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The impacts of leaf damage on the isotopic composition of leaf transpiration

Abstract

John Weddle¹, Matthew Lanning², Remi Ewing¹, Lixin Wang²*

¹Department of Biology, Indiana University-Purdue University Indianapolis (IUPUI), Indianapolis, IN, USA

²Department of Earth Sciences, Indiana University-Purdue University Indianapolis (IUPUI), Indianapolis, IN, USA Stable isotopes of water are useful tracers to study water cycling between the subsurface, plants, and the atmosphere. The development of water vapor based isotope methods has facilitated the direct and continuous measurements of plant transpiration isotopic composition, which is often used to determine plant water sources and partition evapotranspiration. The assumption that isotopic composition of transpiration is equal to the source water is fundamental in such applications. However, it is possible that leaf damage may obscure the transpiration isotopic signature violating this key assumption. We hypothesized that leaf damage would expose isotopically enriched lamina water to the chamber environment resulting in more enriched measurements. We compared the isotopic compositions of transpiration for un-damaged, artificially damaged, and recovered leaves using a leaf chamber and laser isotope analyzer. Our results showed significant enrichment in the transpiration isotope signature after leaf damage and the signature returned to pre-damage values after a few days. This study is the first to evaluate the consequences of damaged leaves on the isotopic composition of transpiration using chamber based methods and has direct applications for source water estimation and evapotranspiration partitioning studies.

Keywords: ecohydrology, isotopes, transpiration, evapotranspiration

1. Introduction

Plants are a dominant force in the global hydrological cycle as transpiration is responsible for up to 90 percent of total evapotranspiration (ET) (Jasechko et al., 2013, Schlesinger and Jasechko 2014, Wang et al., 2014). Detailed knowledge of plant-mediated water fluxes at multiple scales is critical in understanding ecological response to environmental changes.

Stable isotopes offer a suite of tools for tracking the physical and biochemical processes acting on water as it cycles from the ground through plants and to the atmosphere. Unlike radioactive isotopes, stable isotopes do not decay. Stable isotopologues of water (e.g., ¹H²H¹⁶O, ¹H¹H¹⁸O) serve as effective environmental tracers for studying the movement of water through the hydrologic cycle (Bowen and Wilkinson, 2002; Dawson and Ehleringer, 1993; Kaseke et al., 2017, 2016; Tian et al., 2018; Wang et al., 2012; Williams et al., 2004; Zhao et al., 2012). Due to small differences in mass, the isotopologues move through the environment at different rates, causing fractionation to the original signature. For example, vapor of evaporated water will be depleted in the heavier isotopologue because of kinetic fractionation and the remaining liquid more enriched. Isotopic composition can be defined as the relative abundances of isotopes of the same element.¹⁶O is the most abundant isotope of oxygen found on Earth, with ¹⁸O and ¹⁷O accounting for 0.204% and 0.037%, respectively. The great majority of hydrogen exists as ¹H. However, 0.015% exists as ²H (Farguhar et al., 2007). The stable isotope composition for hydrogen or oxygen can be defined with delta notation:

$$\delta(\%_0) = \left(\frac{R}{R_{std}} - 1\right) x 1000$$

where R is the ratio of ${}^{2}H/{}^{1}H$ or ${}^{18}O/{}^{16}O$ present in the sample and R_{std} is the same ratio of the standard. Units are expressed as per mil (‰).

It has been shown that the isotopic composition of xylem water is generally the same as the source water the plant draws from (Dawson, 1993, White et al.,1985), suggesting that little or no fractionation occurs during the uptake and transport of water from root to the evaporation site. The isotopic composition of transpired water vapor (δT) can be assumed to be in steady state with that of source water (δs) under stable environmental and physiological conditions (Cernusak et al., 2016; Craig and Gordon, 1965; Farquhar et al, 2007). Vapor based isotope measurements provide an advantage over traditional cryogenic extraction methods because they require less time and labor, and provide high-frequency data in real time (Cernusak et al., 2016; Wang et al., 2009). Direct and continuous measurement of δT as described by Wang et al., (2012) is particularly useful as it can be paired with real-time measurements other of environmental conditions. These methods are more applicable to *in situ* applications. For example, Lanning et al., (2020) used continuous leaf-scale $\delta \tau$ measurements of maples and oaks at a forested site in conjunction with real-time measures of vapor pressure deficit, soil water content and leaf level parameters. At the canopy scale, Lee at al., (2006) used a vapor isotope measurement technique adapted to a flux gradient approach to understand the

interaction between environmental parameters and temporal variations in $\delta \tau$.

A key assumption in measurements of $\delta \tau$ is that all of the measured water vapor passes through the stomata. While fractionation doesn't tend to occur during transport through xylem, enrichment does occur at the evaporative sites (Cernusak et al., 2016). Back-diffusion of enriched water vapor towards the xylem competes with advection of relatively depleted source water vapor towards the stomata (Cernusak et al., 2016). This process, termed the Péclet effect, results in enrichment of lamina water that increases both with decreased transpiration rate, and increased effective path length (Cernusak and Kahmen, 2013). Leaf damage could violate the assumption that all water passes through the stomata in a more significant way than cuticular conductance does. If leaf damage is present, water from different pools in the leaf could evaporate and mix with vapor conducted via stomata. The mixed signature of transpired water and enriched leaf lamina water could confound interpretations of $\delta \tau$ measurements.

The objective of this study was to determine the effect of leaf damage on vapor based isotope measurements. Measurements of transpired water vapor were conducted on artificially damaged leaves before damage and after a recovery to test for transient and permanent effects. We hypothesized that leaf damage would cause the isotopic composition of the measured water vapor to be enriched, due to mixing of evaporated lamina water and transpiration. The potential for leaf damage to complicate the interpretation of vapor based isotope measurements makes understanding such impacts important for

their use in ecohydrological studies.

2. Materials and Methods

To determine the impact of leaf damage on vapor based isotope measurements, we measured $\delta \tau$ on a *Euonymus alatus* plant, deciduous shrub in the family a Celastraceae (Brand et al., 2012). The plant was acclimated to artificial light for two weeks and had been watered consistently with tap water of approximately -50‰. Source water was measured using a water vapor isotope standard source coupled to a triple water vapor isotope analyzer (T-WVIA-45-EP, Los Gatos Research Inc. (LGR), Mountain View, CA, USA) following Tian et al., (2016). The leaves were divided into three layers: L1, L2 and L3 based on their distances to light source. For all the three isotopic layers. compositions of transpiration were measured in three groups: treatment control group (undamaged leaves), damaged group (artificially damaged with a hole punch) and recovered group (damaged leaves after a 72-hour recovery period). For the damaged group, major veins were avoided to prevent leaf death. The recovery period allowed leaves to callus over to test if δT returned to pre-treatment values (Fig. 1). The isotopic compositions of transpiration of the three treatment groups were compared against each other and the source water.

Isotopic measurements were made using a chamber based method following Lanning et al., (2020). An acrylic leaf chamber provided an isolated environment to capture leaf transpiration, enclosing the leaf with a neoprene gasket which forms a seal around the leaf petiole. Two small ports prevented the formation of a vacuum by the pump and allowed ambient air into the chamber. A fan mixed ambient air with the transpired leaf vapor in the chamber and prevented condensation from forming inside the chamber. The chamber was plumbed to T-WVIA- 45- EP using a short length of Teflon tubing. The water vapor mixture was drawn through the T-WVIA using an external pump (Fig. 2). The instrument was allowed to warm up for ~30 min before measurements began. Each leaf was placed into the chamber and measurement continued until water vapor concentration reached a plateau, taking 90-160s. Data was collected at a frequency of 1 Hz. The water vapor concentration was allowed to return to baseline values before the next measurement began.

δτ was calculated using the Keeling plot method. In this method the inverse of water vapor concentration is plotted against the delta value of ambient-airtranspiration mixture and the y- intercept is calculated from a linear regression of this plot (Fig. 3, 4). The value of the yintercept represents an estimate for δT (Good et al., 2012). Only stable portions of the measurement period were used for our analysis as a direct linear relationship between δ_T and the water vapor concentration is a prerequisite for the method. Any measurement taken under highly variable conditions was excluded from our analysis. A coefficient of determination of $R^2 > 0.65$ was used as criteria for acceptable regressions. A three point moving average was performed on all data to allow some otherwise unusable measurements to meet the data quality threshold. The three-point moving average did not alter the $\delta \tau$ estimation in a way that would impact our conclusions (Fig. 3). The final values of δT were subjected to a Grubb's test ($\alpha = 0.05$), to identify and eliminate outliers. A severalsample ANOVA (Mann-Whitney) test was conducted to test for statistical difference among control, damaged, and recovered groups. Standard deviations were calculated for each of the three groups in Microsoft Excel and are reported with the mean of each group. Both the Grubb's test and ANOVA were performed using the software Past v4.03 (Lanning et al., 2019).

Water vapor concentrations between groups were compared by plotting a representative concentration from the same three leaves in control, damaged, and recovered groups. Concentrations representative of the middle of each measurement were calculated by averaging the five values at the center of each range. The ambient water vapor concentration before each measurement was then subtracted to give provide a comparable estimate the water vapor attributable to the leaf.

3. Results

3.1 The isotopic composition of leaf transpiration ($\delta \tau$)

The objective of this study was to determine if leaf damage affects the vapor-based results of isotopic measurements. Three experimental groups, consisting of control (predamaged, and recovered, damage), provided data for $\delta \tau$. A $\delta^2 H$ value of -50‰ was observed for source water. While R^2 > 0.65 was considered acceptable, every plot included here had $R^2 > 0.90$ after 3point moving average was applied (Table 1).

The isotopic composition of control and recovered groups were not statistically different (p > 0.05). Mean δT for the control group $(-44.05 \pm 1.43\%)$ nearly equaled that for the damaged group $(-45.33 \pm 10.0\%)$ (Table 1). The damaged group was statistically more enriched than both the control and recovered groups (p <0.05) having a mean of $-23.81 \pm 8.51\%$ (Table 1). Mean δT of the control and recovered groups reflected δs (~ -50‰), although both groups were slightly more enriched than δs (~5%, Table 1). Little variation in $\delta \tau$ occurred within the control group. However, a wide range was observed in the damaged (-11.99 \pm 8.51 and $-34.76 \pm 8.51\%$) and recovered (-25.12‰ and -57.64‰) groups (Table 1).

3.2 Water vapor concentrations of experimental groups

Water vapor concentrations between measurement groups were compared at a consistent point during the leaf measurement. Increased water vapor contribution by the leaf after damage and decreased vapor contribution after the recovery period was observed in all three leaf groups (Fig. 5). The magnitude of leaf water contribution varied based on proximity to the light source where L1 (1671.53, 2044.04, and 1305.60 ppm) was consistently higher than L2 (1316.61, 1669.23, and 1083.47 ppm), which was higher still than L3 (744.436 and 517.616 ppm) (Fig. 5). This was presumably a result of higher transpiration in the leaves with greater light exposure. The transpiration rate was not directly measured in this study.

4 Discussion

The results of this experiment indicate that leaf damage could have a significant effect on the outcome of vapor-based isotopic measurements. Leaf damage caused an enrichment of $\delta \tau$, rising from -44.15± 5.34‰ in un-damaged leaves to -23.81 \pm 8.51‰ after damage (Table 1). Water vapor contributed by the leaf also increased after the leaf was damaged (Fig. 5). Enrichment of the isotope signature and increased total water vapor is consistent with leaf lamina water evaporating into the chamber along with the transpired vapor. The subsequent decrease in δ^2 H (-23.81 ± 8.51‰ to-45.33 10.0‰) and in water \pm vapor concentration indicated that post recovery, leaf lamina water was no longer contributing to the total vapor isotope composition (Fig. 5; Table 1).

The change in signature caused by exposing leaf lamina water to the atmosphere fluctuated greatly between measurements of the damaged leaves (Table 1). L1 of the damaged group initially had a $\delta \tau$ of -11.99‰, whereas ~12 minutes later a signature of -34.77‰ was measured (Table 1). This variance may have been a result of non-steady-state dynamics associated with the damage or a rapid physiological response. Variation among the recovered leaves appeared to be less pronounced (Table 1), suggesting that such non-steady-state dynamics are associated with the immediate effects of leaf damage. However, without additional measurements we cannot confirm this.

4.1 Isotopic heterogeneity of leaf water

One implication of the variation in δ values of leaf water is that damage to leaves could expose pools of water that differ in isotopic composition from that of source water. Leaf lamina water is typically enriched compared to xylem or source water due to the enrichment that occurs at the evaporative sites, whereas δ T tends to reflect the signature of the source water (Cernusak et al., 2016; Leany et al., 1985). Furthermore, the leaf isotopic composition is not homogenous, and is more enriched from the basal to the distal end of the leaf, as well as outwards from the midrib (Bariac et al. 1994; Cernusak et al. 2016; Gan et al. 2002; Helliker & Ehleringer 2000; Santrucek et al. 2007; Wang & Yakir 1995). In any case, the other water pools in the leaf are generally enriched relative to source water. If leaf damage occurred during or before a measurement, vapor based estimated of $\delta \tau$ could appear more enriched than it actually is (Table 1).

4.2 Implications of leaf damage for ET partitioning and water sourcing studies

One of the critical assumptions made when conducting isotope measurements of water vapor from plants is that transpired vapor comes only through stomata (relatively depleted) and evaporation comes from surfaces (relatively enriched) (Jasechko et al., 2013; Schlesinger and Jasechko, 2014; Wang et al., 2012). The presence of leaf damage violates this assumption by introducing isotopically enriched vapor to the measurement, causing δT to appear closer to δE (Fig. 6). When partitioning ET with stable isotopes, the effect could cause an overestimation of the contribution from evaporation. Without knowing the degree to which leaf damage is contributing to vapor enrichment, such enrichment could lead to a number of misinterpretations (identification of vapor source or proportion of ET partitioning). Canopy scale ET partitioning studies like Lee et al., (2007), may also be complicated by leaf damage if the damage is extensive and its effects are compounded by other variables such as low air turbulence or low transpiration rates. However, leaf damage is likely to have greater effects as the scale of measurement decreases. Leaf damage may present obstacles to water sourcing studies as well. Research questions involving the sourcing of plant waters rely on the production of a local soil isotope profile to compare against the isotopic ratio of transpired water vapor (Lanning et al. 2020). Assuming steady state, δT will equal δs , which can be used to predict soil depth as water generally becomes more depleted at greater depths (Oerter et al. 2014). If measurements of $\delta \tau$ are made at the leaf scale, leaf damage could skew the measurement to reflect a more enriched signature.

5. Conclusions

The principle objective of this study was to determine the impact that leaf damage has on vapor-based stable isotope measurements. We hypothesized that leaf damage could lead to a more enriched vapor composition, obscuring the true value of isotopic signature of δT when measured using the chamber method. Our results showed a significantly enriched signature for the damaged group compared to the control group, supporting the original hypothesis. We express some caution in the applicability of these results, however, as it is the first study of this kind and draws from a relatively small pool of data. Future experiments would benefit from a larger dataset and coupling with other physiological measures. Detailed quantification of leaf damage impacts could be used to develop more complete non-steady-state models for certain applications. Use of live insects could provide realistic more damage incorporating other the various

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physiological changes associated with their impact. It may also be productive to evaluate the effect of natural insect damage at canopy scale.

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Measurements	Control		Damaged		Recovered	
	δт	R ²	δт	R ²	δτ	R ²
1	-42.53‰	0.98	-11.99‰	0.99	-25.12‰	0.99
2	-43.64‰	0.99	-34.77‰	0.99	-53.22‰	0.98
3	-45.97‰	0.95	-24.68‰	0.99	-57.64‰	0.98
4			-17.53‰	0.92	-47.97‰	0.97
5					-38.28‰	0.98
6					-39.68‰	0.99
7					-41.14‰	0.93
Mean ± Standard Deviation	-44.05 ± 1.43‰		-23.81 ± 8.51‰		$\textbf{-45.33} \pm 10.0\%$	

Table 1: The isotopic compositions of transpiration (δ_T) values for the control, damaged, and recovered leaves.



Fig. 1 (A): Diagram of potted *E. alatus* under artificial light, showing sample leaves L1 (1), L2 (2), L3 (3). **(B):** Diagram of leaf damage procedure and experimental groups. Edges of holes in "Recovered" group were observed to be callused over. Major veins were avoided when hole-punching to avoid killing leaves.



Fig. 2: Diagram of stable isotope measurement apparatus including leaf chamber, isotope analyzer (T-WVIA) and pump.



Fig. 3: Keeling plot regressions for a measurement from the recovered group before (red) and after (black) a three-point moving average was applied.



Fig. 4: Keeling plots for (A) control group; (B) damaged group; and (C) recovered group. All of them are from L2.



Fig. 5: Water vapor concentrations for control, damaged, and recovered leaves. Bar color and pattern corresponds, from left to right, to L1, L2, and L3 so that black bars represent L1 during each of the three measurement groups.



Fig. 6: Schematic of proposed mechanism for isotopic enrichment of $\delta \tau$ measured in the chamber. White arrow represents normal transpiration through the stomata, while blue arrows indicate evaporation from the damaged sites. Decrease in opacity/blue color in the recovered group indicates lower contribution of that source to measured vapor in the chamber. Isotopic gradient across leaf was not verified and is only a proposed feature following Cernusak et al., (2016).