Direct quantification of leaf transpiration isotopic composition

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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 D and δ 18O 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.

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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 δ = (R/Rstd − 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 Rstd 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 (δ T,wa ) within leaves (Ogée et al., 2007; Welp et al., 2008). The δ T,wa represents a critical boundary condition to estimate 18O–CO 2 photosynthetic discrimination and to help constrain the atmospheric 18O–CO 2 budget (e.g., Farquhar et al., 1993). The δ T,wa 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,wa 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 ET remains rare, which may significantly hinder the accuracy of isotopic-based ET partitioning. In principle, δ ET 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.
The use of stem water as a proxy for the value of $\delta_T$ is based on the assumption that leaves operate under isotopic steady state so that $\delta_T$ 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 $\delta_T$ 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 $\delta_T$ at the timescales of minutes to hours because $\delta_T$ is rarely directly and continually quantified. The paucity of continuous $\delta_T$ 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$_2$O exchange) and to reconstruct paleoclimate and productivity parameters (e.g., $\delta_{13}C$ estimates). The fact that indirect methods of quantifying $\delta_T$ 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 $\delta_T$ 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 $\delta^2$H 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 $\delta^2$H allows for the possibility of direct quantification of $\delta_T$ 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 $\delta_T$ 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 $\delta_T$ values, so there remains a need for a method to make rapid observations of $\delta_T$ under ambient environmental conditions at high precision. To address this urgent need for $\delta_T$ 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 $^2$H) 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 Gaemmerer 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

$$\frac{dC_C}{dt} = q_A C_A + A_T T - q_M C_M.$$  (1)

Air flow 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

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

$$T = \frac{q_M C_M}{A_L} \left( 1 - \frac{q_M C_A}{q_M C_M} - \frac{C_A}{C_M} + \frac{q_M C_A}{q_M C_M} \exp \left( -\frac{q_M t}{V_C} \right) \right),$$  (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 ($^2$H and $^{18}$O) of water vapor inside the leaf chamber, changes with time are described by

$$\frac{dC}{dt} = q_A C_A R_A + A_T R_T - q_M C_M R_M,$$  (4)

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 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, $dC_C/dt = 0$ and combining Eqs. (4) and (2) results in

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

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

$$\delta_T = \frac{C_M \delta M q_M - C_A \delta A q_A}{C_M q_M - C_A q_A}.$$  (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 $\delta t$:

$$\delta t = \frac{V_c \delta C_A}{\delta t} + V_c \frac{d M}{d t} - q A \delta A + q M C_{M} \delta M.$$  \hspace{1cm} (7)

To solve Eq. (4) analytically, the expanded expression can be combined with Eq. (1). The resulting equation, Eq. (A4), 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

$$R_T = \frac{q_A C_A (R_M - R_A) + A_T (R_M - R_d \exp (-q_M C_M t))}{A_T (1 - \exp (-q_M C_M t))},$$  \hspace{1cm} (8)

which can be expressed in delta notation as

$$\delta T = \frac{q_A C_A (\delta M - \delta A) + A_T (\delta M - \delta A \exp (-q_M C_M t))}{A_T (1 - \exp (-q_M C_M t))}.$$  \hspace{1cm} (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

$$T = \frac{q_A}{A_T} (C_M - C_A).$$  \hspace{1cm} (10)

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

$$T = \frac{q_M C_M}{A_T} \left( \frac{1 - (C_A/C_M)}{1 - \exp (-q_M C_M t)} \right).$$  \hspace{1cm} (11)

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

$$\delta T = \frac{C_M \delta M - C_A \delta A}{C_M - C_A}.$$  \hspace{1cm} (12)

Thus under steady state conditions, only isotopic ratios and water vapor concentrations are required to estimate the $\delta T$ 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 ($C_A, q_A, \delta A$) was drawn into a small chamber with a transpiring leaf inside. Within the chamber the ambient water vapor mixed with transpired water vapor ($A_T, T, \delta T$) and this mixture ($C_M, q_M, \delta_M$) 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 dewpoint generator approach followed Wang et al. (2009) and values of $C_A, C_M, \delta A$ and $\delta_M$ 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).

![Field Measurement Setup](image1.png)

![Laboratory Validation Setup](image2.png)

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$^2$/min inline flow meter (Cole-Parmer, Vernon Hills, IL, USA).

2.3. Verification of $\delta T$ 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 $\delta$ values of DPG generated water vapor (variable with time)
leaves to inspiration from prior calculated vapor going two generated the mixture is 5-min qM, qA, qD, qM, qD and qM values. The modeled 1 Hz δM from Eq. (A.8) was compared with observed δM to verify Eq. (9).

2.4. Observations of the δT response to rapid environmental changes

Six mature tropical foliage plants (Spathiphylum 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 δT of three individuals was measured under full light (400 W/m²) and immediately after the light was shut off to test our system's ability to observe rapid δT 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 tortilla (Forsk.) Hayne) were selected for field measurements. Six midday measurements were made on three A. tortilla 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 δT using Eq. (12). The 1 Hz δT estimates were calculated using Eq. (7) and are shown in Fig. 8A. Ten-second moving averages were applied to the d18O and d34S 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 δ18O and δ34S 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 δT measurements.

3. Results and discussion

The laboratory verification generally showed satisfactory results for using this method to directly quantify δT. Fig. 3A–C depicts observed water vapor dynamics before and after the insertion of “artificial” leaves (DPG output tubing) inside the leaf chamber.
Observations closely matched the predicted values ($C_M$) from Eq. (3) ($R^2 = 0.99, p < 0.01, \text{Fig. 3A}$). Changes in $\delta^{18}O$ and $\delta^2H$ compositions of the water vapor ($\delta_M$) including the transient stage immediately after the DPG tube insertion were also successfully modeled ($R^2 = 0.99, p < 0.01, \text{Fig. 3B and C}$). \text{Fig. 3D–F} provides comparisons between 1 Hz observations and predictions for water vapor concentration ($C_M$, \text{Fig. 3D}$), $\delta^2H$ ($\delta_M$, \text{Fig. 3E}$) and $\delta^{18}O$ ($\delta_M$, \text{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 $\delta_A$), we were able to predict the $\delta^{18}O$ and $\delta^2H$ composition of simulated leaf transpired water vapor at a 1 Hz sampling rate (see \text{Fig. 8A} for calculated 1 Hz $\delta_T$ 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 ($\delta_M$): 0.7‰ for $\delta^2H$ (\text{Fig. 4A}) and 0.4‰ for $\delta^{18}O$ (\text{Fig. 4B}). Under steady state conditions as described by Eq. (12), the departure of measured $\delta^{18}O$ composition from true values ranged from $-0.4\%$ to $-1.7\%$, with a standard deviation of 1.0‰ (Table 1, \text{Fig. 3E}). While the departure of $\delta^2H$ composition from true values ranged from $-0.4\%$ to $2.3\%$, with a standard deviation of 1.6‰ (Table 1, \text{Fig. 3F}).

The laboratory light experiment using hydroponic plants revealed a rapid leaf response to light for both $\delta_T$ and transpiration.

**Table 1**

<table>
<thead>
<tr>
<th>Trial number</th>
<th>$\delta^{18}O$ (‰)</th>
<th>Measured</th>
<th>Diff (‰)</th>
<th>$\delta^2H$ (‰)</th>
<th>Measured</th>
<th>Diff (‰)</th>
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<td>1.9</td>
</tr>
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<td>0.13 (1.6)</td>
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rate (Fig. 5). Both $\delta^{18}$O and $\delta^2$H of $\delta_T$ 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 $\delta_T$ responses – which can only be captured using direct $\delta_T$ 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 $\delta^{18}$O and $\delta^2$H. 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
vapor concentrations reached steady state after only 1 min (Fig. 6A). Changes in δ18O and δ2H 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 δ18O and δ2H composition of leaf transpired water, which was −16.9‰ and −4.1‰ (Fig. 7) for δ2H and δ18O, respectively. The isotopic composition of rainfall between May and July 2010 ranged from −30.5‰ to 25.4‰ for δ2H and ranged from −6.4‰ to 3.5‰ for δ18O (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‰ < δ2H < −4.5‰ and −4.5‰ < δ18O < −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 δ2H and δ18O (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 δT by 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 H2O-free and CO2-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 δT 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 δT and steady state δT 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 δT estimates. The combination of rapid, direct observations of transpiration isotopic composition with minimal disturbance to the transpiring leaf will significantly increase the efficiency of δT measurements in the future. The estimated precision of δT measurements is 1.0‰ and 1.6‰ for δ18O and δ2H respectively (Table 1). This precision is close to other laser-based systems (Kim and Lee, 2011; Lee et al., 2007). However, the precision of our δT measurements are lower than the WVIA itself (0.1‰ for δ18O and 1 for δ2H), as reported earlier (Wang et al., 2009) especially for δ18O. We are unable to determine the exact cause for the lower precision of δ18O but suspect that it may be linked to the different adsorption of oxygen atoms to the leaf chamber wall, compared with hydrogen atoms.

The current δT quantification method relies on the assumption that ambient conditions (C4 and δL) do not change during the mixing measurement period. Although we believe this will be true in most cases as the measuring period is short (5–5 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 δT measurements (1 Hz) under field conditions following the leave enclosure (Fig. 8A). As the pattern showed, the δT 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 δT following Eqs. (13) and (14), based on Farquhar and Cernusak (2005) and Cuntz et al. (2007):

\[ \Delta \delta(t + dt) = \Delta \delta_{L} = \left( \Delta \delta_{L}(t) - \Delta \delta_{L} \right) \exp \left\{ -\frac{g_{w} W c_{L}}{\alpha L c_{L} dt} \right\}, \]  

\[ \frac{d(W \cdot \Delta \delta)}{dt} = -T \Delta \delta. \]  

In these equations, t is time, ΔL is the isotope ratio of leaf water relative to source water, ΔδL is the steady state isotope ratio of leaf

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**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.

**Fig. 8.** The observed continuous (1 Hz) δT measurements (δ18O) after the closure of leaf cuvette (A), in field conditions (central Kenya) using Eq. (7) and the modeled result of the transpiration enrichment (δ18O) due to changes in relative humidity with leaf temperature of 30 °C (B). The dashed line in (A) represents the mean.
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$^{-1}$)), $\alpha$ is the equilibrium water vapor fractionation factor, and $\Delta T$ is the kinetic fractionation factor, $\Delta T$ is the isotopic ratio of transpiration relative to source water, and $T$ is transpiration rate (mol m$^{-2}$ s$^{-1}$). The term $c_i$ is a constant, and was taken as $1 - (1 - P)/P$, where $P$ is a Péclet number. This definition of $c_i$ assumes constant leaf water volume ($\text{Cuntz et al.}, 2007$).

To evaluate the effect of the leaf chamber on $\delta_T$ 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 $\delta^{18O}$. A possible explanation is that 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 $w_r/w_i$, 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 $\delta_T$ 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 $\delta_T$ 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 $\delta_T$ 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 ($\delta_E$) 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 hydrological 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 $C_T$ (mol m$^{-3}$) are given by Eq. (1):

$$\frac{dC_T}{dt} = q_A C_A + A_T T - q_M C_M.$$  

where $C_T$ is the volume of the chamber [m$^3$] and $C_T$ is the water vapor concentration in the chamber [mol m$^{-3}$]. The ambient airflow rate and water vapor concentrations are given by $q_A$ (mol s$^{-1}$) and $C_A$ (mol m$^{-3}$), and the flow rate and concentration of mixed air leaving the chamber to the analyzer are $q_M$ (mol 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 $C_T = C_M$. With these assumptions Eq. (1) is rearranged in terms of $C_M$ such that

$$\frac{dC_M}{dt} = -q_M \frac{dC_T}{dC_M} = \frac{-q_M dC_T}{dC_M} = \frac{-q_M dC_T}{dC_M}.$$  

(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

$$C_M(t) = \frac{q_A C_A + A_T T}{q_M} + \left( C_A - \frac{q_A C_A + A_T T}{q_M} \exp \left( \frac{-q_M t}{C_M} \right) \right).$$  

(A.2)

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

$$T = \frac{q_M C_M A_L}{A_T} \left( \frac{C_A - \frac{q_A C_A + A_T T}{q_M}}{C_M - \frac{q_A C_A + A_T T}{q_M}} \exp \left( \frac{-q_M t}{C_M} \right) \right).$$  

(A.2)

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

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

$$\frac{d(C_T R_C)}{dt} = \frac{q_A C_T R_A + A_T T R_T - q_M C_M R_M}{A_T},$$  

where $R_C$ is the ratio between rare and abundant isotopes ($^2H$/$^1H$ or $^{18O}$/$^{16O}$) 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 $C_T = C_M$ and $R_C = R_M$. Using the product law and the same assumptions as above, Eq. (4) becomes:

$$\frac{d(C_M R_M)}{dt} + \frac{d(C_M R_A)}{dt} = \frac{q_A C_T R_A + A_T T R_T - q_M C_M R_M}{A_T}.$$  

(A.3)
Combining Eqs. (1) and (A.3), we obtain
\[
R_M(q_{CA} A + A_T T - q_{CM} M) + V_C M = \frac{dR_M}{dt}
\]
which is rearranged and integrated to form
\[
\ln \left( R_M - \frac{q_{CA} R_A + A_T T}{q_{CA} + A_T T} \right) = \frac{q_{CA} A_T}{V_C M} t + \xi,
\]
where \(\xi\) is the constant of integration. We then raise with base \(e\) and use a new constant of integration \(\xi = e_0\) so that
\[
R_M - \frac{q_{CA} R_A + A_T T}{q_{CA} + A_T T} = e^{\frac{q_{CA} A_T}{V_C M} t + \xi}.
\]
When \(t = 0\), we solve for \(\xi\) and obtain
\[
\xi = R_M - \frac{q_{CA} R_A + A_T T}{q_{CA} + A_T T}.
\]
We note that when \(t = 0\), we have \(R_M = R_A\). Thus we combine Eqs. (A.6) and (A.7) to arrive at
\[
R_M = \frac{q_{CA} R_A + A_T T}{q_{CA} + A_T T} + \left( R_A - \frac{q_{CA} R_A + A_T T}{q_{CA} + A_T T} \right) e^{\frac{q_{CA} A_T}{V_C M} t},
\]
which is then solved for \(R_T\), resulting in Eq. (7):
\[
R_T = \frac{q_{CA} (R_M - R_A) + A_T T}{A_T T \left( 1 - \exp \left( -\frac{q_{CA} A_T}{V_C M} t \right) \right)}.
\]

References