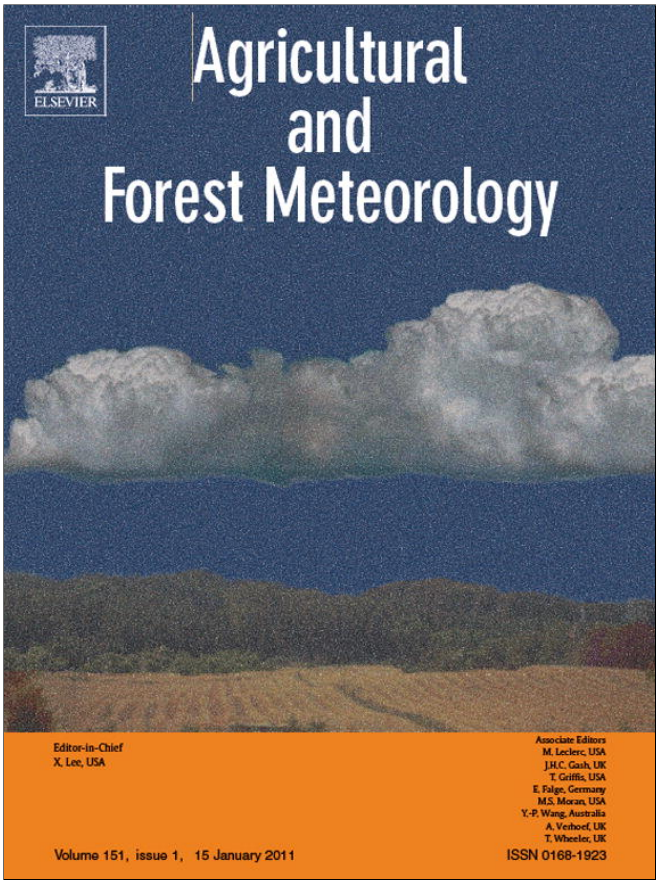

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Direct quantification of leaf transpiration isotopic composition

Lixin Wang[a,b,*], Stephen P. Good[a], Kelly K. Caylor[a], Lucas A. Cernusak[c]

[a] Department of Civil and Environmental Engineering, Princeton University, Princeton, NJ 08544, USA
[b] School of Civil and Environmental Engineering, University of New South Wales, Sydney, NSW 2052, Australia
[c] Research School of Biology, Australian National University, Acton, ACT 0200, Australia

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ABSTRACT

The stable isotopic composition of plant transpired water (δ_T) is a powerful tracer used to characterize plant processes in the fields of ecology, plant physiology and hydrology. However, δ_T is rarely directly measured due to the general difficulty in traditional water vapor isotopic measurements. We report a new direct method with the potential to continuously monitor δ_T utilizing a commercially available laser-based isotope analyzer coupled to a transparent leaf chamber in a flow-through system arrangement. The method is based on the mass balance of both water vapor and water vapor isotopes inside the chamber. We present the theoretical bulk water vapor and isotope mixing equations, which we verify using simulated water vapor of known isotopic compositions, producing a precision of 1.6‰ and 1.0‰ for δ^2H and δ^{18}O respectively. We demonstrate the applicability of our method to field observations and capture rapid (minute time scale) δ_T responses to shifts in transpiration driven by variation in irradiance.

1. Introduction

Stable isotopes of water are powerful tracers that carry useful information regarding ecology (Dawson, 1993), plant physiology (Dongmann et al., 1974; Flanagan et al., 1991), meteorology (Griffis et al., 2011; Yakir and Wang, 1996) and hydrology (Wang et al., 2010; Williams et al., 2004). The stable isotope composition of plant transpired water (δ_T) is defined as $\delta = (R/R_{std} - 1)$, where R is the ratio of rare and common isotope (^{2}H/^{1}H or ^{18}O/^{16}O) of instantaneous transpired water vapor, and R_{std} is the ratio of the international standard on the *V-SMOW* (Vienna Standard Mean Ocean Water)-*SLAP* (Standard Light Antarctic Precipitation) scale. The instantaneous value of δ_T is the result of complex interactions between liquid water at the evaporation site, water vapor in ambient air, and the environmental conditions inside and outside the leaf (Farquhar et al., 2007; Ogée et al., 2007; Welp et al., 2008). The δ_T value has a strong effect on and is affected by isotopic compositions of atmospheric water vapor (Farquhar et al., 2007). These interactions have been used to aid the spatial and temporal reconstruction of environmental parameters such as ambient temperature and relative humidity (e.g., Helliker and Richter, 2008) and primary productivity (Welp et al., 2011). The δ_T value is often linked to the isotopic compositions of liquid water at the site of evaporation ($\delta_{L,e}$) within leaves (Ogée et al., 2007; Welp et al., 2008). The $\delta_{L,e}$ represents a critical boundary condition to estimate ^{18}O–CO$_2$ photosynthetic discrimination and to help constrain the atmospheric ^{18}O–CO$_2$ budget (e.g., Farquhar et al., 1993). The $\delta_{L,e}$ can also be linked to organic matter isotopic compositions due to the fact that ^{18}O exchange occurs between water and carbonyl oxygen in triose phosphates (Sternberg et al., 1986).

More directly, knowledge of δ_T, together with the isotopic composition of evapotranspiration (δ_{ET}) and evaporation (δ_E), is vital for the success of evapotranspiration (*ET*) partitioning methods that depend on stable isotopes (Newman et al., 2010; Wang et al., 2010; Yepez et al., 2003). Direct estimates of δ_{ET} at ecosystem to landscape scale emerged in recent literature (Griffis et al., 2010; Wang et al., 2010; Welp et al., 2008; Williams et al., 2004), but direct estimation of δ_T remains rare, which may significantly hinder the accuracy of isotope-based *ET* partitioning. In principle, δ_T can be estimated using models developed for evaporating pools of water by Craig and Gordon (1965), who describe the isotopic composition of evaporation as a function of humidity, kinetic and equilibrium isotope fractionation, the isotopic composition of water of the evaporation surface, and atmospheric vapor. Practically, the isotopic composition of transpiration is usually assessed using stem water measurements, leaf water measurements including corrections for leaf enrichment (Yepez et al., 2003) or through biophysical modeling (Lai et al., 2005). Some exceptions include Harwood et al. (1998), who provided probably the first direct – but temporally coarse – δ_T observations using traditional isotope ratio mass spectrometry (IRMS) and cold trap method during a two-day campaign.

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* Corresponding author at: School of Civil and Environmental Engineering, University of New South Wales, Sydney, NSW, Australia. Tel.: +61 02 9385 5227.
E-mail address: w.lixin@gmail.com (L. Wang).

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The use of stem water as a proxy for the value of δ_T is based on the assumption that leaves operate under isotopic steady state so that δ_T is usually equal to the isotopic composition of plant source water. This assumption is generally valid for timescales much greater than the turnover time of water in the leaves and in the absence of rapidly changing environmental conditions because mass balance constraints require that the δ_T should be equal to that of the soil water in the rooting zone. However, on small timescales of minutes to hours, non-steady state isotopic enrichment is also common in many natural systems from leaf to canopy scales, especially during early morning and late afternoon (Dongmann et al., 1974; Flanagan et al., 1991; Griffis et al., 2011; Lai et al., 2005; Welp et al., 2008), when transpiration rates are lower. At present, little is known about how biotic and abiotic factors influence the temporal variations of δ_T at the timescales of minutes to hours because δ_T is rarely directly and continually quantified. The paucity of continuous δ_T observations under varying environmental conditions undermines the use of isotopes to understand vegetation–atmosphere interactions (e.g., $^{18}O–CO_2$ and $^{18}O–H_2O$ exchange) and to reconstruct paleoclimate and productivity parameters (e.g., $\delta_{L,e}$ estimates). The fact that indirect methods of quantifying δ_T are widely used is primarily due to the intensive labor and time involved in cryogenic water vapor collection and measurement using IRMS method and therefore the lack of fast-response sensors capable of resolving the isotopic composition of water vapor in ambient air. Water vapor samples are usually collected using cold-trap methods, which attempt to completely condense water vapor contained within an air sample for laboratory analysis. Even with recent advances for faster sampling (Helliker et al., 2002), traditional IRMS based cold-trap methods are still not capable of resolving values of δ_T at minute to hourly resolutions.

Over the past several years, laser-based isotope instruments capable of making continuous measurements of water vapor $\delta^{18}O$ and δ^2H have become available, with precision similar to traditional cryogenic-based mass spectrometry methods (Lee et al., 2005; Wang et al., 2009; Wen et al., 2008). The continuous measurement of water vapor $\delta^{18}O$ and δ^2H allows for the possibility of direct quantification of δ_T at time scales that are constrained only by the rate of vapor flux from leaves and size of observation chambers used to take leaf water vapor flux measurements.

Wang et al. (2010) reported the first continuous δ_T measurements using a customized leaf chamber and off-axis integrated cavity output spectroscopy (OA-ICOS) water vapor isotope analyzer within an atmosphere of pure nitrogen, which was used as purging gas. However, the water-free and CO_2-free inline environment used by Wang et al. (2010) can affect leaf stomatal behavior in ways that are difficult to interpret because humidity and CO_2 have opposite effects on stomatal function (Morison and Gifford, 1983). These shifts in leaf function would then likely alter δ_T values, so there remains a need for a method to make rapid observations of δ_T under ambient environmental conditions at high precision. To address this urgent need for δ_T monitoring approaches, we present a new method that provides – for the first time – direct and potentially continuous quantification of the isotopic composition of leaf transpiration. Our method is targeted towards application in field settings, and is based on the mass balance of water vapor isotopes inside a leaf chamber within a flow-through chamber system.

2. Materials and methods

2.1. Theoretical development

Our method is based on the mass balance of both bulk water vapor and the isotopes of water vapor (^{18}O or ^2H) within a leaf chamber and OA-ICOS system configured as a flow-through open system. The method fundamentally follows the basic gas exchange principles developed by Caemmerer and Farquhar (1981) and is similar to those used in prior CO_2 isotope mass balance approaches (Barbour et al., 2007; Wingate et al., 2010) and laboratory evaporation isotopic composition quantification (Kim and Lee, 2011). For bulk water vapor within a confined chamber, the change in concentration C_C [mol m^{-3}] with time inside a constant volume V_C [m^3] is described by

$$V_C \frac{dC_c}{dt} = q_A C_A + A_L T - q_M C_M. \tag{1}$$

Ambient air flows into the chamber with a flow rate q_A [m^3 s^{-1}] and concentration C_A [mol m^{-3}], with C_A assumed invariant before and during the measuring period. The incoming ambient water vapor mixes with transpired water vapor leaving a leaf surface of area A_L [m^2] with flux rate T [mol m^{-2} s^{-1}]. The mixed air then enters the analyzer with flow rate q_M [m^3 s^{-1}] and concentration C_M [mol m^{-3}]. Finally, high flow rates, small chambers, and small tubing sizes and lengths allow us to assume that the leaf chamber and laser chamber form a well mixed volume such that $C_C = C_M$, with V_C the combined volume of both chambers.

Measurements are made by observing ambient conditions with an empty chamber, then placing a transpiring leaf sample in the chamber and allowing the system to come to a new steady state. Under the steady state conditions, $dC_c/dt = 0$ and Eq. (1) is simplified to

$$T = \frac{1}{A_L}(q_M C_M - q_A C_A). \tag{2}$$

Thus the transpiration rate of a leaf in the chamber (T) can be fully resolved with knowledge of the leaf area, ambient conditions and chamber conditions.

Eq. (1) can be integrated for the non-steady state by assuming that when the leaf is initially inserted into the chamber ($t = 0$), the chamber vapor concentration is equal to that of ambient air ($C_M = C_A$). Integrating Eq. (1) with this initial condition and rearranging leads to

$$T = \frac{q_M C_M}{A_L}\left(\frac{1 - \frac{q_A C_A}{q_M C_M} - \left(\frac{C_A}{C_M} - \frac{q_A C_A}{q_M C_M}\right)\exp\left(\frac{-q_M}{V_C}t\right)}{1 - \exp\left(\frac{-q_M}{V_C}t\right)}\right), \tag{3}$$

where t is time after the leaf has been inserted into the chamber. Full development of non-steady state equations is presented in Appendix A.

For the rare isotopes (^2H and ^{18}O) of water vapor inside the leaf chamber, changes with time are described by

$$V_C \frac{d(C_C R_C)}{dt} = q_A C_A R_A + A_L T R_T - q_M C_M R_M, \tag{4}$$

where R_C is the ratio between rare and abundant isotopes ($^2H/^1H$ or $^{18}O/^{16}O$) of water vapor inside the chamber, R_A is the isotope ratio of ambient water vapor, R_T is the isotope ratio of leaf transpired water, and R_M is the isotope ratio of the mixed water vapor.

As with bulk vapor, we assume that the leaf chamber and laser chamber form a well-mixed volume such that $C_C = C_M$ and $R_C = R_M$ within a volume V_C. For steady state conditions, $dR_C C_C/dt = 0$ and combining Eqs. (4) and (2) results in

$$R_T = \frac{C_M R_M q_M - C_A R_A q_A}{C_M q_M - C_A q_A}, \tag{5}$$

which can be expressed in delta notation using the definition $R = R_{std}(1 + \delta)$:

$$\delta_T = \frac{C_M \delta_M q_M - C_A \delta_A q_A}{C_M q_M - C_A q_A}. \tag{6}$$

For non-steady state conditions, Eq. (4) can be expanded using the product law. The expanded equation can be rearranged, combined with Eq. (1), and expressed in delta notation to attain instantaneous estimates of δ_T:

$$\delta_T = \frac{V_C \delta_M \frac{dC_M}{dt} + V_C C_M \frac{d\delta_M}{dt} - q_A C_A \delta_A + q_M C_M \delta_M}{V_C \frac{dC_M}{dt} - q_A C_A + q_M C_M}. \qquad (7)$$

To solve Eq. (4) analytically, the expanded expression can be combined with Eq. (1). The resulting equation, Eq. (A.4), is then integrated following a similar derivation to that of bulk water vapor, using initial condition at $t = 0$ of $C_M = C_A$. The result (full development of non-steady state equations is presented in Appendix A) is then rearranged to obtain

$$R_T = \frac{q_A C_A (R_M - R_A) + A_L T \left(R_M - R_A \exp\left(-\frac{q_A C_A + A_L T}{V_C C_M} t \right) \right)}{A_L T \left(1 - \exp\left(-\frac{q_A C_A + A_L T}{V_C C_M} t \right) \right)}, \qquad (8)$$

which can be expressed in delta notation as

$$\delta_T = \frac{q_A C_A (\delta_M - \delta_A) + A_L T \left(\delta_M - \delta_A \exp\left(-\frac{q_A C_A + A_L T}{V_C C_M} t \right) \right)}{A_L T \left(1 - \exp\left(-\frac{q_A C_A + A_L T}{V_C C_M} t \right) \right)}. \qquad (9)$$

By combining Eq. (3) with (9) we are able to identify the isotopic composition of transpired water vapor directly, utilizing only knowledge of the chamber conditions before and after a leaf sample is inserted.

Because system volume is conserved and chamber pressure remains constant, the flow rate of air into the chamber is equal to the flow rate of air out of the chamber ($q_A = q_M$) during leaf measurements. Eq. (2) may then be simplified to

$$T = \frac{q_A}{A_L} (C_M - C_A). \qquad (10)$$

Similarly, Eq. (3) may also be simplified to

$$T = \frac{q_M C_M}{A_L} \left(\frac{1 - \left(\frac{C_A}{C_M} \right)}{1 - \exp\left(\frac{-q_M}{V_C} t \right)} \right). \qquad (11)$$

Finally, using the same set of assumptions, Eq. (6) can be simplified to

$$\delta_T = \frac{C_M \delta_M - C_A \delta_A}{C_M - C_A}. \qquad (12)$$

Thus under steady state conditions, only isotopic ratios and water vapor concentrations are required to estimate the δ_T signal.

2.2. Equipment configuration

During measurements, the transpiration chamber, water vapor isotope analyzer (WVIA) and vacuum pump were connected in series. Ambient air containing water vapor (C_A, q_A, δ_A) was drawn into a small chamber with a transpiring leaf inside. Within the chamber the ambient water vapor mixed with transpired water vapor (A_L, T, δ_T) and this mixture (C_M, q_M, δ_M) was drawn into the water vapor isotope analyzer. The experimental setup, used for both field and laboratory measurements, is shown in Fig. 1. The WVIA was continuously running and the chamber was manually opened and closed to obtain ambient and mixed air measurements with 10 min/measurement. The calibration of WVIA using a dew-point generator approach followed Wang et al. (2009) and values of C_A, C_M, δ_A and δ_M were measured using the calibrated WVIA.

All of the field and laboratory measurements were conducted using a commercially available OA-ICOS water vapor isotope analyzer (WVIA, DLT-100, Los Gatos Research, Mountain View, CA, USA) and a transparent leaf chamber modified from a LI-COR conifer chamber (part no. 6400-05, LI-COR Biosciences, Lincoln, NE, USA).

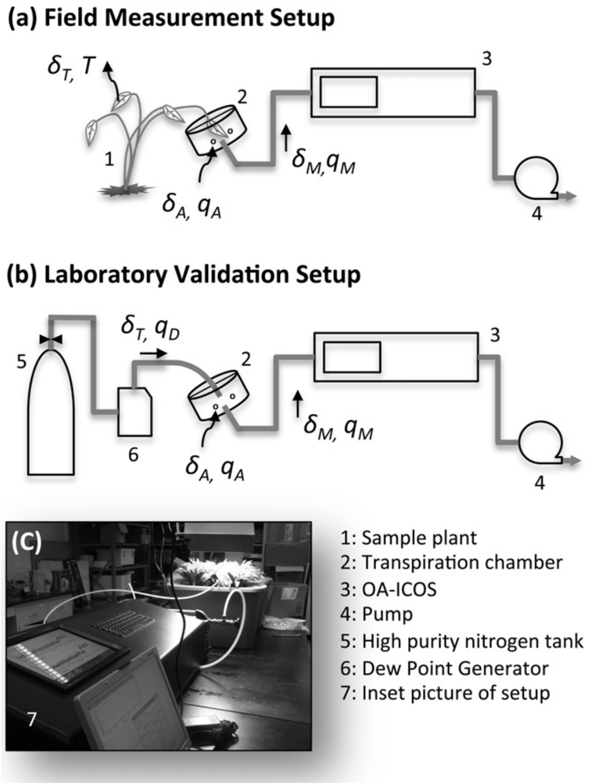

Fig. 1. Schematic of the experimental setup for directly quantifying leaf transpiration isotopic composition in field and laboratory settings. During field measurements (a) leaves of the plant to be sampled (1) are placed in the transpiration chamber (2), which is connected directly to the water vapor isotope analyzer (off-axis integrated cavity output spectroscopy, OA-ICOS) (3), and a vacuum pump (4). During laboratory validation measurements (b), a high purity nitrogen tank (5); is connected to a portable dew point generator (6). The output of the dew point generator is then placed into the transpiration chamber, mimicking a transpiring leaf. Panel c shows a photo of experimental setup.

The chamber is made of Teflon lined transparent plastic and has a volume of 150 cm^3, for a residence time of 18 s at a flow rate of 500 cm^3/min. The chamber is made of two half cylinders that join at a neoprene gasket, allowing for leaf samples to be placed inside the chamber while still connected to plant stems. The base plate of the chamber was removed and a 1/4″ brass bulkhead was installed to allow the WVIA inlet to connect to the chamber base (Fig. 2). The chamber has two air vents at the base that allow for ambient air to enter the chamber and mix with water vapor exiting the leaf stomata. Teflon tubing with an inner diameter of 1/8″ and tubing length of less than three feet was utilized. Therefore, the volume of tubing (less than 1.5 cm^3) was negligible for the volume calculations. Values of q_A and q_M were measured using a 1000 cm^3/min inline flow meter (Cole-Parmer, Vernon Hills, IL, USA).

2.3. Verification of δ_T methodology with a known vapor source

We verified that our experimental system was capable of matching predictions from Eqs. (3) and (9) by using a dew point generator (DPG, Licor 610, LICOR Biosciences, Lincoln, NE, USA) as an artificial leaf vapor source. As shown in Wang et al. (2009), the DPG can generate a continuous water vapor source with specific isotopic compositions using different combinations of dew point temperature setting and liquid water source. This setup is depicted in Fig. 1. The δ values of DPG generated water vapor (variable with time)

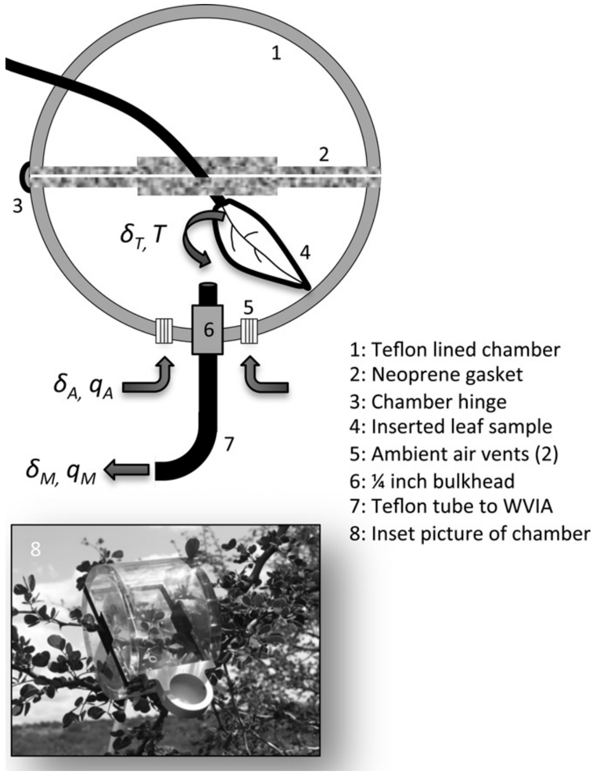

Fig. 2. Schematic of the leaf transpiration chamber. The chamber (1) is made up of two half cylinders made of transparent plastic with a Teflon coating. The two halves join at a neoprene gasket (2) and are connected by a hinge (3). Plant samples (4) are passed into the chamber through the gasket. Transpired water vapor from sample leaves mixes with ambient water vapor entering through two air vents (5). Chamber air is sucked out through a bulkhead (7) where Teflon tubing connects the chamber to the water vapor isotope analyzer. The part (8) shows a photo of the chamber setup.

were considered as true values and were compared with measured δ_T.

The DPG is designed to produce a constant flow of water vapor at specified saturation levels. Therefore the incoming vapor stream from the DPG includes both air and water. The simulated DPG transpiration (F_D, which is equivalent to A_LT for leaf measurements) is composed of a flow rate q_D of concentration C_D, resulting in a water vapor flow of $A_LT = F_D = q_DC_D$, with isotopic composition of δ_T (DPG generated "transpiration" isotopic composition). Because the transpiration signal is associated with a mass flow of air, the equality of $q_A = q_M$ observed under field conditions is not applicable. Instead, the flow rate from the DPG and the ambient air must equal that going into the WVIA (i.e., $q_A + q_D = q_M$). The q_A was estimated by measuring the difference between q_M and q_D.

The isotopic composition of DPG output water vapor was calculated using Rayleigh distillation theory and the details of such calculations were previously reported in Wang et al. (2009). The DPG was connected to an ultra-high purity nitrogen tank, which provided water-free input to the DPG. To determine δ_T, the ambient air water vapor concentration (C_A), air flow rate ($q_A = q_M - q_D$) and water vapor isotopic compositions (δ_A) were measured for 5 min prior to addition of the water vapor flux generated by the DPG. After the 5-min ambient condition reading, the outlet tube from the DPG was inserted into the chamber as if it were a leaf on a live stem. The mixture of DPG generated water vapor and ambient moisture then entered the WVIA, resulting in an increase in the H_2O level and changes in the isotopic composition. The mixed water vapor concentration (C_M), air flow rate (q_M) and water vapor isotopic compositions (δ_M) were then measured. The last 2 min of each 5-min period were used to estimate concentrations and isotopic compositions. Six independent measurements were conducted. The steady state δ_T was calculated with Eq. (12) using measured C_A, C_M, δ_A, δ_M, q_D and q_M values. The modeled 1 Hz δ_M from Eq. (A.8) was compared with observed δ_M to verify Eq. (9).

2.4. *Observations of the δ_T response to rapid environmental changes*

Six mature tropical foliage plants (*Spathiphyllum spp.*) were transplanted into a hydroponic system including multi-spectrum lighting (SH Hydroponics, Inc., Saline, MI, USA) in November 2010. The system has a capacity of 16-gallons to minimize isotopic changes in source water caused by evaporation. Bi-weekly monitoring of water isotopic composition within the 16-gallon hydroponic reservoir indicated negligible changes during the period from October to December, 2010. The δ_T of three individuals was measured under full light (400 W/m^2) and immediately after the light was shut off to test our system's ability to observe rapid δ_T responses to environmental perturbations. For each measurement, in a manner similar to the DPG measurements, 5 min were used to collect ambient conditions, and then the chamber was placed on the leaves for 5 min. The averages over the last 2 min of each 5-min period were used to estimate concentrations and isotopic compositions. Transpiration rates were also measured for light and dark conditions on the same leaves using a Licor 6400 (LI-COR Biosciences, Lincoln, NE, USA). All measurements were taken between 2 and 4 pm over two days in January 2011.

2.5. *Kenyan savanna field measurements*

A set of field measurements was obtained in July 2010 in an African savanna ecosystem located at the Mpala Research Center in central Kenya (36°52′ E, 0°29′ N). Two common tree species (*Acacia mellifera* and *Acacia tortollis* (Forskk.) Hayne) were selected for field measurements. Six midday measurements were made on three *A. tortollis* and four measurements were made on two *A. mellifera*. The selected trees had basal diameters ranging from 8 to 15 cm. The field procedure was very similar to the laboratory verification process, with 5-min ambient (chamber open) and 5-min mixed (chamber closed with leaves inside) measurements taken from sun-lit leaves. The average over the last 2 min of each 5-min period was used to estimate steady state δ_T using Eq. (12). The 1 Hz δ_T estimates were calculated using Eq. (7) and are shown in Fig. 8A. Ten-second moving averages were applied to the dC_M/dt and $d\delta_M/dt$ values. Small branches were selected to avoid vapor saturation inside the leaf chamber and subsequent condensation. Samples of ten individual rainfall events were collected between May and July 2010 at the field site and were measured for δ^2H and $\delta^{18}O$ compositions using the WVIA and a water vapor isotope standard source (Los Gatos Research, Mountain View, CA), which completely vaporizes a droplet (<1 μL) of water (e.g., without inducing fractionation). Trees in this landscape do not have access to near-surface groundwater. Therefore, their water source is likely from rainfall, and the rainfall isotopic compositions in these two months should approximate the isotopic compositions of vegetation source water, providing a constraint for δ_T measurements.

3. Results and discussion

The laboratory verification generally showed satisfactory results for using this method to directly quantify δ_T. Fig. 3A–C depicts observed water vapor dynamics before and after the insertion of "artificial" leaves (DPG output tubing) inside the leaf chamber.

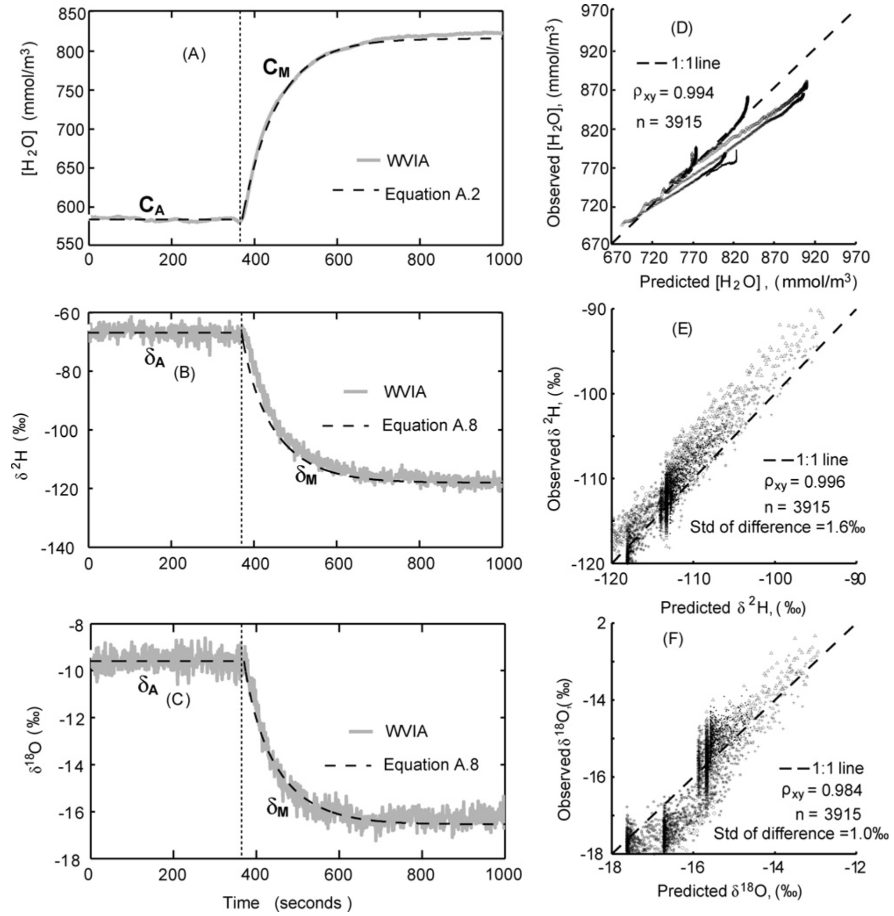

Fig. 3. Typical measurements (gray solid line) and modeling results (black dashed line) of the dynamics in water vapor concentrations (A), δ^2H (B) and δ^{18}O (C) values before and after putting artificial leaves (dew point generator) inside leaf chamber at the laboratory settings at 1 Hz sampling rate. The dotted lines indicate the timing of "leaf" insertion. Observations vs. predicted values and 1:1 lines for water vapor concentrations (D), δ^{18}O (E) and δ^2H (F) under laboratory conditions for all the trials.

Observations closely matched the predicted values (C_M) from Eq. (3) ($R^2 = 0.99$, $p < 0.01$, Fig. 3A). Changes in δ^{18}O and δ^2H compositions of the water vapor (δ_M) including the transient stage immediately after the DPG tube insertion were also successfully modeled ($R^2 = 0.99$, $p < 0.01$, Fig. 3B and C). Fig. 3D–F provides comparisons between 1 Hz observations and predictions for water vapor concentration (C_M, Fig. 3D), δ^2H (δ_M, Fig. 3E) and δ^{18}O (δ_M, Fig. 3F) for all the runs. The correlations between the 1 Hz true and predicted values were all larger than 0.98 ($p < 0.001$). More importantly, with the assumption of constant ambient conditions (C_A and δ_A), we were able to predict the δ^{18}O and δ^2H composition of simulated leaf transpired water vapor at a 1 Hz sampling rate (see Fig. 8A for calculated 1 Hz δ_T based on the field data). The observed values agreed well with the true values based on expected values of water vapor isotopic composition from the DPG. Across all DPG runs, only minimal bias was found between observed and predicted values (δ_M): 0.7‰ for δ^2H (Fig. 4A) and 0.4‰ for δ^{18}O (Fig. 4B). Under steady state conditions as described by Eq. (12), the departure of measured δ^{18}O composition from true values ranged from −0.4‰ to −1.7‰, with a standard deviation of 1.0‰ (Table 1, Fig. 3F), while the departure of δ^2H composition from true values ranged from −0.4‰ to 2.3‰, with a standard deviation of 1.6‰ (Table 1, Fig. 3E).

The laboratory light experiment using hydroponic plants revealed a rapid leaf response to light for both δ_T and transpiration

Table 1
Expected and measured isotopic values (δ_T) during validation with a known vapor source (mean μ, and standard deviation σ, also reported).

Trial number	δ^{18}O (‰)		Diff (‰)	δ^2H (‰)		Diff (‰)
	True	Measured		True	Measured	
1	−20.5	−18.8	−1.7	−142.3	−140.7	−1.6
2	−20.3	−19.2	−1.2	−140.9	−140.5	−0.4
3	−20.0	−19.1	−0.9	−138.3	−138.0	−0.3
4	−19.7	−18.9	−0.7	−140.9	−139.7	−1.1
5	−19.6	−20.8	1.2	−140.3	−142.5	2.3
6	−19.4	−19.0	−0.4	−139.2	−141.1	1.9
μ (σ)			−0.6 (1.0)			0.13 (1.6)

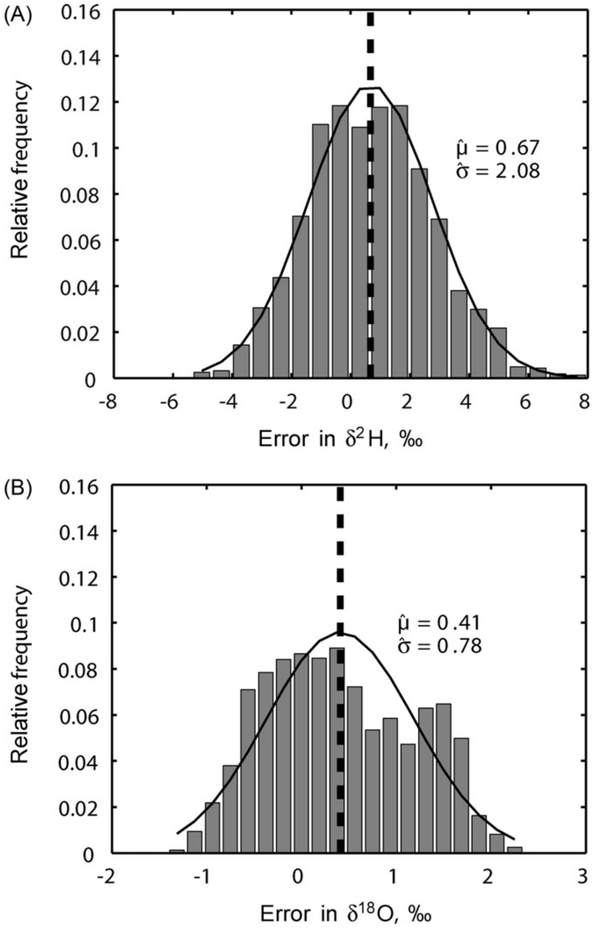

Fig. 4. The error distributions of the transpiration isotopic measurements (δ_M) for δ^2H (A) and δ^{18}O (B) respectively for all trials. The solid lines are fittings of normal distribution with mean value μ and standard deviation σ. The dashed vertical lines are average error between observed and predicted values.

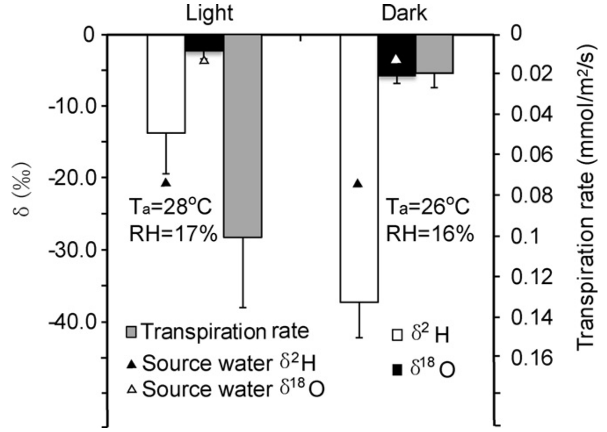

Fig. 5. Rapid responses of δ_T (white boxes for δ^2H and black boxes for δ^{18}O) and transpiration rates (gray boxes) to lighting conditions under laboratory settings. The isotopic compositions of source water (black triangles for δ^2H and white triangles for δ^{18}O) were also shown. The plants were growing in hydroponic system between November 2010 and January 2011.

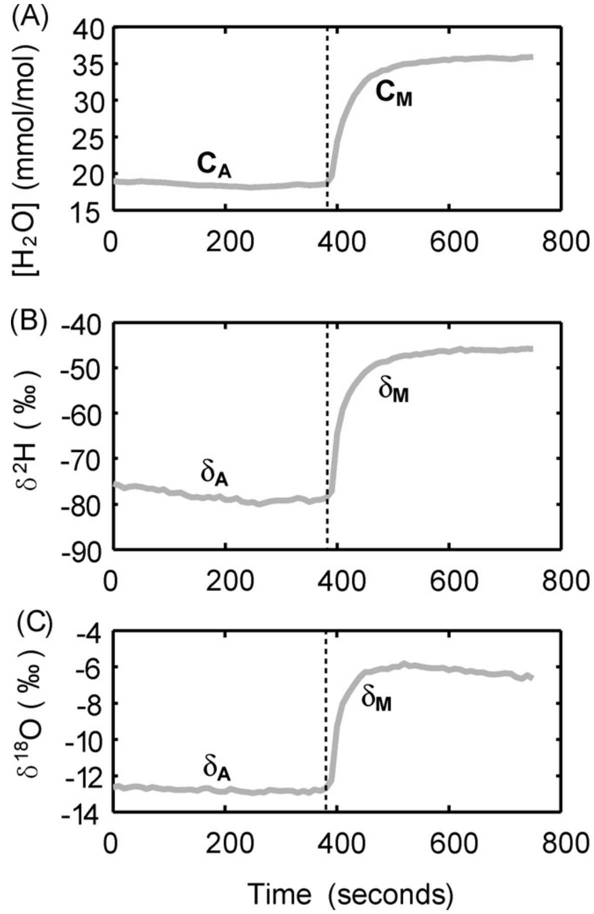

Fig. 6. Typical measurements of the changes in water vapor concentrations (A), water vapor δ^{18}O (B) and δ^2H (C) before and after leaf insertion inside the leaf chamber at the Kenya field settings. The dotted lines indicate the timing of leaf insertion.

rate (Fig. 5). Both δ^{18}O and δ^2H of δ_T were slightly enriched compared to source water and became more depleted as transpiration rates decreased after turning the light off (Fig. 5). A likely explanation for this pattern is related to the changes in vapor concentration of the leaf cuvette. Under the light, transpiration rate is higher and therefore the increase in vapor concentration surrounding the leaf is larger when the leaf is placed in the cuvette. Under these conditions, the leaf water begins depleting in heavy isotopes and the transpired vapor will therefore be enriched. When the lights are turned off, the transpiration rate decreases, vapor concentration in the cuvette decreases, and the leaf water begins enriching in heavy isotopes. At this time the transpired vapor is necessarily depleted in heavy isotopes compared to source water. Such rapid δ_T responses – which can only be captured using direct δ_T measurements – will likely occur in response to other environmental perturbations which affect leaf water isotope composition over short time scales (e.g., changes in incoming radiation).

Field based measurements of the isotopic composition of transpired water also demonstrated success. Fig. 6A–C depicts a typical field measurement of water vapor δ^{18}O and δ^2H. Fig. 7 shows all the field results and rainfall isotopic compositions in the corresponding period. As expected, an increase of water vapor concentrations was observed after the insertion of leaves inside the leaf chamber (Fig. 6A). Due to the small size of the leaf chamber, water

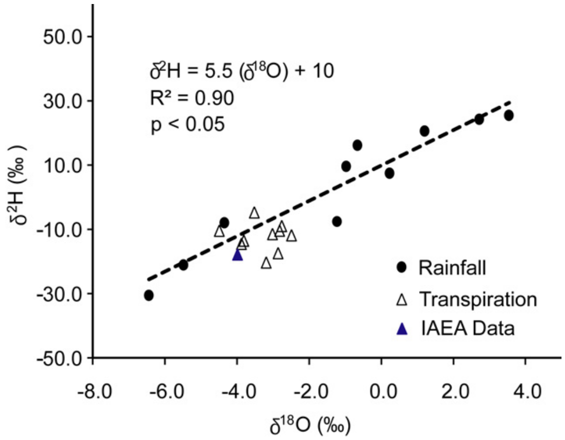

Fig. 7. Measured isotopic compositions of rainfall (black dots) and leaf transpired water (open triangles) in central Kenya. The dashed black line is the Local Meteoric Water Line determined via least-squares fitting of the rainfall isotope values. The blue triangle indicates annual volume-weighted rainfall isotopic compositions from IAEA sites in central Kenya.

vapor concentrations reached steady state after only 1 min (Fig. 6A). Changes in δ^{18}O and δ^2H compositions of the water vapor were also observed after the insertion of leaves inside the leaf chamber (Fig. 6B and C). Based on the isotopic changes and water vapor concentration changes, we were able to directly calculate the steady state δ^{18}O and δ^2H composition of leaf transpired water, which was −16.9‰ and −4.1‰ (Fig. 7) for δ^2H and δ^{18}O, respectively. The isotopic composition of rainfall between May and July 2010 ranged from −30.5‰ to 25.4‰ for δ^2H and ranged from −6.4‰ to 3.5‰ for δ^{18}O (Fig. 7). The values of isotopic composition of field measured leaf transpiration generally followed the Local Meteoric Water Line (Fig. 7), but were within a much smaller range than rainfall ($-20.8‰ < \delta^2H < -4.5‰$ and $-4.5‰ < \delta^{18}O < -2.5‰$). Though these results do not completely validate our method, these measurements provide a proof-of-concept of the application of our method under field conditions. In addition, annual volume-weighted rainfall isotopic compositions from IAEA sites in central Kenya, which likely represent long-term plant source water, are −18‰ and −4‰ for δ^2H and δ^{18}O (www-naweb.iaea.org) and are very similar to our transpiration measurements (Fig. 7). This supports our assumption that these two tree species exclusively rely on rainfall during the May–July period.

As far as we know, Wang et al. (2010) provided the first report of continuous measurements of δ_T using an ICOS system inside a greenhouse-like facility (Biosphere 2 in Arizona, US). Though direct, the prior method has two shortcomings: (1) the method relies on ultra-purity nitrogen as purging gas to provide a water-free environment before the measurements, which may not be readily available for field applications, especially at rural sites; (2) the H_2O-free and CO_2-free inline environment used by Wang et al. (2010) will affect stomata openings, which could impact the isotopic composition of transpiration. The new approach we present here allows for measurements to be taken under ambient conditions, making it much more suitable than past approaches for field applications and for understanding the instantaneous responses of δ_T to natural ranges of environmental variability. Because our approach is based on a flow-through system, the leaf environment does not change significantly (relative humidity will increase) during the short measuring period (5–10 min), which results in more accurate measurements. The similarity of initial instantaneous calculations of δ_T and steady state δ_T calculations validates this conclusion. Our new method also does not require extraction of stem water for IRMS measurement to attain both steady state and instantaneous δ_T estimates. The combination of rapid, direct observations of transpiration isotopic composition with minimal disturbance to the transpiring leaf will significantly increase the efficiency of δ_T measurements in the future. The estimated precision of δ_T measurements is 1.0‰ and 1.6‰ for δ^{18}O and δ^2H respectively (Table 1). This precision is close to other laser-based systems (Kim and Lee, 2011; Lee et al., 2007). However, the precisions of our δ_T measurements are lower than the WVIA itself (0.1‰ for δ^{18}O and 1 for δ^2H), as reported earlier (Wang et al., 2009) especially for δ^{18}O. We are unable to determine the exact cause for the lower precision of δ^{18}O but suspect that it may be linked to the different adsorption of oxygen atoms to the leaf chamber wall, compared with hydrogen atoms.

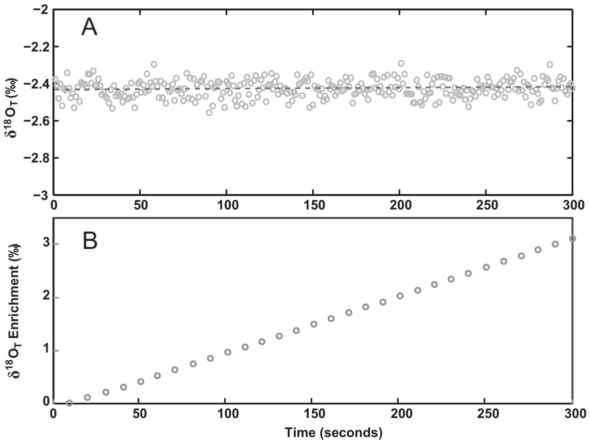

Fig. 8. The observed continuous (1 Hz) δ_T measurements (δ^{18}O) after the closure of leaf cuvette (A), in field conditions (central Kenya) using Eq. (7) and the modeled result of the transpiration enrichment (δ^{18}O) due to changes in relative humidity with leaf temperature of 30°C (B). The dashed line in (A) represents the mean.

The current δ_T quantification method relies on the assumption that ambient conditions (C_A and δ_A) do not change during the mixing measurement period. Although we believe this will be true in most cases as the measuring period is short (~5 min), it would be relatively easy to remove this limitation by alternating the ambient and mixed air measurements more frequently. Another uncertainty of this method is the effect of the leaf cuvette on leaf transpired isotopic compositions, that is, how similar the chamber measured transpired water vapor isotopic compositions are to the transpired water vapor isotopic compositions for leaves not enclosed in the chamber. To address this issue, we reported the continuous δ_T measurements (1 Hz) under field conditions following the leaf enclosure (Fig. 8A). As the pattern showed, the δ_T did not change significantly during the 5-min measurement (Fig. 8A). We also used a modeling approach to address this issue. The major change that takes place when the leaf is enclosed in the cuvette is the increase in water vapor concentration inside the cuvette. In addition, the isotopic composition of the vapor the leaf is exposed to can change, as can leaf temperature. To demonstrate the predicted dynamics, we therefore modeled the effect of increasing chamber water vapor concentration on δ_T following Eqs. (13) and (14), based on Farquhar and Cernusak (2005) and Cuntz et al. (2007):

$$\Delta_L(t+\mathrm{d}t) = \Delta_{Ls} + [\Delta_L(t) - \Delta_{Ls}]\,\exp\left\{-\frac{gw_i}{\alpha^{+}\alpha_k W c_1}\mathrm{d}t\right\}, \qquad (13)$$

$$\frac{\mathrm{d}(W\cdot\Delta_L)}{\mathrm{d}t} = -T\Delta_T. \qquad (14)$$

In these equations, t is time, Δ_L is the isotope ratio of leaf water relative to source water, Δ_{Ls} is the steady state isotope ratio of leaf water relative to source water, W is leaf water volume (mol m^{-2}), g is total conductance to water vapor (mol m^{-2} s^{-1}), w_i is water vapor mole fraction in the stomatal cavity (mol (H$_2$O) mol (air)$^{-1}$), α^+ is the equilibrium water vapor fractionation factor, α^k is the kinetic fractionation factor, Δ_T is the isotope ratio of transpiration relative to source water, and T is transpiration rate (mol m^{-2} s^{-1}). The term c_1 is a constant, and was taken as $(1-e^{-P})/P$, where P is a Péclet number. This definition of c_1 assumes constant leaf water volume (Cuntz et al., 2007).

To evaluate the effect of the leaf chamber on δ_T values we first calculated leaf water enrichment using Eq. (13) and then used Eq. (14) to calculate transpired water vapor enrichment. We set up a linear increase in air relative humidity surrounding the leaf from about 36% to about 42% over 5 min, assuming a constant leaf temperature of 30 °C. The leaf water was initially at isotopic steady state in the model and so transpired water vapor enrichment was zero. The model predicted increasing enrichment of transpired water up to about 3.5‰ after 5 min with increasing water vapor concentration in air surrounding the leaf (Fig. 8B). We did not observe a similar pattern in the 1 Hz estimates of transpired water vapor δ^{18}O. A possible explanation is that as the vapor concentration inside the chamber increased, the leaf temperature may also have increased. In this case, the water vapor mole fraction inside the leaf would also increase, and the net effect may have been that w_a/w_i, the ratio of water vapor mole fraction in the cuvette to that inside the leaf, may have remained approximately constant. Unfortunately, key parameters for testing this explanation, such as leaf temperature and photosynthetically active radiation, were not measured in the field along with the leaf transpiration isotopic composition. However, we have set the stage for rigorous comparisons and the development of a more advanced cuvette system capable of characterizing these data in concert with leaf transpiration isotopic composition measurements will allow for much more rigorous comparisons between model predictions and observations. At the same time, we did not see clear enrichments during our measurements, indicating the current method is suitable for obtaining estimates of δ_T that are not biased by the enclosure of the leaf in the cuvette.

In summary, we have presented a novel method to directly and efficiently quantify δ_T that is targeted at obtaining measurements under field settings and ambient conditions. The method is based on fundamental solutions to the isotopic mass balance within a chamber containing a transpiring leaf and has been verified using artificial leaves. We applied our method to obtain direct measurements of the isotopic composition of transpiration under field conditions in central Kenya, and also demonstrated that the method can capture rapid δ_T changes in response to environmental fluctuations that occur on the time scale of seconds to minutes. Our method requires only a commercially available laser-based water vapor isotope analyzer and a transparent chamber and provides an efficient and simple way to directly measure leaf transpiration isotope signals in the field. A simple extension of our approach would allow the same concept to be applied for direct soil evaporation signal (δ_E) measurements. Potential future development would include: (1) adding a buffer volume before air intake to dampen the natural variation of water vapor concentrations and isotopic compositions in ambient air for more stable signal, and (2) adding a solenoid switch to alternate the ambient and mixed air sampling more frequently for more accurate background monitoring. We believe the more general application of our approach will enhance our ability to address a wide range of questions in ecohydrological research regarding how evapotranspiration is partitioned at the land surface, how the isotopic composition of transpiration fluxes depends on environmental factors, and the degree to which plants operate at isotopic steady state under typical environmental conditions.

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Appendix A. Non-steady state equation derivation

A.1. Dynamics of bulk water vapor

The dynamics of the concentration of bulk water within the chamber C_C, [mol m^{-3}] are given by Eq. (1):

$$V_C \frac{\mathrm{d}C_C}{\mathrm{d}t} = q_A C_A + A_L T - q_M C_M,$$

where V_C is the volume of the chamber [m^3] and C_C is the water vapor concentration in the chamber [mol m^{-3}]. The ambient airflow rate and water vapor concentrations are given by q_A [m^3 s^{-1}] and C_A [mol m^{-3}], and the flow rate and concentration of mixed air leaving the chamber to the analyzer are q_M [m^3 s^{-1}] and C_M [mol m^{-3}]. Water vapor flux from a leaf of area A_L [m^2] into the chamber is given by T [mol s^{-1} m^{-2}]. We assume that the leaf chamber and laser chamber form a well mixed volume such that $C_C = C_M$. With these assumptions Eq. (1) is rearranged in terms of C_M such that

$$\frac{\mathrm{d}C_M}{C_M + \frac{q_A C_A + A_L T}{-q_M}} = \frac{-q_M}{V_C}\mathrm{d}t. \tag{A.1}$$

For the non-steady state condition, Eq. (A.1) is integrated with initial condition such that when the leaf is inserted (at time $t = 0$) the chamber concentration is that of ambient air ($C_A = C_M$) so that

$$C_M(t) = \frac{q_A C_A + A_L T}{q_M} + \left(C_A - \frac{q_A C_A + A_L T}{q_M}\right)\exp\left(-\frac{q_M}{V_C}t\right), \tag{A.2}$$

where t is time after the leaf has been inserted in the chamber. Eq. (A.2) is rearranged to obtain Eq. (3):

$$T = \frac{q_M C_M}{A_L}\left(\frac{1 - \frac{q_A C_A}{q_M C_M} - \left(\frac{C_A}{C_M} - \frac{q_A C_A}{q_M C_M}\right)\exp\left(\frac{-q_M}{V_C}t\right)}{1 - \exp\left(\frac{-q_M}{V_C}t\right)}\right)$$

A.2. Dynamics of rare isotopes (^{18}O and ^{2}H)

For the rare isotopes (^{18}O and ^{2}H) of water vapor inside the leaf chamber, the isotopic changes with time inside the leaf chamber are described by Eq. (4):

$$V_C \frac{\mathrm{d}(C_C R_C)}{\mathrm{d}t} = q_A C_A R_A + A_L T R_T - q_M C_M R_M,$$

where R_C is the ratio between rare and abundant isotopes (^{2}H/^{1}H or ^{18}O/^{16}O) of water vapor inside the chamber, R_A is the ratio of ambient water vapor, R_T is the ratio of leaf transpired water, and R_M is the ratio of the mixed water vapor. As with bulk vapor, we assume that the leaf chamber and laser chamber form a well mixed volume such that $C_C = C_M$ and $R_C = R_M$. Using the product law and the same assumptions as above, Eq. (4) becomes:

$$V_C R_M \frac{\mathrm{d}(C_M)}{\mathrm{d}t} + V_C C_M \frac{\mathrm{d}(R_M)}{\mathrm{d}t} = q_A C_A R_A + A_L T R_T - q_M C_M R_M. \tag{A.3}$$

Combining Eqs. (1) and (A.3), we obtain

$$R_M(q_AC_A + A_LT - q_MC_M) + V_CC_M\frac{\mathrm{d}(R_M)}{\mathrm{d}t} = q_AC_AR_A + A_LTR_T - q_MC_MR_M, \tag{A.4}$$

which is rearranged and integrated to form

$$\ln\left(R_M - \frac{q_AC_AR_A + A_LTR_T}{q_AC_A + A_LT}\right) = \frac{q_AC_A + A_LT}{V_CC_M}t + \xi, \tag{A.5}$$

where ξ is the constant of integration. We then raise with base e and use a new constant of integration $\xi = e_\xi$ so that

$$R_M - \frac{q_AC_AR_A + A_LTR_T}{q_AC_A + A_LT} = \xi' e^{-\frac{q_AC_A + A_LT}{V_CC_M}t}. \tag{A.6}$$

When $t = 0$, we solve for ξ and obtain

$$\xi' = R_M - \frac{q_AC_AR_A + A_LTR_T}{q_AC_A + A_LT}. \tag{A.7}$$

We note that when $t = 0$, we have $R_M = R_A$. Thus we combine Eqs. (A.6) and (A.7) to arrive at

$$R_M = \frac{q_AC_AR_A + A_LTR_T}{q_AC_A + A_LT} + \left(R_A - \frac{q_AC_AR_A + A_LTR_T}{q_AC_A + A_LT}\right)e^{-\frac{q_AC_A + A_LT}{V_CC_M}t}, \tag{A.8}$$

which is then solved for R_T, resulting in Eq. (7):

$$R_T = \frac{q_AC_A(R_M - R_A) + A_LT\left(R_M - R_A\exp\left(-\frac{q_AC_A + A_LT}{V_CC_M}t\right)\right)}{A_LT\left(1 - \exp\left(-\frac{q_AC_A + A_LT}{V_CC_M}t\right)\right)}.$$

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