support legs. To enclose a target plant canopy into the chamber for measurement, the main stem of the plant is placed between two soft rubber-foam gaskets in the center of the two base halves, which are subsequently screwed together. The rubber foam forms a seal around the stem, which can be made air-tight, if necessary, with a small amount of soft paraffin. A mixing fan was installed inside the chamber to ensure sufficient gas mixing. Also installed within the chamber was an air temperature sensor (Onset HOBO UX100-011, Bourne, MA, USA), and a separate RH probe (Vaisala HMP110, Helsinki, Finland) that was programmed to communicate with the RH stabilizer for RH control purposes as detailed below.

During measurement, pressurized ambient air supplied from the air compressor was delivered to a self-regenerative desiccant drying unit (LIDU-DS1, Xiamen, China), which operates through two pressure-swing, miniature towers filled with molecular sieves as desiccant, capable of providing complete and continuous removal of moisture from the compressed air flow with minimal operator attention (i.e., its capacity for desiccant self-regeneration without heat means there is no need for desiccant replacement even after weeks of continuous use). The dry air flow out of the drying unit was regulated back to the ambient air pressure by a pressure regulator and subsequently split into three streams. Each air stream was then passed via tubing through one of the three mass flow controllers (MFC) housed within the RH stabilizer, and afterwards was delivered to one of the three whole-plant chambers. After exiting the chamber, the air flow was sent via a Los Gatos multiport inlet unit (MIU) to a laser spectrometer (IWA-45-EP, Los Gatos Research, San Jose, CA, USA) for online monitoring of transpiration isotope signals. The MIU was programmed to allow sequential sampling from each chamber on 10-min intervals.

The RH stabilizer, purposely designed to communicate with the RH sensor located inside each chamber, served to stabilize the chamber RH to a pre-set target value (i.e., 40% to 70% depending on species/transpiration rate) by actively regulating rate of the incoming air flow via MFC (regulatory range being as wide as 2 to 10 L min^{-1}) through a feedback control mechanism. According to the isotope theory, stability in RH (together with a stable air temperature) is critical for leaf water and leaf transpired water vapor to reach and maintain at the ISS (i.e., an achieved ISS could be disrupted if RH changes) (Simonin *et al*. 2013; Cernusak *et al*. 2016), and hence constitutes a prerequisite for fulfilling our goal of obtaining accurate δ_{T_SS} measurement. In this context, it should be noted that our employment of a RH stabilizer differs from previous studies in which air flow rate is conventionally set at a fixed value (meaning RH was not actively regulated) when it comes to transpiration isotope measurement in a laser-chamber coupled system (Wang *et al*. 2012; Simonin *et al*. 2013; Dubbert *et al*. 2014; Song *et al*. 2015; Kuhnhammer *et al*. 2020). In theory, a "fixed flow rate" mode is sufficient to keep the chamber RH stable if the plant inside the chamber is transpiring at a constant rate (and assuming that stability in within-chamber air temperature is also met). However, this mode would provide no guarantee for a constant transpiration rate (E) in our study that used diverse plant species for measurement, as some species, due to their inherently large τ (the "time constant" parameter determining the speed with which transpiration approaches ISS in a stable environment) values, would inevitably require enclosure within the chamber for long duration of time (i.e., up to 10 hours), over which we would expect circadian rhythm to manifest to cause fluctuations in E (i.e., in spite of a stable environmental condition). Such a complexity necessitated our use of an active RH control setup for correcting for potential instability in chamber moisture content as caused by fluctuations in E.

Cryogenic vacuum extraction of stem and soil water

The cryogenic vacuum distillation line used in the present study is a manual system, bearing a design similar to that described in Ehleringer *et al.* (2000) and West *et al.* (2006). The line consisted of six independent glass units all attached to a stainless-steel vacuum manifold with 2.8-cm in diameter. For water extraction, the glass arm of each unit was attached with a Pyrex collection tube at one end and an ignition (sample) tube containing plant or soil samples (together with the sampling glass vial) at the other end. The extraction line was subject to a baseline vacuum of 0.1 pa. During the extraction, samples in the ignition tubes were heated at 100 ℃ for at least 2 hr following the recommendations made in West *et al.* (2006). The evaporated water was collected into the collection tubes that were submerged in liquid nitrogen cold traps. In practice, an extraction was deemed as being complete if no further moisture condensation occurred on the inside of the collection tube even after the liquid nitrogen level was raised up along the tube for a few centimeters. After extraction, samples were always subjected to over-drying at 100 ℃ for 24 hr, and sample dry weights determined after oven-drying were compared against their counterparts determined right after extraction, so as to further validate the achievement of complete extraction.

Possible explanations for no significant presence of organic contamination in the cryogenically-extracted water samples

The spectral contamination identification software (Spectral Contamination Identifier or SCI, Los Gatos Research Inc.) integrated with the Los Gatos isotope analyzer was used to identify potential spectral interference by organic compounds that may co-distill with water during cryogenic extraction. In the present study, none of the soil and stem samples was flagged by the SCI as being organic contaminated. The soil samples all belong to sandy soil type, which is known to be organically less complicated than other soil types, and hence is expected to be less prone to organic contamination. With regard to stem water samples, spectral contamination is a frequently reported phenomenon in literature (Martín-Gómez *et al*. 2015); as such our observation of a general lack of stem-water related organic contamination in various species may need further explanation. We postulate this may be related to the fact that our samples were all collected from tree saplings that were growing under favorable, non-stressed greenhouse conditions. As well recognized, biogenic volatile organic compounds (BVOC) mainly function as protective and stress-coping molecules in plants, and their production is often closely related to the types and levels of abiotic/biotic stresses plant experience (Niinemets 2010). Further, it is worth emphasizing that bark and phloem were all removed during our stem sample collection, -- this procedure could also help minimize organic contamination given that bark tissues are known as a potentially important source of BVOC emissions in plants (Šimpraga *et al*. 2019).

Recalculating xylem water deuterium offset from precipitation based on the data of Evaristo *et al.* (2015)

Source data used for calculating globally-averaged xylem water offset from precipitation (hereafter denoted by d_{xylem}) in Evaristo *et al*. (2015) were downloaded from https://www.nature.com/articles/nature14983#Sec11. The data were further organized into a new dataset classified by biome and stem-water extraction method.

In Evaristo *et al*. (2015), d_{xylem} was calculated as:

$$d_{xylem} = [\delta^2H_{xylem} - a\,\delta^{18}O_{xylem} - b] / S \quad \text{Eqn S1}$$

where a and b are the slope and intercept of LMWL respectively, and S denotes the measurement uncertainty for both δ^2H_{xylem} and $\delta^{18}O_{xylem}$, which according to Evaristo *et al.* (2015) is 1.02.

Out of a total of 1,451 compiled data entries, 1,079 (i.e., comprising *ca.* 74% of the entire dataset) involved the use of the stem-water cryogenic extraction technique for determining xylem water isotope ratios. For each of these 1,079 data entries, we made correction to d_{xylem} by taking cryogenic extraction caused bias in δ^2H_{xylem} into consideration. The corrected d_{xylem}, hereafter denoted as d_{xylem_c}, can be described using the following equation:

$$d_{xylem\_c} = [(\delta^2H_{xylem} - \varepsilon) - a\,\delta^{18}O_{xylem} - b] / S \quad \text{Eqn S2}$$

where ε denotes the deuterium offset between cryogenically extracted stem water and true xylem water.

Combining Eqns S1 and S2 yields:

$$d_{xylem\_c} = d_{xylem} - \varepsilon/S \quad \text{Eqn S3}$$

We used Eqn S3 to convert d_{xylem} into d_{xylem_c}. We note that an ideal way of parameterizing ε would be to use stem relative water content to correct for each individual data point based on the regression function revealed in Fig. 4. However, as stem relative water content data

was not available in Evaristo *et al.* (2015), in practice we treated ε as a constant of −8.1‰, which corresponds to the species-averaged value for offset of $\delta^2H_{stem\_CVD}$ from δ^2H_{xylem} as obtained from the current study. After data correction, a global average of d_{xylem} was recalculated and compared against that of groundwater, stream water and soil water offset to re-evaluate whether ecohydrological separation is a widespread, global phenomenon.

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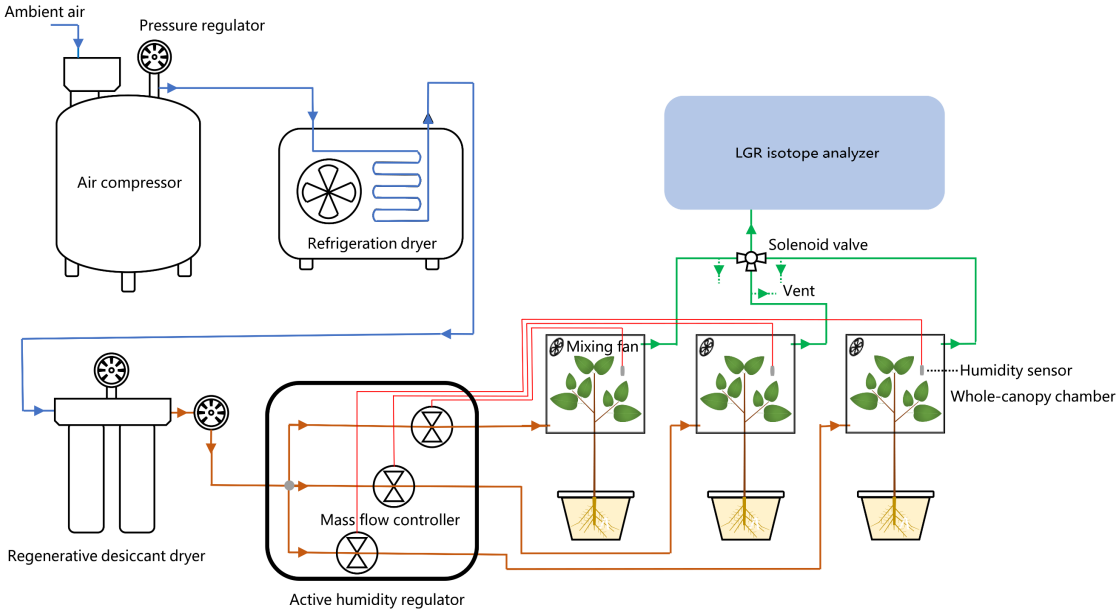

Fig. S1. A schematic overview of the whole-plant transpiration isotope measurement system. Arrows indicate direction of the gas flow. The ambient air (blue lines) is compressed by an air compressor and passed into a regenerative drying unit for complete removal of air moisture under high pressure (~0.4 MPa). The dry air flow (orange lines) out of the drying unit is regulated back to the ambient air pressure by a pressure regulator and subsequently split into three streams, each of which then passing through an individual MFC-equipped line within a RH stabilizer, and further to one of the three whole-plant chambers. The air stream out of the chamber (green lines) is then sent through a Los Gatos multiport inlet unit (MIU) to a Los Gatos vapor isotope analyzer for online $\delta^{18}O$ and $\delta^{2}H$ monitoring of plant transpired water vapor. See *SI Appendix* for a more detailed description.

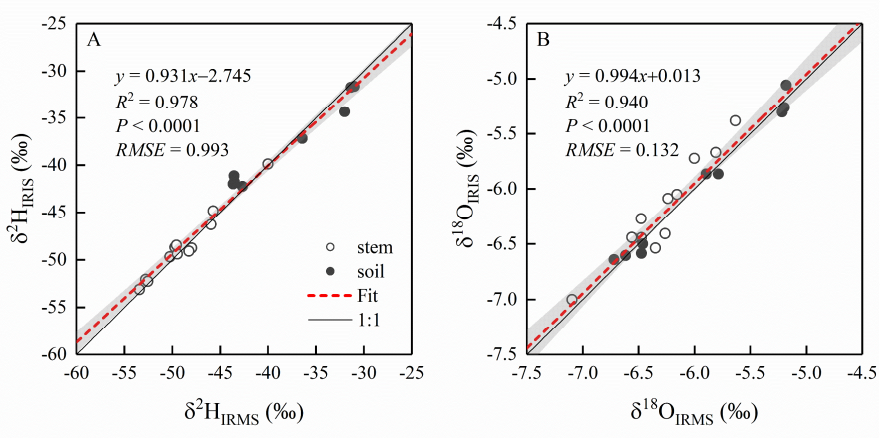

Fig. S2. δ^2H (A) and $\delta^{18}O$ (B) values of cryogenically extracted stem and soil samples measured by both IRIS and IRMS methods. Samples subject to measurements by both methods contain 21 stem and 9 soil water samples. They were randomly selected from a total of 54 samples collected from the SS transpiration experiment. Red dashed line represents fitted line, solid line represents 1:1 line, and light gray shaded area represents 95% confidence intervals. Pair-Sample t-Test showed no significant difference between the IRMS and IRIS measurements for either δ^2H ($P = 0.335$) or $\delta^{18}O$ ($P = 0.095$).

Table S1. Results of a preliminary test of the degree of vertical variability in isotope compositions of soil water under the growth treatment similar to that applied in the current study. Two species (*F. microcarpa* and *A. canescens*) were used in this test. For each species, plants were grown in sandy-soil filled pots with the soil surfaces fully covered with tinfoils to minimize evaporative enrichment. After daily irrigation with water of the same isotope compositions for two weeks, we collected soil samples from upper (2 cm below soil surface), middle (9 cm below soil surface) and lower portions (16 cm below soil surface) of each pot for water extraction and subsequent isotopic analysis. Neither of the species showed a significant difference among soil positions for either hydrogen or oxygen isotope data.

Treatment	Soil Position	$\delta^{2}H$ of soil water (‰)	$\delta^{18}O$ of soil water (‰)
Irrigation water	\	-34.5±0.1	-5.39±0.09
F. macrocarpa (n = 3)	Upper	-34.7±0.1	-5.49±0.17
	Middle	-34.5±0.4	-5.37±0.06
	Lower	-34.7±0.6	-5.30±0.12
A. canescens (n = 3)	Upper	-35.4±0.3	-5.49±0.08
	Middle	-34.9±0.3	-5.42±0.06
	Lower	-34.5±0.5	-5.30±0.09

Table S2. Values (species mean ± SE) for δ^2H and $\delta^{18}O$ of xylem water, cryogenically extracted stem water, and source water in all nine species subjected to the SS transpiration experiment. Data provided in this table was used calculating isotope offsets as shown in Fig. 2.

Species	δ^2H_{xylem} (‰)	$\delta^2H_{stem\_CVD}$ (‰)	δ^2H_{source} (‰)	$\delta^{18}O_{xylem}$ (‰)	$\delta^{18}O_{stem\_CVD}$ (‰)	$\delta^{18}O_{source}$ (‰)
A.canescens	-41.6 ± 0.2	-52.3 ± 0.4	-41.4 ± 0.2	-6.44 ± 0.07	-6.60 ± 0.21	-6.60 ± 0.02
P.euphratica	-41.9 ± 0.5	-50.9 ± 1.2	-41.1 ± 0.1	-6.20 ± 0.01	-5.98 ± 0.10	-6.27 ± 0.04
T. chinensis	-41.3 ± 0.1	-48.6 ± 0.4	-41.6 ± 0.4	-6.21 ± 0.03	-6.25 ± 0.22	-6.49 ± 0.07
A.corniculatum	-36.7 ± 0.2	-47.8 ± 0.2	-36.9 ± 0.3	-5.59 ± 0.05	-5.21 ± 0.11	-5.37 ± 0.06
A. marina	-31.2 ± 1.1	-39.2 ± 0.4	-31.7 ± 0.1	-5.47 ± 0.15	-5.04 ± 0.17	-5.27 ± 0.02
K.obovata	-33.1 ± 1.1	-40.9 ± 1.5	-32.9 ± 0.8	-5.00 ± 0.35	-4.91 ± 0.03	-5.17 ± 0.14
E.robusta	-41.7 ± 0.3	-48.9 ± 0.3	-42.7 ± 0.4	-6.47 ± 0.05	-6.44 ± 0.01	-6.49 ± 0.08
F. microcarpa	-42.3 ± 0.4	-48.1 ± 0.8	-43.0 ± 0.4	-6.37 ± 0.12	-6.03 ± 0.03	-6.47 ± 0.02
T. distichum	-38.8 ± 0.3	-45.2 ± 0.6	-37.4 ± 0.2	-5.96 ± 0.05	-5.57 ± 0.13	-5.91 ± 0.05