An Isotopic Approach to Partition Evapotranspiration in a Mixed Deciduous Forest

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Running Head: Water Isotopes and Forest Transpiration

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Abstract

Transpiration (T) is perhaps the largest fluxes of water from the land surface to the atmosphere and is susceptible to changes in climate, land use, and vegetation structure. However, predictions of future transpiration fluxes vary widely and are poorly constrained. Stable water isotopes can help expand our understanding of land-atmosphere water fluxes, but are limited by a lack of observations and a poor understanding of how the isotopic composition of transpired vapor (δT) varies. Here, we present isotopic data of water vapor, terrestrial water, and plant water from a deciduous forest to understand how vegetation affects water budgets and land-atmosphere water fluxes. We measured sub-diurnal variations of δ18O_T from three tree species, and use water isotopes to partition T from ET to quantify the role of vegetation in the local water cycle. We find that δ18O_T deviated from isotopic steady-state during the day but find no species-specific patterns. The ratio of T to ET varied from 53% to 61%, and was generally invariant during the day, indicating that diurnal evaporation and transpiration fluxes respond to similar atmospheric and micrometeorological conditions at this site. Finally, we compared the isotope-inferred ratio of T to ET with results from another ET partitioning approach that uses eddy covariance and sap flux data. We find broad mid-day agreement between these two partitioning techniques, in particular the absence of a diurnal cycle, which should encourage future ecohydrological isotope studies. Isotope-inferred estimates of transpiration can inform land surface models and improve our understanding of land-atmosphere water fluxes.

Keywords: water isotopes, evapotranspiration, partitioning, water cycle, forest hydrology, sap flux, eddy covariance

1. Introduction

Evapotranspiration (ET) connects the water and carbon cycles and plays an important role maintaining terrestrial energy balance (Dunn & Mackay, 1995; Ellison et al., 2017; Swann et al., 2012; Worden et al., 2007). Despite its broad significance, estimates of terrestrial water fluxes from reanalysis, upscaled observations, and land surface models (LSMs) differ by up to
50% and predicting future land-atmosphere water fluxes remains a challenge (Mao et al., 2015; Mueller et al., 2013; Vinukollu et al., 2011). Central to this uncertainty are yet unresolved responses of plants to climate and land use change (Frank et al., 2015; Jackson et al., 2001; Massmann et al., 2019; Schlesinger & Jasechko, 2014). In a higher CO₂ world, some predict changes to leaf area index (LAI), stomatal conductance, soil moisture, and terrestrial runoff will intensify the water cycle (Brutsaert, 2017; Ohmura & Wild, 2002; Zeng et al., 2018; Zhang et al., 2016); others anticipate these vegetation-induced changes will decrease water cycling (Gedney et al., 2006; Labat et al., 2004). Consequently, a growing body of ecohydrological research is aimed at studying terrestrial water fluxes to better understand what drives water exchange between the land and the atmosphere, how terrestrial hydrology may change in the future, and how plants regulate freshwater resources.

ET is comprised of ecosystem evaporation (E, including surface evaporation and evaporation of canopy-intercepted water) and plant transpiration (T). The ratio of T to ET, hereafter referred to as \( F_T \), provides insight into the role that vegetation plays in terrestrial water recycling and links plant hydrology with climate and meteorological conditions (Stoy et al., 2019). A complete understanding of this ratio is an important step towards predicting how plants will respond to land use and climate changes and how hydrologic balance may change in the future. To date, there is no consensus about the values of global, regional, and ecosystem \( F_T \) (Anderegg et al., 2019; Bowen et al., 2019; Stoy et al., 2019). In particular, estimates of \( T \) and \( F_T \) from LSMs and remote sensing algorithms, which rely on ecosystem-scale information, do not currently agree with ground-based observations of \( T \) and \( F_T \) that can vary on spatial scales of less than a kilometer (Good et al., 2015; Talsma et al., 2018; Wei et al., 2017). Most LSMs and remote sensing data cannot capture sub-grid cell variations of lateral water flow (Chang et al., 2018; Ji et al., 2017; Maxwell & Condon, 2016), plant water stress (Fang et al., 2017; Matheny, Bohrer, Stoy, et al., 2014), and micrometeorological forcing (Badgley et al., 2015) that are necessary to accurately model \( F_T \). Further complicating our understanding of land-atmosphere water exchange, some ground-based observations of ET may not actually capture conditions at the transpiring or evaporating surfaces. For example, near-surface gradients of water vapor concentrations and vapor pressure deficits can make it difficult to relate ET measurements, most of which are made using eddy covariance above canopies, to leaf and soil fluxes within canopies (Aron et al., 2019; Jarvis & McNaughton, 1986; Kauwe et al., 2017). Therefore, additional leaf-
and soil-level flux measurements are needed to improve estimates of $F_T$ and predictions of terrestrial water fluxes.

Stable water isotopes can improve our understanding of water fluxes from the land to the atmosphere because the component processes, evaporation and transpiration, have distinct isotopic signatures (Yakir & Sternberg, 2000). Evaporation causes a large fractionation that enriches vapor in the lighter isotope. Because plants generally do not fractionate water during uptake and a vast amount of water passes through plants without fractionating, transpiration generally adds vapor with a higher proportion of heavy isotopes to the atmosphere (Ehleringer & Dawson, 1992). Using these fingerprints, many researchers have used water isotopes to measure $F_T$ and learn about land-atmosphere water exchange (Xiao et al., 2018 and references therein).

Isotopic ET partitioning requires knowledge of the isotope ratios associated with evapotranspiration ($\delta_{ET}$), evaporation ($\delta_E$), and transpiration ($\delta_T$). Until recently, isotope-inferred estimates of evapotranspiration were limited to a low temporal resolution (day-to-annual timescales). As a result, the isotopic composition of transpired vapor was not measured and instead was assumed to be in isotopic steady-state (equal to that of source water) (Haese et al., 2013). However, observations from high-resolution laser absorption spectrometers now enable estimates of $\delta_T$ and show that transpiration can deviate from isotopic steady-state when periods of stable environmental conditions are too short to allow $\delta_T$ to reach the isotopic composition of source water (Dubbert et al., 2013, 2017; Dubbert, Cuntz, et al., 2014; Dubbert, Piayda, et al., 2014; Simonin et al., 2013). These $\delta_T$ observations may improve estimates of land-atmosphere water fluxes and our understanding of the role plants play in the water cycle. However, thus far studies of $\delta_T$ have focused only on a small subset of species and environments, and it is still quite challenging to model short term (sub-diurnal) variations of $\delta_T$ (Dubbert, Cuntz, et al., 2014) or incorporate non-steady-state transpiration into isotope-enabled land surface models (Wong et al., 2017). Additional observations of $\delta_T$ from a wide variety of species and environments can inform estimates of $F_T$ and may help reconcile $F_T$ differences between observations and LSMs or remote sensing.

Forests play a critical role in land-atmosphere water exchange, but very few studies have directly used water isotopes to partition forest ET (Lai, Ehleringer, Bond, & Paw, 2006; Lee, Kim, & Smith, 2007; Moreira et al., 1997). Instead, most isotopic ET partitioning studies are based in croplands or grasslands where water management is easy to control and canopy cover is

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low, uniform, and continuous (e.g., Aouade et al., 2016; Lu et al., 2017; Wu et al., 2017). To address this gap, we measured the isotopic composition of transpired vapor from three tree species, bigtooth aspen (*Populus grandidentata*), red oak (*Quercus rubra*), and red maple (*Acer rubrum*), in a mixed deciduous forest in northern lower Michigan. We then used δ_T measurements to estimate forest F_T. Our objectives are to: 1) quantify the temporal and species-specific variability of δ_T, 2) use water isotopes to estimate forest F_T, and 3) evaluate whether measurements of non-steady-state δ_T improve isotopic ET partitioning. Finally, we compare our results from the isotopic ET partitioning with results from another partitioning technique that uses eddy covariance and sap flux data. Taken together, these objectives examine whether water isotopes provide accurate quantitative estimates of forest ET fluxes. If so, isotope-inferred F_T and δ_T may inform isotope-enabled LSMs and improve predictions of land-atmosphere water exchange. Broadly, this work builds upon a growing field of high-resolution isotope ecohydrology studies that seek to understand the role of vegetation in local, regional, and global water budgets.

2. ET Partitioning

2.1 Theoretical Isotopic ET Flux Partitioning

The isotopic two-source model is commonly used to partition evapotranspiration (ET) because evaporation (E) and transpiration (T) fluxes have distinct isotopic compositions. In this framework, ET is defined as

\[ ET = E + T. \]  

Eq. 1

Following isotopic mass balance and using delta (δ) notation, Eq. 1 can be expressed as

\[ δ_{ET} = δ_E + δ_T \]  

Eq. 2

where δ_{ET}, δ_E, and δ_T are the isotopic compositions of evapotranspiration, evaporation, and transpiration, respectively. A list of all symbols and abbreviations used in this study is presented in Table 1. Throughout this manuscript, we use δ notation in per mil (‰), where R is the ratio of
the heavy isotope to the light isotope ($\delta = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$) and the standard is Vienna Standard Mean Ocean Water (VSMOW) (Coplen, 1996; Gat, 1996). Combining Eq. 1 and Eq. 2 yields $F_T$, the ratio of $T$ to ET:

$$
F_T = \frac{T}{ET} = \frac{\delta_{ET} - \delta_E}{\delta_T - \delta_E}
$$

Eq. 3

This linear, two-source mixing model has been used in a number of previous studies to partition water fluxes of evapotranspiration (e.g., Wang and Yakir, 2000; Xiao et al., 2018; Yakir and Sternberg, 2000).

We determined $\delta_{ET}$ with a Keeling mixing model (Keeling, 1958; Yakir and Sternberg, 2000), where $\delta_{ET}$ is estimated as the y-intercept of a linear regression between the isotopic composition of atmospheric water vapor ($\delta_a$) and the reciprocal of the water vapor concentration.

The isotopic composition of transpired vapor ($\delta_T$) is calculated from leaf chamber measurements following Wang et al. (2012). Using this approach, $\delta_T$ is defined as

$$
\delta_T = \frac{q_m \delta_m - q_a \delta_a}{q_m - q_a}
$$

Eq. 4

where $q$ is the water vapor concentration, $m$ refers to measurements when the chamber was closed around a leaf, and $a$ refers to measurements when the chamber was open to ambient vapor (Wang et al., 2012).

The isotopic composition of soil evaporation ($\delta_E$) is estimated using the Craig and Gordon (1965) model:

$$
\delta_E = \frac{\alpha_{eq}^{-1} \delta_s - h \delta_a - \varepsilon_{eq} - (1-h) \varepsilon_k}{(1-h) + 10^{-3}(1-h) \varepsilon_k}
$$

Eq. 5

using meteorological measurements and isotopic values of soil water ($\delta_s$) and atmospheric vapor ($\delta_a$). Here $\alpha_{eq} (> 1)$ is the temperature dependent equilibrium fractionation factor (Majoube, 1971), $\varepsilon_{eq}$ is calculated as $(1 - 1/\alpha_{eq}) \times 10^3$, $\varepsilon_k$ is the kinetic fractionation term, and $h$ is the relative humidity at the temperature of the evaporating surface.
2.2 ET Partitioning from Sap Flux and Eddy Covariance Data

ET partitioning from sap flux and eddy covariance measurements follows the approach described by Williams et al. (2004). In this technique, the latent heat-derived ET is separated into biotic (T) and abiotic (E) components using eddy covariance estimates of latent energy and direct measurements of sap flux. To partition ET, we assumed that transpiration accounted for nearly all of the ET flux on the driest days during the growing season and derived a scaling equation to estimate the ratio of T to ET on days when evaporation was not negligible (Kool et al., 2014). Additional details on this scaling are provided in the Supplement.

3. Methods

3.1 Site Description

This study was conducted at the 46 m AmeriFlux-affiliated eddy covariance tower site at the University of Michigan Biological Station (UMBS) in northern lower Michigan (45.59°N, 84.70°W, AmeriFlux database site-ID US-UMB). The forest at this site has been dominated by bigtooth aspen (Populus grandidentata) and paper birch (Betula papyrifera), but is currently transitioning to a mixed composition dominated by red oak (Quercus rubra), red maple (Acer rubrum), white pine (Pinus strobus), American beech (Fagus grandifolia), and sugar maple (Acer saccharum). As a result of heavy logging in the early 20th century, the forest has a relatively uniform age and canopy structure. Mean canopy height is ~ 22 m and mean peak LAI is 3.9 m² m⁻². The site receives 766 mm of precipitation annually and the mean annual temperature is 5.5°C (Matheny et al., 2017). Soils at the UMBS site are well drained Haplorthods of the Rubicon, Blue Lake, or Cheboygan series and consist of ~ 95% sand and ~ 5% silt (Nave et al., 2011). Additional site details are available in Matheny et al. (2017) and Gough et al. (2013).

3.2 Isotope Measurements

3.2.1 Surface Waters

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We collected a variety of surface waters and shallow groundwaters during the 2017 growing season to characterize the isotopic composition of potential source waters for trees and to examine seasonal hydrologic variability near our study site. We collected event-scale precipitation at the tower site in a plastic bucket lined with mineral oil to prevent evaporation (Friedman et al., 1992; Scholl et al., 1996). We used a needle point syringe to extract precipitation and avoid transferring any oil to the collection vial. The sampling bucket was cleaned, dried, and given a fresh layer of oil between samples. From April to October, we collected monthly samples from the edge of a nearby lake and from the mouth of a groundwater spring. The groundwater spring originates from a seep at the bottom of the lake (Hendricks et al., 2016). We collected shallow (within 3 m of the surface) groundwater in April, June, and November from 15 wells near the mouth of the spring. All liquid water samples were collected in HDPE vials (Wheaton Industries, 986716) and analyzed within a few weeks of collection, so we do not expect any fractionation between the plastic HDPE collection containers and the sampled water (Spangenberg, 2012). We used a Picarro L2130-i cavity ringdown spectrometer (CRDS) with an A0211 high-precision vaporizer and attached autosampler to measure $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of liquid water samples. We used Picarro ChemCorrect software to monitor samples for organic contamination. For liquid samples, precision was better than 0.1‰ and 0.3‰ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively.

### 3.2.2 Vapor

To analyze water vapor isotopes, we deployed two cavity ringdown spectrometers, a Picarro L2120-i and a Picarro L2130-i, in a temperature-controlled shed located next to the 46 m eddy covariance tower. We used a Picarro Standard Delivery Module (SDM, A0101) to deliver liquid laboratory standards to monitor for drift and calibrate isotope data to the VSMOW–SLAP scale (Bailey et al., 2015). Each SDM was setup with a Drierite (26800) column and a Picarro high precision vaporizer (A0211) maintained at 140°C and ambient pressure. We analyzed standards at night in order to minimize interference with data collection during the day when transpiration was higher.

Cavity ringdown spectrometers are known to exhibit an isotope-ratio bias due to changes in cavity humidity (Aemisegger et al., 2012). To correct for this bias, we used version 1.2 of the University of Utah vapor processing scripts to derive cavity-humidity correction equations and

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instrument precision (Fiorella et al., 2018). We present the $1\sigma$ uncertainty at 10,000 ppmv, the lowest measured vapor mixing ratio, and 25,000 ppmv, near the highest measured mixing ratio. For d-excess ($d = \delta^2\text{H} - 8*\delta^{18}\text{O}$ (Dansgaard, 1964)), we assume oxygen and hydrogen errors are independent. $1\sigma$ uncertainty on the L2120-i ranged from $0.28\%o$ for $\delta^{18}\text{O}$, $0.93\%o$ for $\delta^2\text{H}$, and $2.45\%o$ for $d$ at 10,000 ppmv to $0.20\%o$, $0.59\%o$, and $1.68\%o$ (for oxygen, hydrogen, and $d$, respectively) at 25,000 ppm. On the L2130-i, $1\sigma$ uncertainty ranged from $0.13\%o$ for $\delta^{18}\text{O}$, $0.43\%o$ for $\delta^2\text{H}$, and $1.14\%o$ for $d$ at 10,000 ppmv to $0.09\%o$, $0.29\%o$, and $0.78\%o$ (for oxygen, hydrogen, and $d$, respectively) at 25,000 ppm. Additional information about the cavity humidity correction equations is available in the Supplement.

We installed a vapor sampling manifold on the eddy covariance tower and selected three similarly-sized nearby trees – a bigtooth aspen, a red oak, and a red maple – for transpiration measurements. We chose these species because together they account for more than 70% of the LAI and a majority of the sap flux at the site (Figure 1). Leaves and branches from the aspen and oak were accessible from a platform on the eddy covariance tower 15 m above the ground. No maple branches were accessible directly from the eddy covariance tower, so we built a small 5 m tower a few meters from the base of the eddy covariance tower to reach a maple tree. The uppermost extent of all three sampled trees reached the upper canopy and was exposed to full sunlight.

We built two transparent flow-through sampling chambers following the description in Wang et al. (2012) to make $\delta_T$ measurements at 5 and 15 m. Each chamber was approximately 20 cm long, 15 cm wide, and 5 cm tall. This size accommodated large (up to ~15 cm) oak leaves but was kept small to minimize lag or memory effects between switching samples. Just before a closed-chamber transpiration measurement, we manually inserted a live leaf (still attached to the tree) into the chamber and sealed the chamber. Each chamber had two small (~ 2 cm) openings to pull in ambient vapor during closed-chamber measurements. The chamber hung from the tree for the duration of each transpiration measurement period. Occasionally we had to reorient the chamber to prevent the leaf from touching the side of the chamber because any contact points between the leaf and the chamber promoted condensation. Every closed-chamber measurement was made on a different leaf. At the end of the transpiration measurement period, we opened the chamber, removed the leaf, and measured ambient vapor from the open chamber.
Sampling lines extended from the chambers to the Picarro analyzer. The 5 m chamber had two sampling lines, one to measure vapor when the chamber was closed around a leaf and another to measure vapor when the chamber was open. The 15 m chamber had three sampling lines, one for closed oak measurements, one for closed aspen measurements, and one for open chamber measurements. A final ambient-only sampling line extended above the canopy and was collocated adjacent to 34 m meteorological and flux measurements from the eddy covariance tower. All sampling lines were constructed from non-fractionating Bev-A-Line tubing (Simonin et al., 2013), encased in insulation, and wrapped with a warm wire to prevent condensation. The whole sampling manifold was held below ambient pressure by a diaphragm pump that operated at ~5 L/min to maintain constant airflow and minimize memory effects between samples.

Each Picarro analyzer controlled a multi-position valve (VICI/Vako EMT2SD6MWE) to switch between sampling locations. We measured each ambient vapor for 5 minutes and transpired (closed chamber) vapor for 10 minutes. We define a cycle of isotopic measurements as a loop through each port on the multi-position valve, and assume that the average isotopic composition at each sampling location represents the isotopic composition at that location for the full cycle of measurements.

Initially we planned to use the L2120-i to analyze ambient vapor and the L2130-i to analyze transpired vapor. This setup was designed to measure the highest possible temporal resolution of $\delta_T$. However, the L2130-i analyzer malfunctioned after the June sampling campaign, which forced us to reconfigure our approach and use the L2120-i to measure all six locations in August and October. We measured vapor isotopes during three periods in 2017: June 19 (DOY 170); August 14 (DOY 226), August 15 (DOY 227), and August 16 (DOY 228); and October 6 (DOY 279) and October 9 (DOY 282). These days were selected to study transpiration during periods when water fluxes were high (June and August) and low (October). Missing days in October (DOY 280 and 281) are due to technical issues with the Picarro analyzers, poor weather, and other logistical difficulties at the field site.

### 3.2.3 Terrestrial and Biological Waters

We used a soil auger to collect soil from the top 10 cm around noon on June 19, August 16, and October 6. Xylem samples were collected mid-day at breast height using an increment borer on August 16, October 6, and October 9. To avoid disrupting the hydraulics of the trees...
that were monitored for transpiration, we collected xylem samples from trees near the eddy covariance tower. We collected leaves from the transpiration-monitored trees because leaves from other trees were out of reach and the removal of a few leaves from a fully leafed-out tree was not expected to significantly affect plant hydraulics. Leaf samples were collected around 8am, 11am, 2pm, and 5pm on August 15, August 16, October 6, and October 9. To collocate measurements of leaf water and transpired vapor, we collected maple leaves at 5 m and oak and aspen leaves at 15 m. Soil, xylem, and leaf samples were stored in a refrigerator after collection.

Waters from soil, xylem, and leaf matrices were extracted on a cryogenic vacuum distillation line following the methods of West et al. (2006). The midrib was not removed from leaves prior to the distillation. Distilled soil waters were analyzed for oxygen and hydrogen isotopes on a Picarro L2130-i as described earlier. Due to complications arising from the presence of organic compounds (West et al., 2010), leaf and xylem waters were analyzed for $\delta^{18}O$ and $\delta^2H$ using a Thermo Scientific Delta V gas isotope ratio mass spectrometer (TC/EA-IRMS hereafter) that does not suffer from organic contamination. The TC/EA-IRMS was interfaced with a Thermo Scientific FlashIRMS elemental analyzer running in pyrolysis mode. A 0.5 uL aliquot of distilled water was injected into a glassy carbon furnace maintained at 1450°C. The product gases were separated chromatographically on a Restek Molesieve 5A column (60/80 mesh, 2m x 2 mm ID isothermal at 50°C) and were introduced to the IRMS by means of a continuous flow open-split interface (Conflo IV) optimized to each gas for linearity and sensitivity. Each gas was normalized to an injection of internal reference gas, and each batch of samples was then normalized to VSMOW by means of complementary analysis of known standards under these same conditions. Precision of TC/EA-IRMS analyses was better than 0.4‰ for $\delta^{18}O$ and 2.4‰ for $\delta^2H$.

3.3 Sap Flux

Sap flux is considered a proxy for transpiration (Granier & Loustau, 1994; Phillips & Oren, 1998). We used a network of custom-built Granier (1987) style thermal dissipation probes in 60 trees to continuously monitor sap flux at our field site. For this project, we installed six additional sap flux probes in the maple and oak trees that were used to measure transpiration to ensure they were hydrologically similar to others at the site. Sap flux measurements were made...
every minute and reported as 30-minute averages. Additional details about the sap flux sensors and network are available in Matheny et al. (2014b) and Matheny et al. (2017).

3.4 Meteorological and Eddy Covariance Measurements

Temperature and relative humidity (HMP45g, Vaisala, Helsinki, Finland) were measured at 3, 15, and 34 m from the eddy covariance tower. 3 m measurements were reported every minute; 15 and 34 m measurements were reported as 30-minute averages. To facilitate comparison with other meteorological and eddy covariance data, 3 m temperature and relative humidity were averaged to common 30-minute timesteps. Daily precipitation amount was measured approximately 6 km east of our field site at the Pellston Regional Airport. These data are available from the National Oceanic and Atmospheric Administration Climate Data Online archive (Network ID USW00014841).

Eddy covariance CO₂ and H₂O fluxes were measured above the canopy at 34 m. The latent heat flux was measured at high resolution (10 Hz) using the eddy covariance approach: water vapor and CO₂ concentrations were measured using a closed-path infrared gas analyzer (LI7000, LI-COR Biosciences, Lincoln, NE, USA); wind velocity and temperature were measured with a 3-D ultrasonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA). The latent heat flux was corrected by the Webb-Pearman-Leuning correction to account for density fluctuations in water vapor fluxes (Webb et al., 1980). A complete description of the eddy covariance data processing is available in Gough et al. (2013). All eddy covariance variables were reported as 30-minute averages. Spikes in the eddy covariance data were identified using a median filter (Starkenburg et al., 2016) and removed.

3.5 Data Processing: δT Calculations and ET Partitioning

All isotopic, meteorologic, and eddy covariance data were processed to a common time-step to facilitate analysis. The common time of δT measurements was rounded to the nearest half hour of the closed-chamber measurements. Following Eq. 4, δ¹⁸Oₜ was calculated from isotope and humidity measurements when the chamber was open (measuring ambient vapor) and closed (measuring transpired vapor). The Picarro simultaneously measures isotopic compositions and specific humidity; no additional parameters or measurements are needed to calculated δT (Wang et al., 2012). We omit the first 2 minutes of each measurement period to minimize memory...
effects from switching sampling ports and used the average of measurements from minutes 3-5 for the $\delta^{18}$O_T calculation (Aemisegger et al., 2012). Although the closed-chamber measurements continued for 10 minutes, we chose not to use transpired vapor measurements from minutes 5-10 because we observed that condensation occasionally built up in the chambers after 5 minutes.

Air within the canopy is usually poorly mixed (Aron et al., 2019), so we used above-canopy measurements for the Keeling regression to derive ecosystem-scale $\delta_E$. In contrast, $\delta_T$ measurements are separated by species (e.g., $\delta_T$,maple, $\delta_T$,aspen, and $\delta_T$,oak). At UMBS, maple, aspen, and oak account for ~22%, 26%, and 26%, respectively, of the total LAI (Figure 1b). To ensure we did not overpredict the transpiration flux from these three species, we scaled $\delta_T$,maple, $\delta_T$,aspen, and $\delta_T$,oak values by the percentage of total LAI accounted for by each species. This approach can produce species-specific values of $F_T$, although that is not our focus in this study because similar measurements are already done at UMBS from sap flux data (Figure 1a). Instead, in this study, we combine transpiration fluxes from maple, oak, and aspen trees to approximate an ecosystem-wide flux. We refer to $F_T$ calculated from the scaled $\delta_T$ measurements as non-steady-state $F_T$.

To test the effects of assumed steady-state transpiration on isotope-inferred $F_T$, we compare non-steady-state $F_T$ with $F_T$ estimated with two steady-state $\delta_T$ assumptions: a source water assumption that uses the Craig and Gordon (1965) leaf water model and defines $\delta_T$ as xylem water ($\delta_x$) and a precipitation assumption that sets $\delta_T$ as $\delta_p$. A summary of the various techniques and assumptions we use to estimate $F_T$ is presented in Table 2. $\delta_x$ and $\delta_T$ can vary spatially across a landscape (Brooks et al., 2010; McDonnell, 2014) and mostly likely reflect a mixture of water from past precipitation events and other incoming surface and groundwater flows (Barbour, 2007). Preferential flow paths through the porous (> 90% sand) UMBS soil may also bias the isotopic composition of available soil water (Brooks et al., 2010). Neither the source water nor the precipitation assumptions consider these environmental complexities, and a detailed assessment of soil hydrology is beyond the scope of this study. Instead, the steady-state assumptions used in this study are our best attempt to capture a representative transpiration flux from the forest.

4. Results
4.1 Seasonal and Synoptic Scale Variability

Seasonal variations of local meteorology, sap flux, and latent heat flux are shown in Figure 2. Temperature, specific humidity, sap flux, and latent heat flux increased through the spring, reached a maximum in the summer, and decreased in the fall. Soil moisture was greatest in the spring when the soil was moist from winter snowmelt and decreased through the growing season as water percolated through the soil or returned to the atmosphere via evapotranspiration (Figure 2f). Soil moisture increased rapidly after precipitation events, but due to the high sand content, limited storage potential, and increased ET fluxes after rain, decreased quickly after each storm pulse (Figure 2f). Sap flux and latent heat were positively correlated (Pearson’s r > 0.75) throughout the growing season and moderately well correlated with above-canopy VPD (r > 0.53) (Figures 2d and 2e). Imprinted on this seasonal variation, meteorological, eddy covariance, and sap flux measurements varied on 3-4 day timescales as weather systems passed through the study region (Figure 2). Daily precipitation totals varied from 0 to 1.18 cm (Figure 2f). In general, on rainy days, temperature, sap flux, and latent heat were lower and specific humidity was higher.

Monthly variability of terrestrial (rain, lake, soil, and ground) and plant (xylem and leaf) waters $\delta^{18}$O and $\delta^2$H are shown in Figure 3. Precipitation, surface water, and shallow groundwater cluster around the global meteoric water line (GMWL, $\delta^2$H = 8*$\delta^{18}$O + 10‰ (Craig, 1961)). The local meteoric water line (LMWL, $\delta^2$H = 7.9*$\delta^{18}$O + 13.6‰) at UMBS has a slope close to that of the GMWL and an intercept that reflects the high degree of moisture recycling downwind of Lake Michigan (Bowen et al., 2012; Putman et al., 2019). The isotopic compositions of soil ($\delta_s$), xylem ($\delta_x$), and leaf ($\delta_l$) waters generally fall below the GMWL along lines with shallow slopes (~ 2.5‰‰⁻¹) and very low intercepts (~ −37‰), indicative of evaporative enrichment.

Timeseries of meteoric water isotopes through the 2017 growing season are shown in Figure 4. Event-scale $\delta^{18}$O$_p$ generally varied between ~4 to −12‰ (~10 to −80‰ for $\delta^2$H), although a large (~1.2 cm) storm in late June had a particularly low isotopic composition (~17.1 and −120.6‰ for oxygen and hydrogen, respectively, Figure 4a). Precipitation d-excess (~13‰) was relatively consistent from May to October, with the exception of three mid-summer storms that had low d-excess (< 6.1‰, Figure 4b). $\delta^{18}$O of the lake and groundwater spring, which flows...
from a seep at the bottom of the lake, increased 1.2‰ and 0.3‰, respectively, through the growing season (Figure 4a). Together, these trends indicate that some lake water evaporated during the growing season. δ¹⁸O and δ²H of groundwater was almost always less than that of surface water. The groundwater spring (δ¹⁸O = −9.1‰ to −8.5‰) was therefore likely a mixture of lake water (δ¹⁸O = −8.1‰ to −6.9‰) and shallow groundwater (δ¹⁸O = −12.2‰ to −8.1‰). The seasonal trends in δ¹⁸O and d-excess of the spring suggest that the contribution of groundwater to the spring decreased through the growing season.

### 4.2 Diurnal Isotope Variability

Soil and xylem waters were evaporatively enriched relative to precipitation on all the days we measured these pools (Figure 5). In August, δ¹⁸O_p of the rain event just before the measurement period (−9.1‰) was less than that of δ¹⁸O_x for maple, aspen, and oak (−4.2, −6.7, −7.8‰, respectively) (Figures 5a and 5b). Similarly, on October 6, δ¹⁸O_p (−5.5‰) was lower than δ¹⁸O_x (−4.8, −4.3, and −3.9‰, maple, aspen, and oak, respectively, Figure 5c); on October 9 δ¹⁸O_p (−4.7‰) was lower than or nearly equal to δ¹⁸O_x (−4.9, −3.4, and −3.3‰, maple, aspen, and oak, respectively, Figure 5d). Precipitation d-excess in August, October 6, and October 9 was higher (14.5, 17.6, and 25.2‰, respectively) than d-excess of xylem water, suggesting that the difference between δ¹⁸O_x and δ¹⁸O_p is likely related to evaporative enrichment prior to uptake (Figures 5e-5h). δ¹⁸O_δ was never equal to δ¹⁸O_p, which suggests that soil water experienced fractionation by post-depositional processes (likely evaporation), was a mixture of water from multiple previous rain events, and/or was fed by other nearby sources (Figures 5a-5d). Near surface soil water d-excess was lower than that of precipitation, indicating that soil water was also evaporatively enriched relative to the most recent precipitation (Figures 5e-5h).

Observed δ¹⁸O_l of all three species exhibited a pronounced (> 10‰) daily pattern with the most evaporative enrichment (highest δ¹⁸O_l values) in the afternoon when temperature was at a maximum, relative humidity was at a minimum, and sap flux was high (Figures 5a-5d). As expected, d-excess of leaf water exhibited the opposite diurnal pattern with the greatest values in the morning and the lowest values in the mid-afternoon (Figures 5e-5h). Observed δ¹⁸O_l are generally lower than estimated steady-state δ¹⁸O_l, which may result from a discrepancy between observed δ¹⁸O_l, which includes midrib and vein water, and modeled δ¹⁸O_l, which estimates water
at the evaporation sites. Alternatively, the offset between observed and estimated δ18O_l may suggest that, even at midday when the transpiration flux was high (Figure 1a) and leaf-water turnover time was quickest, leaves were not at isotopic steady-state (Figures 5a-5d).

Although the diurnal pattern of leaf water isotopes was consistent between maple, oak, and aspen, the magnitude of diurnal δ1 change and values of δ18O_l and δ18O_x varied between species. For example, in August morning (8am) δ18O_x and δ18O_l of oak were lower than δ18O_x and δ18O_l of either maple or aspen (Figure 5a). Additionally, minimum morning δ18O_l varied on consecutive sampling days, with lower δ18O_l,maple and δ18O_l,aspen on August 16 than August 15 (Figures 5a and 5b). In contrast, October δ18O_x,maple, δ18O_x,oak, and δ18O_x,aspen were within 1‰ of each other (~−4‰), but δ18O_l,maple was ~5‰ lower than δ18O_l,oak and δ18O_l,aspen (Figures 5c and 5d).

δ18O_T varied between −15 and 6‰ and frequently deviated from δ18O_x, δ18O_o, or δ18O_p, indicating that transpiration was not at isotopic steady state on sub-diurnal timescales (Figure 6). In general, δ18O_T was lower in the morning when relative humidity was high and increased through the day as transpiration increased. δ18O_T was always greater than δ18O_a (−23.6 to −16.7‰, Figure 6) and therefore pushed the isotopic composition of atmospheric water vapor to higher values. No consistent species-specific δ18O_T trend emerged, and δ18O_T,aspen, δ18O_T,oak, and δ18O_T,maple varied considerably day-to-day and on sub-diurnal timescales (Figure 6). δ18O_E varied between −38.3 and −31.2‰ and pushed δ18O_a to lower values (Figure 6).

4.3 Diurnal ET Partitioning

A summary of ET partitioning results is presented in Table 2. Using Eq. 3 and the measured values of δ18O_T, transpiration from maple, oak, and aspen accounted for 37 ± 2% of the ET flux. This value, referred to as non-steady-state F_T, did not exhibit a consistent diurnal cycle (Figure 7). We compare non-steady-state F_T with F_T calculated from two steady-state isotope assumptions: that δ_T is equal to xylem water (source water assumption) and that δ_T is equal to δ_p of the most recent storm event (precipitation assumption). The precipitation assumption, which assumes that the only available source water is recent precipitation, allows us to estimate a transpiration flux from all species in the forest, including ones from which we did not measure δ_x. The precipitation assumption is our best attempt to quantitatively estimate a plot-
level transpiration flux; it does not address the timescale over which plants access available soil water or the complexities of preferential flow paths through soils, both of which affect $\delta_x$ and $\delta_T$ (Allen et al., 2018; Brooks et al., 2010; Evaristo et al., 2015).

$F_T$ estimated from the source water assumption ($36 \pm 2\%$, Figure 7) is nearly identical to non-steady-state $F_T$. The precipitation assumption produces a higher estimate of $F_T$ ($53 \pm 3\%$, Figure 7). The offset between these $F_T$ values arises because the precipitation assumption includes a water flux from all tree species at the site while the source water assumption only includes only the species from which we measured $\delta_x$ (maple, oak, and aspen) and accounts for ~70% of the site LAI. Correcting for this LAI discrepancy (scaling $F_T$ results from the precipitation assumption to include only 70% of the trees) and assuming that each species produces a similar amount of transpiration per unit leaf (Jarvis & McNaughton, 1986), we find that the source water assumption (36%) and the precipitation assumption (37%) produce nearly identical estimates of $F_T$. Agreement between the two steady-state $\delta_T$ assumptions suggests that at this site either technique is a precise approach to measuring forest $F_T$. The plot-level $F_T$ results ($53 \pm 3\%$) agree with other estimates of forest $F_T$ (Berkelhammer et al., 2016; Matheny, Bohrer, Vogel, et al., 2014; Sun et al., 2014; Tsujimura et al., 2007; Zhou et al., 2016). Like non-steady-state $F_T$, $F_T$ calculated using the either source water or precipitation assumptions exhibits no diurnal variation (Figure 7).

Finally, we compare isotopic ET partitioning results with $F_T$ estimated using eddy covariance and sap flux data (Figure 7). The sap flux network at this site is extensive and, coupled with eddy covariance data, provides a wide range of information about forest water fluxes including an estimate of $F_T$. For simplicity, we refer to $F_T$ calculated using eddy covariance and sap flux data as the ecohydrologic ET partitioning technique. Plot-level ecohydrologic $F_T$ was 65 ± 12%; ecohydrologic $F_T$ scaled to include only the transpiration flux from maple, oak, and aspen was 43 ± 9% (Figure 7). Agreement between the isotopic and ecohydrologic partitioning techniques was stronger midday (10am to 4pm, 61 ± 8% plot-level $F_T$; 40 ± 7% $F_T$ for maple, oak, and aspen) when water fluxes were high and weaker in the morning and evening when water fluxes were lower. When $F_T$ from the isotopic and ecohydrologic ET partitioning techniques diverged, the ecohydrologic partitioning technique...
tended to estimate higher $F_T$ than the isotopic technique (Figure 7). Neither partitioning approach revealed a consistent or pronounced daytime $F_T$ cycle.

5. Discussion

5.1 Isotope Data as an Indicator of Local Hydrology

5.1.1 Observations of Non-Steady-State $\delta_T$

It has long been recognized that on timescales longer than the plant-water turnover time the isotopic composition of vapor that is transpired from a leaf must equal the water that enters the leaf from the source (Dongmann et al., 1974). Accordingly, most isotope models assume that transpiration is a non-fractionating process, at least on longer timescales (Farquhar & Cernusak, 2005; Flanagan et al., 1991; Haese et al., 2013; Wang & Yakir, 1995). However, on short timescales (sub-diurnal to a few days) recent observations have revealed that $\delta_T$ deviates from steady-state conditions because environmental conditions change quicker than the turnover of plant water (Dubbert et al., 2017; Dubbert, Piayda, et al., 2014; Harwood, Gillon, Griffiths, & Broadmeadow, 1998; Simonin et al., 2013; Wang & Yakir, 1995; Yakir, Berry, Giles, & Osmond, 1994). $\delta_T$ varies with abiotic and biotic conditions including stomatal conductance, temperature, humidity, and $\delta_a$ (Simonin et al., 2013). At the leaf level, $\delta_T$ is also controlled by the transpiration rate, stomatal density, and leaf water content (Buckley, 2019; Dubbert et al., 2017). The Craig and Gordon (1965) model predicts that temperature and humidity are correlated with $\delta_T$ (Dongmann et al., 1974; Farquhar et al., 1993; Farquhar & Cernusak, 2005; Farquhar & Lloyd, 1993; Farris & Strain, 1978; Flanagan et al., 1991), which Simonin et al. (2013) confirmed in a leaf-cuvette study and we find to be true in naturally varying conditions (Figure 6).

We measured $\delta_T$ from three broadleaf deciduous trees but did not find consistent species-specific $\delta_T$ patterns (Figure 6). In contrast, in a controlled greenhouse, Dubbert et al. (2017) measured $\delta_T$ from a variety of herbs, shrubs, and trees and linked $\delta_T$ variations to species-specific differences in the transpiration rate, stomatal aperture, stomatal density, and leaf water content. At our field site, oak have an extensive rooting structure and can access a deeper, isotopically more depleted soil water pool than maple, which are shallow rooting (Matheny et al., 2017),

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although these uptake dynamics may be site-specific (Lanning et al., 2020). We therefore expected that the isotopic composition of xylem, leaf, and transpired water from oaks would be less than that from maples and aspen, but this was only true of xylem and leaf water in August when soil moisture was low. Rain storms on October 4 and October 7 moistened the soil and provided near-surface moisture for the maple, oak, and aspen trees to transpire. When the soil was drier during the August sampling period, the oak favored a more abundant, deeper, isotopically more negative water source (Matheny et al., 2017). Taken together, these results suggest that when broadleaf deciduous trees are not water stressed species-specific effects on local isotope signals are difficult to identify and distinguish. In contrast, when these trees are water stressed, species-specific differences may be evident in water isotope signals.

5.1.2 Surface, Terrestrial, and Biologic Water Isotope Variability

The isotopic composition of precipitation at UMBS reflects the dominant fractionation processes in northern Michigan, Rayleigh distillation and ‘lake-effect’ precipitation (Bowen et al., 2012). Previous estimates suggest that up to 32% of precipitation in this region is derived from evaporation over Lake Michigan (Bowen et al., 2012; Gat et al., 1994; Machavaram and Krishnamurthy, 1995). This high degree of moisture recycling explains the high (~ 13‰) observed precipitation d-excess. The seasonal increase (decrease) of $\delta^{18}O_{lake}$ (d-excess$_{lake}$) indicates that evaporation of local surface water likely also added vapor with a high d-excess to the atmosphere (Figure 4).

The dome-shaped pattern of diurnal $\delta_1$ has been observed in many studies and is related to the changes in vapor pressure deficit and transpiration rate (Cernusak et al., 2016 and references therein). Among the broadleaf deciduous trees in this study, the shape and magnitude of the diurnal $\delta^{18}O_1$ pattern was independent of species type and are broadly consistent with common isotopic leaf water models (Farquhar & Cernusak, 2005). The initial, morning isotopic composition of $\delta^{18}O_1$ did, however, vary between the three species and is particularly notable on August 16 ($\delta^{18}O_{oak}$, Figure 5 b and 5e) and October 6 ($\delta^{18}O_{maple}$, Figure 5c and 5f). These differences may be related to rooting strategy when the soils are dry (Matheny et al., 2017) or may arise due to the high sand content and low moisture retention of soils that can cause high spatial variability of $\delta_s$ or $\delta_x$ at the site (He et al., 2013; Nave et al., 2011).
5.2 ET Partitioning

ET partitioning distinguishes the evaporation and transpiration components of the ET flux and helps provide a quantitative understanding of ecological processes within the water cycle (Jasechko et al., 2013; Kool et al., 2014). Isotopic ET partitioning is predicated on E and T fluxes of distinct isotopic compositions and accurate estimates of $\delta_{ET}$, $\delta_E$, and $\delta_T$. Currently, there is no consensus on the best approach to measure the isotopic composition of the ET flux, and researchers use either Keeling mixing models or the flux-gradient technique (Good et al., 2012). The flux-gradient method works best over smooth, homogenous surfaces such as lakes and grasses (Xiao et al., 2017); we chose the Keeling approach to avoid complications of canopy turbulence that may limit the flux-gradient method (Good et al., 2012; Yakir & Wang, 1996).

Other ET partitioning studies (e.g., Berkelhammer et al., 2016; Sun et al., 2014; Tsujimura et al., 2007) have also successfully used the Keeling method to calculate $\delta_{ET}$ in forested environments, which further justifies our approach to estimating $\delta_{ET}$.

We used the Craig and Gordon (1965) model (Eq. 5) to calculate $\delta_E$. Here the challenging factors are an accurate and representative value for the isotopic composition of soil water at the evaporation front and the soil kinetic fractionation factor (Wang et al., 2013; Xiao et al., 2018). We collected soil from the top 10 cm and used $\delta_s$ from a single location to estimate the evaporative flux over the entire tower footprint. This approach does not capture the spatial heterogeneity of $\delta_s$ (Gazis & Feng, 2004; Hsieh et al., 1998), but is a common approach in most ET partitioning studies (e.g., Aouade et al., 2016; Dubbert et al., 2014; Yepez et al., 2005; Zhang et al., 2011). The closed, thick canopy cover at our field site (Aron et al., 2019) likely reduces spatial variation in $\delta_s$. The kinetic fractionation factor in soil evaporation studies has long been a point of debate and varies with soil tortuosity, soil moisture, and atmospheric conditions (Quade et al., 2018; Xiao et al., 2018). In our study, diurnal soil water content was relatively consistent (varied by less the 0.5 % (m$^3$ m$^{-3}$) per day) so we elected to use the constant value for $\varepsilon_k$ provided by Quade et al. (2018).

Most isotope-based ET studies assume transpiration is in isotopic-steady state and estimate that $\delta_T$ is equal to $\delta_s$ or $\delta_s$ (e.g., Aouade et al., 2016; Wang and Yakir, 2000; Yepez et al., 2003; Zhang et al., 2011). Instead, in this study we measured $\delta_T$ using a leaf chamber to 1) observe any non-steady-state transpiration isotope patterns and 2) evaluate whether direct $\delta_T$...
measurements affect isotopic ET partitioning. The technical and methodological advancements for this type of measurement have only recently been developed (e.g., Wang et al., 2012) and to date only a handful of studies have used a leaf chamber to measure δT and partition FT (Dubbert, Cuntz, et al., 2014; Good et al., 2014; Lu et al., 2017; Wang et al., 2010, 2013; Wu et al., 2017). However, nearly all of this work has been done in agricultural fields or grasslands, and still relatively little is known about δT (Lanning et al., 2020) and isotope-inferred FT in forests.

The daytime, plot-level values of FT reported in this study (53% from the precipitation assumption; 61% from the ecohydrologic technique, Figure 7) agree well with other estimates of forest FT. Berkelhammer et al. (2016) and Tsujimura et al. (2007) used water isotopes to calculate forest FT values of 49-62% and 60-73%, respectively. Non-isotope ET partitioning techniques reveal similar FT and range from 52% (Zhou et al., 2016) to ~ 70-80% (Matheny, Bohrer, Vogel, et al., 2014; Sulman et al., 2016) in deciduous broadleaf forest sites. At our field site, Matheny et al. (2014b) and Aron et al. (2019) demonstrated that ET partitioning is sensitive to forest structure and LAI, with a greater transpiration flux from closed forest canopies and a greater evaporation flux from open forest canopies. The positive relationship between LAI and FT is also observed in a variety of non-forest environments (Scott & Biederman, 2017; Wang, Good, & Caylor, 2014; Wei et al., 2017), although it is poorly parameterized in most LSMs, with estimates of FT that are typically lower than expected (Bowen et al., 2019).

In this study, mid-day FT did not exhibit a consistent cycle regardless of species, steady-state assumption, or partitioning technique (Figure 7). Because LAI sets FT, Wang et al. (2014) proposed that FT should be relatively consistent throughout the growing season. Although FT can vary with passing weather systems and precipitation (e.g., Aron et al., 2019; Wen et al., 2016), periods of water stress (Good et al., 2014; Matheny et al., 2017), and the removal of biomass (e.g., harvesting or cutting grass) (Wang, Yamanaka, Li, & Wei, 2015), Berkelhammer et al. (2016) demonstrated that forest FT was generally invariant on seasonal timescales. We come to the same conclusions on sub-diurnal timescales (Figure 7), although this observation may be dependent on vegetation type, aridity, and soil moisture. For example, in arid sites with very low soil moisture, diurnal increases in the transpiration flux may not be accompanied by a concurrent evaporation flux and FT may increase mid-day (Zhou et al., 2018). However, the absence of a diurnal FT cycle at our broadleaf deciduous forest site suggests that similar ecological processes and environmental conditions drive the component ET fluxes in this environment as both
Evaporation and transpiration fluxes are controlled by external environmental factors including vapor pressure deficit, incoming solar radiation, temperature, humidity, wind speed, water availability, and ambient CO₂ concentration as well as a number of internal soil or plant factors (e.g., tortuosity, available surface area, water potential) (Ball, 1988; Cernusak et al., 2016; Penman, 1948; Sperry et al., 2002; Tyree & Zimmerman, 2002).

Finally, we compare Fₜ from the isotopic and ecohydrologic partitioning techniques. Isotopic and ecohydrologic derived Fₜ were similar during the day when ET was high, but results from the two techniques diverged in the early morning and late afternoon when water fluxes were lower. The timing of diurnal sap flux is usually well correlated with incoming solar radiation, temperature, and vapor pressure deficit (Ling et al., 2008). It is therefore possible that the high ecohydrologic Fₜ in the morning and evening reflects differences in the initiation and termination of early morning and late afternoon diurnal evaporation and transpiration fluxes.

However, both steady-state isotopic Fₜ estimates remained invariant during these times (field logistics and low water fluxes prohibited direct δ¹⁸O measurements in the early morning and evening), suggesting that the high morning and afternoon ecohydrologic Fₜ may be an artefact of sap flux or eddy covariance measurements. To this point, sap flux measurements are known to be biased and prone to errors when water fluxes are low (Ewers & Oren, 2000; Granier, 1987). High ecohydrologic Fₜ may also be explained by the refilling of dehydrated xylem tissues that does not necessarily result in the release of water to the atmosphere at that time. The mid-day agreement between isotopic non-steady-state, isotopic steady-state, and ecohydrologic partitioning techniques highlights the precision of these different approaches. Despite a multitude of assumptions and simplifications, these techniques capture the same water fluxes that are driven by incoming solar radiation, water availability, and plant hydraulics. Additional ET partitioning techniques such as solar-induced fluorescence (SIF) (Lu et al., 2018; Shan et al., 2019) may soon be available at this site and may yield new insights into the divergent partitioning results in the early morning and late afternoon.

### 5.3 Caveats and Experimental Considerations

Forests play a critical role in the water cycle and imprint a distinct signature on the isotopic composition of local and regional water cycles. However, measuring forest water fluxes is difficult because forests are heterogeneous, turbulent environments. Accordingly, studies of
forest $\delta_T$ (e.g., Lanning et al., 2020) and isotopic ET partitioning have lagged behind similar studies in greenhouses or homogenous environments such as croplands and grasslands (e.g., Dubbert et al., 2017; Good et al., 2014). While our experimental approach mitigates this gap, this study was affected by field logistics. For example, we were only able to reach three trees for transpiration measurements. As a result, $F_T$ from $\delta_T$ measurements, the source water assumption, and sap flux scaled to include only the transpiration flux from maples, oaks, and aspen are biased low.

Limitations of the experimental setup are also an important consideration. First, direct $\delta_T$ calculations require that a leaf be manually inserted and removed from a sampling chamber, which limits the number of measurements. We likely missed water fluxes before and after our measurement periods. Second, the different measurement heights (5 m for maple, 15 m for aspen and oak) may complicate species-specific observations of $\delta_T$. Although vertical light-induced differences in stomatal conductance and leaf temperature can balance each other (Bögelein et al., 2017), even small differences in measurement location and microclimate within the canopy can strongly affect transpiration and $\delta_T$ (Baldocchi et al., 2002; Chen et al., 1999; Jarvis & McNaughton, 1986). Third, scaling isotopic ET partitioning from local measurements to a plot or regional scale remains a challenge given soil heterogeneity, diversity of plant ecophysiology, and a variety of vegetative and canopy structures. Sap flux measurements suffer from similar scaling challenges (Schaeffer et al., 2000), however our field site has an unusually robust sap flux network that has been successfully statistically-scaled to plot-level water fluxes (Matheny, Bohrer, Vogel, et al., 2014). Scaling individual soil and tree isotope measurements to the plot-level remains difficult (Sutanto et al., 2014).

### 5.4 Implications and Directions of Future Work

Moving forward, we show that continuous analysis of $\delta_a$ and routine measurements of $\delta_x$ or $\delta_p$ can efficiently record $F_T$. Researchers should make measurements for the source water ($\delta_x$) or precipitation ($\delta_p$) approaches based on site-specific characteristics such as species distribution, expected $\delta_a$ heterogeneity, and the frequency of precipitation events. Neither approach requires laborious leaf chamber measurements and both are founded on a steady-state assumption about $\delta_T$ that is valid for mid-day (Figure 7) and seasonal (e.g., Wei et al., 2015) isotopic ET
partitioning. In contrast, assumptions of steady-state $\delta_T$ may not suffice for questions related to isotope and water cycles on sub-diurnal timescales (e.g., Aron et al., 2019; Simonin et al., 2013; Welp et al., 2012). On this relatively short timescale, non-steady-state $\delta_T$ measurements inform how transpiration forces the isotopic composition of atmospheric water vapor and may help validate the Craig and Gordon (1965) model that is commonly used to estimate $\delta_T$ and $\delta_E$ (e.g., Dubbert et al., 2013; Dubbert et al., 2014; Good et al., 2012; Hu et al., 2014). Additionally, studies that measure and model $\delta_T$ can partition species-specific $F_T$ to learn about species-specific hydrology and responses to environmental conditions. Observation of $\delta_T$ may also improve the parameterization of kinetic isotope effects during evaporation and transpiration, which remains a major challenge in isotope ecohydrology research (Quade et al., 2018).

Overall, continued efforts to accurately measure and understand local transpiration are critical to expand our knowledge of continental water recycling and understand the role that plants play in regulating water budgets. This study examines forest ET fluxes; additional observations from environments such as wetlands and tundra are still needed to assess how hydrologic processes are represented in land surface models and to monitor how water and energy fluxes respond to climate and land use change. Currently, almost all LSMS underestimates $F_T$. Recent and ongoing efforts to incorporate water isotopes into land surface models (e.g., Wong et al., 2017) may improve our understanding of land-atmosphere water fluxes, but these models must be validated with measurements of local $\delta_T$ and $F_T$.

6. Conclusions

We present direct, species-specific measurements of $\delta^{18}O_T$ from three broadleaf deciduous trees and estimate the contribution of transpiration to the ET flux in a mixed deciduous forest. The methodology to make $\delta_T$ measurements in a field setting is new, and these are among the first $\delta_T$ results obtained from a forest environment. $\delta^{18}O_T$ deviated from isotopic steady-state on sub-diurnal timescales but did not exhibit a clear species-specific pattern. Using water isotopes, we found that the $F_T$ was invariant during the day, which indicates similar atmospheric and micrometeorologic conditions control evaporation and transpiration fluxes at this site. We find strong mid-day agreement between isotopic steady-state, isotopic non-steady-state, and ecohydrologic (eddy covariance and sap flux) estimates of $F_T$, which suggests that assumptions of steady-state $\delta_T$ may suffice for other forest ET partitioning studies. Agreement
between the isotopic and ecohdrologic partitioning techniques, in particular the absence of a diurnal cycle using either approach, should encourage use of the isotopic ET partitioning method in environments where it is impossible or logistically impractical to install sap flux sensors. Transpiration and evapotranspiration remain challenging fluxes to measure, model, and predict, but water isotopes can help improve our understanding of these important hydrological processes. Future work on non-steady-state $\delta_T$ will improve the utility water vapor isotopes as a tool to study land-atmosphere water exchange while steady-state assumptions of $\delta_T$ and isotopic ET partitioning can provide insight into the role of plants in terrestrial water cycling.

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**Data Availability Statement:**

Data associated with this study are archived and freely available from the University of Michigan Biological Station Data Repository (http://biostation.lsa.umich.edu/data).

**Conflicts of Interest**

The authors declare no conflicts of interest.

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**Work Cited**


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Table 1. Description of symbols and subscripts used in this study.

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<th>Symbol</th>
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<td>Atmospheric vapor</td>
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<td>s</td>
<td>Soil</td>
</tr>
<tr>
<td>h</td>
<td>Relative humidity</td>
<td>T</td>
<td>Transpiration</td>
</tr>
<tr>
<td>q</td>
<td>Specific humidity</td>
<td>x</td>
<td>Xylem</td>
</tr>
<tr>
<td>R</td>
<td>Isotope ratio (e.g., ( ^{18}O/^{16}O ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Transpiration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Summary of \( F_T \) methods, species, assumptions, and results.

<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>Assumptions</th>
<th>( F_T ) Explanation</th>
<th>( F_T )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta_T ) measurements (non-steady-state)</td>
<td>Aspen, maple, oak</td>
<td></td>
<td>Direct leaf-level measurements of ( \delta_T )</td>
<td>37 ± 2%</td>
</tr>
<tr>
<td>Source water assumption (steady-state ( \delta_T ))</td>
<td>Aspen, maple, oak</td>
<td>( \delta_x = \delta_T )</td>
<td>( \delta_T ) scaled to LAI of aspen, maple, oak</td>
<td>36 ± 2%</td>
</tr>
<tr>
<td>Aspen, maple, oak ecohydrologic</td>
<td>Aspen, maple, oak</td>
<td></td>
<td>Sap flux scaled to LAI of aspen, maple, oak</td>
<td>43 ± 9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(40 ± 7% midday)</td>
<td></td>
</tr>
<tr>
<td>Precipitation assumption (steady-state ( \delta_T ))</td>
<td>Aspen, beech, birch, maple, oak, pine</td>
<td>( \delta_p = \delta_T )</td>
<td>( \delta_T ) scaled to LAI of all non-oak species + ( \delta_{x,oak} ) scaled to the LAI of oak*</td>
<td>53 ± 3%</td>
</tr>
<tr>
<td>Plot-level ecohydrologic</td>
<td>Aspen, beech, birch, maple, oak, pine</td>
<td>Total plot level sap flux</td>
<td>65 ± 12% (61 ± 8% midday)</td>
<td></td>
</tr>
</tbody>
</table>

* Matheny et al. (2017) demonstrated that oak at our study site have a deeper rooting structure and can access soil water that is more depleted in heavy isotopes than other tree species at the site. As a result, $F_T$ from the precipitation assumption is calculated from the sum of $\delta_p$ scaled to the LAI of all non-oak species plus $\delta_{x,oak}$ scaled to the LAI of oak.
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