Partitioning overstory and understory evapotranspiration in a semiarid savanna woodland from the isotopic composition of water vapor

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Abstract
The relative contributions of overstory and understory plant transpiration and soil evaporation to total evapotranspiration (ET) in a semiarid savanna woodland were determined from stable isotope measurements of atmospheric water vapor. The savanna overstory was dominated by the deeply rooted, woody legume Prosopis velutina ("mesquite"), and the understory was dominated by a perennial C4 grass, Sporobolus wrightii. "Keeling plots" (turbulent mixing relationships) were generated from isotope ratios (δD and δ18O) of atmospheric water vapor collected within the tree (3–14 m) and understory (0.1–1 m) canopies during peak (July) and post-monsoon (September) periods of 2001. The unique regression intercepts from upper and lower profiles were used to partition the ET flux from the understory layer separately from that of the whole ecosystem. Although ET partitioning was problematic during the first sampling period in July, our results in September provided support to the validity of this method for measuring and understanding the dynamic behavior of water balance components in this semiarid savanna woodland.

During the post-monsoon period (22nd September), transpiration accounted for 85% of ecosystem ET. Transpiration by the grass layer accounted for 50% of the understory ET over the same period. The total ecosystem ET estimated by eddy covariance (EC) on 22nd September was 3.5 mm per day. Based on partitioning by the isotope method, 2.5 mm per day (70%) was from overstory ET and 0.5 mm per day (15%) was from transpiration by the grass layer. Independent estimates of overstory and understory ET partitioning from distributed understory EC measurements were remarkably consistent with our isotope approach.

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1. Introduction
The exchange of energy, water, and carbon between the land surface and atmosphere in many arid and semiarid environments is temporal and spatially heterogeneous during the summer growing season.
due to the episodic and localized nature of monsoonal precipitation. Infrequent precipitation inputs during the growing season in these monsoon-dominated environments generally promote rapid shifts in the magnitude and partitioning of mass and energy exchange between the ecosystem components and the atmosphere. A thorough understanding of the links between this heterogeneously distributed precipitation and ecosystem mass and energy exchange is of particular interest because ecosystem function (i.e. productivity) in these environments is highly sensitive to changes in the pattern and amount of precipitation (Noy-Meir, 1973; Ehleringer et al., 1991, 1999).

Although accurate estimates of ecosystem evapotranspiration (ET) are useful in water balance studies, partitioning of ET into its components is required to better understand processes that control ecosystem CO₂ exchange and productivity. Several methods are available to measure ET or some of its components (Wilson et al., 2001; Kostner, 2001; Sala et al., 2000), but each approach has limitations. Chamber gas exchange and sap flow methods are used to describe plant transpiration as a function of physiological and environmental controls (Peary et al., 1989; Jackson et al., 2000), but poor spatial representation often precludes their application at ecosystem or larger spatial scales (Jarvis, 1995; Ehleringer and Field, 1993). Soil weighing lysimeters and soil water budgets are used to measure soil evaporation, but these measurements are difficult and expensive to implement, and often also have poor spatial representation (Dunin, 1991). Modeling approaches have been used to assign probabilistic estimates of transpiration and evaporation in semiarid ecosystems (Paruelo and Sala, 1995; Reynolds et al., 2000). Such simulations are useful for assessing potential year-to-year variability in response to climate, but field validation techniques are needed to refine these models at the appropriate scales.

Ecosystem ET is routinely estimated with micrometeorological methods such as eddy covariance (EC) or Bowen ratio (Moncrieff et al., 2000; Baldocchi et al., 1988). These methods account for the net evapotranspiration flux but do not reflect the contribution from different components of the ecosystem.
Attempts to estimate soil evaporation with micrometeorological techniques have been successful in forest floors free of understory vegetation (Wilson et al., 2000; Baldocchi et al., 2000). However, when a vegetated understory is present, this approach will not distinguish between soil evaporation and understory plant transpiration (Scott et al., 2003). The strategy to overcome inherent limitations in techniques has been to combine methods (Kostner, 2001; Wilson et al., 2001), but spatial resolution remains a topic of concern (Wilson and Meyers, 2001).

Stable isotopes methods offer great promise for partitioning the ET flux at the ecosystem scale (Brunel et al., 1992; Moreira et al., 1997; Yakir and Wang, 1996; Wang and Yakir, 2000). When transpiration is at isotopic steady state (ISS) then the isotopic composition of the transpired vapor (δT) equals that of the water used by the plants (Flanagan et al., 1991). In contrast, water evaporated from soil surfaces is strongly fractionated and depleted in heavy isotopes (Craig and Gordon, 1965; Gat, 1996). Consequently, there is often a significant difference between the isotopic composition of the highly fractionated evaporation flux (δE) from the soil and the non-fractionated transpiration flux from the plants. Identifying these differences and their interaction with the vapor in the ecosystem boundary layer is the basis for partitioning fluxes using isotopic analysis, since the isotopic composition of a vapor sample reflects the mixture of the contributing sources and the background air (Yakir and Wang, 1996). Moreover, if the different vegetation layers access different zones of water that are isotopically unique, an overstory and understory ET partitioning may also be possible.

In this paper, we present a field study where the stable isotopes of atmospheric water vapor were used to partition the ET flux within a semiarid savanna woodland in southeastern Arizona, USA. We produced Keeling plots (isotope mixing relationships) in upper and lower profiles inside the canopy boundary layer; one representing the understory layer and one integrating the whole ecosystem isotopic flux. Our goals were to (1) partition the total evapotranspiration flux into soil evaporation and plant transpiration and (2) test the validity of this approach for partitioning understory and whole ecosystem ET separately.

2. Theoretical overview

Water naturally contains stable isotopes of oxygen (18O, 16O) and hydrogen (2H, 1H). Isotopic concentrations are expressed as the difference between the measured ratios of the sample and an international standard (V-SMOW) over the measured ratio of the standard. This is expressed using delta (δ) notation in per mil (%): (Ehleringer et al., 2000):

\[ \delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \]  

where \( R \) is the molar ratio of the heavy to light isotopes in the sample and the appropriate standard.

2.1. Isotopic signal of soil evaporation

Evaporated water is depleted in heavy isotopes compared to the water at the evaporating surface in the soil. This depletion is a function of the isotopic composition of the vapor in the atmosphere, relative humidity and equilibrium and kinetic fractionations associated with phase change and diffusion (Craig and Gordon, 1965; Gat, 1996). The fractionation is explained by (Moreira et al., 1997):

\[ R_E = \left( \frac{1}{\alpha_k} \right) \left( \frac{R_s(\alpha^*) - R_ah}{1 - h} \right) \]  

where \( R_E \) is the molar ratio of heavy to light isotopes of the water evaporated from the soil, \( R_s \) the molar ratio from the liquid water at the evaporating surface, and \( R_ah \) the ratio of the atmospheric vapor. \( \alpha^* \) the temperature dependent equilibrium fractionation factor (9.3‰ for \( \delta^{18}O \) and 76.4‰ for \( \delta^2H \) at 25°C; Majoube, 1971), \( \alpha_k \) the kinetic fractionation factor for molecular diffusion in air, 1.0285 and 1.025 for oxygen and hydrogen, respectively (Merlivat, 1978) or 1.0189 (≈19‰) for oxygen and 1.017 (≈17‰) for hydrogen in a turbulent boundary layer (Flanagan et al., 1991; Wang and Yakir, 2000) and \( h \) is the relative humidity of the air. The model assumes that no isotopic fractionation occurs during fully turbulent transport further away from the surface (Gat, 1996).

2.2. Transpiration

There is no isotopic fractionation during water uptake by roots and transport to sites of evaporation in
leaves (Ehleringer and Dawson, 1992; Brunel et al., 1995; but see Lin and da Sternberg, 1993). In the leaf, an isotopic enrichment may occur due to the effect of equilibrium and kinetic fractionations that take place during evaporation from leaf surfaces (Flanagan and Ehleringer, 1991; Flanagan et al., 1991). During transpiration an isotopic steady state can be attained, in which the vapor leaving the leaf has the same isotopic composition of water moving into the leaves from the stems (Flanagan et al., 1991). The attainment of isotopic steady state is gradual. Consequently, over the short-term (hourly), the vapor exiting the leaf can deviate from the steady state.

The magnitude of variation from ISS and the time required to attain ISS is variable among species and is dependent on the humidity surrounding the leaf and the turnover time of leaf water (Wang and Yakir, 1995). As a result, the isotopic composition of water vapor from transpiration can be lower (relative to the atmosphere) in the morning and higher in the afternoon, as shown in a tropical broadleaved species by Harwood et al. (1998). Under field conditions, Harwood et al. (1999) found that the isotopic composition (δ18O) of transpired water from oak leaves had a extreme variation from −15.7‰ to 7.4‰ (source −7.4‰), depending on light availability and vapor pressure deficit (VPD) surrounding the leaf, although when all the values measured were averaged over the long-term, the isotopic value of the transpired water matched that from the source. Under stable conditions, this deviation is however more likely to be in the range of 1−3‰, as shown under more controlled conditions (Wang and Yakir, 1995; Flanagan et al., 1991). In partitioning the ET flux, the isotopic values of water vapor from transpiration are determined by analyzing the isotopic composition of water inside the xylem of the transpiring vegetation under the assumption of isotopic steady state (Moreira et al., 1997; Wang and Yakir, 2000).

2.3. Flux partitioning

Flux partitioning is possible by comparing the isotopic values of the evapotranspiration flux with those of the ecosystem vapor sources. The isotopic value of the evapotranspired vapor (δET) is identified using Keeling plot analysis (Keeling, 1961). In ecosystem studies, Keeling plots are mass balance mixing relationships where the isotopic values of air samples collected at different heights above the ground are plotted against the inverse of the concentration of the substance of interest at the time of collection (Flanagan and Ehleringer, 1998; Yakir and da Sternberg, 2000; Moreira et al., 1997). This relationship is linear, and when used with water vapor the y-intercept reflects the source isotopic composition of the evapotranspiration flux:

\[ \delta_{ET} = C_{ebl} \frac{1}{C_{ebl}} + \delta_{ET0} \]  

where \( \delta_{ET} \) is the isotopic composition of vapor collected from the ecosystem boundary layer, \( C_{ebl} \) the atmospheric vapor concentration, \( C_{ebl} \) the vapor concentration in the ecosystem boundary layer, \( \delta_{T} \) is the isotopic composition of the atmospheric background and \( \delta_{ET0} \) indicates the isotopic composition of the evapotranspiration flux. The Keeling plot approach is based on the assumption that the atmospheric concentration of vapor in an ecosystem combines the inputs of two major sources: the background vapor from the atmosphere and vapor added by the sources in the ecosystem. It is further assumed that the only loss of water vapor from the ecosystem is by turbulent mixing with the background atmosphere (i.e. there is no condensation).

The fractional contribution of transpiration \( F_T(\%) \) to the evapotranspiration flux, is calculated by (Yakir and da Sternberg, 2000):

\[ F_T(\%) = \frac{\delta_{ET} - \delta_T}{\delta_T - \delta_{ET0}} \times 100 \]  

where \( \delta_{ET} \) is the isotopic composition of evapotranspiration vapor, \( \delta_T \) the isotopic composition of vapor from soil evaporation (Eq. (2)) and \( \delta_T \) is the isotopic composition of the transpiration vapor sources. Furthermore, the standard error of these estimates can be calculated based on the uncertainty produced by the variability of the sources and the regression coefficients of the Keeling plots (Phillips and Gregg, 2001).

Keeling plots integrate the contributing fluxes of different ecosystem sources. Their spatial resolution varies depending on the heights where samples are collected (Flanagan and Ehleringer, 1998; Wang and Yakir, 2000; Dawson et al., 2002). Collection profiles from lower and upper heights may generate information on particular vegetation layers and thus potentially allow a separate isotopic analysis of each layer. In
two-layered ecosystems with poor mixing conditions, Keeling plots produced from data collected near the ground surface may integrate the flux generated from soil evaporation and the understory vegetation. Keeling plots produced with measurements from higher profiles (hence, affected by the rest of the vegetation) will integrate the isotopic flux from the whole ecosystem.

3. Materials and methods

3.1. Site description

The study site was located along the upper San Pedro River within the San Pedro National Riparian Conservation Area in southeastern Arizona, USA (N31°39′49″, W110°10′39″, 1191 m elevation). A location map is given in Scott et al., 2003. Vegetation at the site was a mesquite (Prosopis velutina Wooton) savanna woodland on an upper floodplain terrace of the river. The understory was dominated by the perennial C4 caesiptose grass (Sporobolus wrightii Munro ex Scribn). The overstory had an average height of 7 m, reaching a maximum height of approximately 12 m, with the lower main crown starting at approximately 4.6 m. The average overstory LAI in July of 2001 was 1.6 m² m⁻². In the understory, dense patches of the perennial S. wrightii and a mixture of annual herbs (Viguiera dentata (Cav.) Spreng, Lepidium thurberi Wooton and Chenopodium album L.) become active following the monsoon precipitation beginning in July and senesced towards the end of the rainy season. The average plant cover in the understory was about 80% in July and declined with the senescence of the herbs.

The climate in this region is semiarid with a bimodal distribution of precipitation. About 60% of the rainfall occurs from July to September during the North American monsoon (Adams and Comrie, 1997) and about 23% occurs from December to March. The annual average precipitation recorded near the site (Tombstone, AZ) is 343 mm, but rainfall in this region has a high spatial and temporal variability. Mean monthly temperatures range between 24.8 °C in July and 9.9 °C in January (Scott et al., 2000).

The site was instrumented with three eddy covariance systems. A permanent system was mounted at the top of a 14 m tower. During the periods of this study, two EC systems were deployed below the mesquite canopy at 2 m height. Estimations of total and understory ET fluxes are described in (Scott et al., 2003). Estimates of total ET and understory fluxes from the “more closed” patch on 22nd September 2001 were used for comparison with isotope measurements during the corresponding period of the present study. We used the estimation from the more closed patch because it has physiognomic characteristics similar to the patch where the vapor collection was conducted (see later).

The transpiration trend of mesquite trees was measured by the heat pulse method (Burgess et al., 2001) and scaled to leaf area (Cable and Hultine, personal communication). Precipitation was gauged at the top of the 14 m tower. VPD was calculated from temperature and relative humidity data measured with temperature-relative humidity probes (HMP35c, Helsinki, Finland) at 14 m. Wind speeds were measured at 3 and 14 m.

3.2. Stable isotope ratios of soil and plant water

Samples of soil and plants for isotope measurements were taken during two field periods corresponding to the peak monsoon season (25th July) and the post-monsoon dry down (22nd September). The average isotopic composition of six stem water samples per species was used to estimate the isotopic value of water vapor from transpiration (δTW). Water was extracted from 2 to 3 stems (~5 cm length) of six different individuals of P. velutina and from 2 to 3 basal portions of the stem of the same number of individuals of S. wrightii. Additionally, in July basal stem sections from the conspicuous herbs V. dentata and L. thurberi were collected. Water was extracted from six soil samples collected from 0 to 10 cm depth. Plant and soil samples were placed in 25 ml glass vials sealed with parafilm and stored in the lab at ~2°C. Water was extracted by cryogenic vacuum distillation (Ehleringer et al., 2000). Because δTW was sub-divided into tree transpiration (δTm) and understory transpiration (δTu), we calculated a weighted average for the isotopic composition of bulk vegetation (δTv) = 0.7 δTm + 0.3 δTu and used this value in the partitioning of the total ET. This average reflects the average canopy cover (70%) of the mesquite overstory.
Soil surface temperature (0.05 m depth) and relative humidity from 0.1 m height was measured along four 100 m transects radiating away from the tower in the four cardinal directions. Measurements were made every 10 m along these transects during each vapor collection period during both campaigns. The isotopic composition of evaporated water (δE) was calculated with Eq. (2). This equation required the knowledge of α*, Rs, h, Ra and αk.

The average soil temperature from the four transects for each collection period was used to calculate the equilibrium fractionation factor (α*) based on the regressions provided by Majoube (1971; cited in Clark and Fritz, 1997):

\[
\begin{align*}
18O\alpha^* &= 1.137(10^6/T^2) - 0.4156(10^3/T) - 2.0667 + 1 \\
18D\alpha^* &= 24.844(10^6/T^2) - 76.248(10^3/T) + 52.612 + 1
\end{align*}
\]

With \( T \) in K.

The average isotopic value of water from the six soil cores (δs) and the average value for vapor collected at 0.1 m (δa) were used for Rs and Ra, respectively. For the kinetic fractionation, we used values previously reported that account for the effects of molecular diffusion through a boundary layer. The αk values for oxygen and hydrogen were 1.0189 and 1.017, respectively (Flanagan et al., 1991) and the average relative humidity (h) was calculated based on the measurements from the four transects during each collection period (Table 1).

### 3.3. Vapor collection

Vapor was collected during each field campaign from 10 heights (14, 12, 10, 8, 6, 4.6, 3, 1, 0.5 and 0.1 m). We were able to collect vapor from five heights at a time, therefore sample heights were separated in two groups (A) 14, 10, 6, 3 and 0.5 m and (B) 12, 8, 4.6, 1 and 0.1 m. For each group vapor was collected for 30 min, alternating groups to complete two 30 min periods per group. The 2h collection period in July was between 09:00 and 11:00h and from 09:00 to 11:00h and 14:00 to 16:00h in September.

Air was simultaneously drawn through low-absorption plastic tubes (Bev-a-Line IV, Thermoplastic Inc. Stirling, NJ, USA) attached to the tower. Flow was regulated to 300 ml min\(^{-1}\) by a set of flow meters. The air was circulated through a set of 25 cm long glass traps (modified from Helliker et al., 2002) submerged in a \(-80^\circ\)C alcohol bath. Traps were made of 9 mm Pyrex glass framed in 9.5 mm Tee and 6.4–9.5 mm Ultra-torr Cajon fittings with an inner tube of 6mm filled with 3 mm glass beads. Traps were sealed with Parafilm after collection and transported to the laboratory and stored at 2°C prior to extraction. Samples were transferred in a vacuum line to 6 mm o.d. flame-sealed Pyrex tubes.

Air was analyzed for its vapor concentration (mmol mol\(^{-1}\)) before entering the traps on intervals of 6 mm per height during July with an infrared gas

### Table 1

| Parameters used to estimate the isotopic composition of the evaporation flux (δE) with Eq. (2) |
|-----------------------------------|---|---|---|---|
| Soil temperature (°C) | h (%) | δs | δa | α* | αk | δE |
| 25th July Morning | 24.5 | 46.3 | -1.15 | -10.5 | 1.0094 | 1.0189 | -27.4 |
| | | | -23.75 | -137 | 1.0768 | 1.017 | -120.9 |
| 22nd September Morning | 20.9 | 20.5 | -3.57 | -19.4 | 1.0097 | 1.0189 | -29.8 |
| | | | -61.44 | -137 | 1.0806 | 1.017 | -144.6 |
| Afternoon | 23.6 | 13.5 | -3.57 | -19 | 1.0094 | 1.0189 | -36.3 |
| | | | -61.44 | -137 | 1.0778 | 1.017 | -141.9 |

Soil temperature and h are averages of four 100 m transects, δs represents the average isotopic values of water at the soil surface (0–10 cm) and δa is the average value for vapor collected at 0.1 m above the ground, α* the equilibrium fractionation factor based on temperature and αk is the kinetic fractionation factor for molecular diffusion including the effects of a boundary layer.
analyzer (IRGA) (LI 6262; Li-Cor Inc., Lincoln, NE, USA). Vapor concentrations in September were measured at 1.5 min intervals per height with an IRGA LI 7000 (Li-Cor Inc., Lincoln, NE, USA). Data were discarded when evidence of condensation was found in the lines. This occurred only for a single collection height (8 m) on the morning of the September period.

Keeling plots of $^{18}$O and $^D$ were generated by plotting the inverse of vapor concentration ($1/[H_2O]$) of each height, averaged over the 30 min of vapor collection, against the isotopic composition of the vapor collected at the corresponding height. As described in Eq. (3), the $y$-intercept of this relation indicated the source isotopic composition of the evapotranspiration flux ($\delta^{18}$ET). The fractional contribution from transpiration was calculated with Eq. (4). To analyze the total and understory fluxes independently, Keeling plots were developed separately for understory and upper canopy profiles. The selection of heights was based on the vegetation structure. For the understory, vapor was analyzed from 3 heights (0.1, 0.5 and 1 m), with 1 m representing the upper height limit of the S. weightii layer. The upper profile (3, 4.6, 6, 8, 10, 12 and 14 m) integrated the whole ecosystem flux. Data were collected only during the morning period in July because of afternoon cloudiness and rainfall (Figs. 1 and 2). Stable conditions in September (Fig. 2) allowed a second collection period in the afternoon and data from both periods were plotted together to integrate the daily trend.

3.4. Mass spectrometry analysis

A gas-tight micro-syringe was used to carefully place 1 $\mu$L of water in the bottom of 2 mm $\times$ 5 mm silver capsules (Exeter Analytical Inc., Chelmsford, MA, USA). Evaporation was avoided by sealing the capsules with indium foil. A small piece of indium foil ($\sim$8 mg) was placed in the center of the capsule at the time of crimping the edges. Once the capsule was crimped the protruding portion of foil was smashed to fill the upper cavity formed in the capsule. Such arrangement prevented evaporative

Fig. 1. Total daily precipitation (mm) during the growing season of 2001. Collection dates are indicated in the inset panels.
Fig. 2. Variation in environmental conditions during two collection periods: 25th July (left) and 22nd September (right). Top panels show solar radiation (W m$^{-2}$), middle panels are for vapor pressure deficit (kPa) and transpiration rate (mmol m$^{-2}$ s$^{-1}$) for mesquite trees, bars indicate the vapor collection periods on which the ET partitioning was based. The bottom panels are wind speeds (m s$^{-1}$) recorded at two heights, 3 m (○) and 14 m (▲).

loss during the time needed to complete the analysis. Nitrogen contamination was avoided by loading the samples in a nitrogen poor atmosphere produced by the continuous flow of argon in the area of preparation.

The stable isotope ratio analysis was performed on a continuous flow Finnigan MAT Delta + mass spectrometer (San Jose, CA, USA). Silver capsules were loaded in a standard multisampling carousel connected to a thermal combustion elemental analyzer (Finnigan MAT TC-EA, San Jose, CA, USA). Capsules were dropped into the reaction furnace (1350 °C) which had a ceramic column packed with coarse fragments of glassy carbon and a graphite crucible. Samples were pyrolized to produce carbon monoxide and diatomic hydrogen. The CO and H$_2$ then passed through a short packed 5 Å molecular sieve gas chromatographic (GC) column, which separated CO and H$_2$. The GC column was operated at 30 °C to create a 3 min separation between H$_2$ and CO at a flow rate of 50 ml min$^{-1}$. Once separated, the sample was carried on-line through an open split into the mass spectrometer. The separation allowed the analysis of δD and δ$^{18}$O from the same sample by peak jumping between the focus of the two gases. Three hundred seconds were allowed to complete the two reference peaks for H$_2$ and the unknown. A full cycle per sample was completed in 11 min. Precautions for a similar method suggested by Sharp et al. (2001) were followed here. The vapor collection on individual vapor traps varied between 0.2 and 0.4 ml, depending on the stage of the season but only 1 μl of water was required for both water isotopes in the CF-IRMS analysis. This advantage, combined with the indium-sealed capsules, allowed a semi-automatic analysis of multiple samples without sacrificing precision. The standard deviation for repeated analysis of known standards with this method was 0.2‰ for δ$^{18}$O and 1.2‰ for δD.
**4. Results**

The collection period on 25th July was preceded by a 6.4 mm precipitation event that occurred in the afternoon of the previous day. Solar radiation was uniform during the collection period and no wet surfaces other than soil were apparent. A smaller precipitation event occurred in the afternoon of 25th July, vapor generated by plant transpiration \( (\delta_T) \) was isotopically enriched relative to that generated by soil evaporation \( (\delta_s) \). The isotopic values of water from stems of mesquite trees were remarkably similar during the periods studied, suggesting that this species had the same water source throughout the season. The isotopic values of the transpiring vegetation from the understory were different in July and September, possible due to variation imposed by the mixing of soil surface water with recent precipitation (Table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Average isotopic values ( (\delta^{18}O \text{ and } ID \pm \text{S.D.)} ) of vapor from soil evaporation ( (\delta_s) ), average stem water values for mesquite trees ( (\delta_{Tw}) ), bulk understory stem water ( (\delta_u) ) ( (S. \text{ wrightii}) ) and herbs in July and ( S. \text{ wrightii} ) only in September, and the weighted average isotopic value for bulk transpiration ( (\delta_T = 0.7 \delta_{Tw} + 0.3 \delta_u) )</th>
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| **25th July** | \( \delta^{18}O (\%) \)
| ID (‰) | \(-27.36 \pm 0.6\) | \(-6.44 \pm 0.2\) | \(-3.55 \pm 1.9\) | \(-5.1 \pm 0.5\) |
| **22nd September** | \( \delta^{18}O (\%) \)
| ID (‰) | \(-143.2 \pm 1.9\) | \(-63 \pm 2\) | \(-60 \pm 15\) | \(-62.2 \pm 6\) |

<table>
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<th>Table 3</th>
<th>Regression analysis of daytime Keeling plots of vapor collected at different levels above the ground</th>
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| **25th July** | Total ET
| 9–11 h | \( \delta^{18}O \)
| ID | \( y = (-56.71 \pm 35.3) - 11.64 \pm 1.22\) | 0.19 | 0.13 | 13 | 8.9 | 0.9 |
| Understory | \( y = (-117.64 \pm 1876) - 92.34 \pm 6.5\) | 0.03 | 0.54 | 13 | -106 | -78 |
| 9–11 h | \( \delta^{18}O \)
| ID | \( y = (-303 \pm 9.68) - 79.14 \pm 30.34\) | 0.02 | 0.76 | 6 | -163 | -5.1 |
| **22nd September** | Total ET
| 9–16 h | \( \delta^{18}O \)
| ID | \( y = (-97.64 \pm 9.41) - 9.16 \pm 1.06\) | 0.82 | 0.0001 | 25 | -11.3 | -6.9 |
| Understory | \( y = (-638.80 \pm 38.71) - 74.47 \pm 4.37\) | 0.92 | 0.0001 | 25 | -83 | -65 |
| 9–16 h | \( \delta^{18}O \)
| ID | \( y = (-411.66 \pm 53.99) - 98.34 \pm 5.66\) | 0.46 | 0.0001 | 11 | -18.4 | -11.9 |

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<th>( r^2 )</th>
<th>( P )</th>
<th>( \alpha )</th>
<th>C.I. (95%)</th>
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<tr>
<td>Lower</td>
<td>Upper</td>
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Total ET includes vapor collected at seven levels above the ground (from 3 to 14 m), and the understory vapor collected at three heights from 0.1 to 1 m. Collection periods in September were from 09:00 to 11:00 h and from 14:00 to 16:00 h and in July from 09:00 to 11:00 h. The \( y \)-intercept (bold) indicates the isotopic composition of evapotranspiration flux \( (\delta_{ET}) \).
and evaporation and transpiration fractions calculated from the y-intercepts of $\delta^{18}O$ and $\delta D$ Keeling plots were widely different for the July period (Fig. 3; Table 3). For these reasons, we placed little confidence in the partitioned values of $E$ and $T$ for this period. By contrast, all the Keeling plot regression slopes in September were significantly different than zero, and both isotopes ($\delta D$ and $\delta^{18}O$) yielded similar results for partitioned ET. Moreover, contrasting values of $\delta ET$ were found between the understory (0.1–1.0 m) and overstory (3–14 m) profiles. During this period, the $\delta ET$ values in the upper profile for both $\delta^{18}O$ and $\delta D$ Keeling plots were close to those of the transpiration source (Fig. 4a and b). The understory Keeling plots indicated $\delta ET$ values more depleted in heavy isotopes and closer to the modeled values for soil evaporation (Fig. 4c and d).

The high correlation coefficients of the Keeling plots and the correspondence of $\delta ET$ to the transpiration source (Fig. 4) were reflected in the fractional...
Fig. 4. Daytime Keeling-type plots of water vapor collected at different levels above the ground in 22nd September 2001. (a) and (c) are for $^{18}$O and (b) and (d) for D. Top panels represent mixing in the whole ecosystem and bottom panels represent mixing in the understory (below 1 m). Closed symbols represent the collection from 9 to 11 h and open symbols the collection from 14:00 to 16:00 h. Regression coefficients are shown in Table 3.

Estimates of transpiration. On 22nd September, 6 days after the last precipitation event of the monsoon season, the estimates based on Eq. (3) indicated that transpiration represented between 85 ± 6% ($^{18}$O) and 88 ± 5% ($D$) of the total ET flux. In contrast, the contribution of transpiration represented only between 54 ± 6 and 60 ± 4% of the understory ET flux based on $D$ and $^{18}$O, respectively. During this period, both isotopes indicated similar proportions of transpiration when the uncertainty proposed by Phillips and Gregg (2001) was considered (error bars in Fig. 5a).

Total evapotranspiration at the site for 22nd September based on the eddy covariance estimates was 3.5 mm per day. The independent estimate of ET from the eddy covariance system deployed under the canopy...
Fig. 5. (a) Fractional contribution of transpiration (%) to ET in different ecosystem layers of a semiarid savanna woodland in 22nd September 2001. Black bars are estimates based in $^{18}$O and white bars in $^{2}$H according to Eq. (4). Error bars in (a) are standard errors based on the variation in the sources according to Phillips and Gregg (2001). (b) Total ecosystem ET portioned into tree transpiration, understory transpiration and soil evaporation. Estimates are based in the combination of isotopic measurements of water vapor and ecosystem water fluxes measured by the eddy covariance experiment (dotted line indicates the tree transpiration from Scott et al., 2003). Numbers in parenthesis indicate the estimated fraction (in %) of each ecosystem component ± the 95% confidence interval after Phillips and Gregg (2001).

5. Discussion

The application stable isotope measurements and Keeling plots provided means of partitioning the ecosystem evapotranspiration in this semiarid mesquite savanna woodland. Moreover, height selection enabled Keeling plots to identify the isotopic composition of the understory flux and partition the...
Fractional contributions of understory transpiration to total ET are common and measured using micrometeorological methods. Our results, based on stable isotope analysis, indicate that transpiration is a significant component of ET. Using Keeling plots from upper and lower profiles, we were able to partition the total ecosystem ET flux into its components. The understory ET flux during 22nd September was about equally divided between evaporation from the soil and transpiration from the herbaceous layer. This was consistent with the isotopic composition of the ET flux. We observed little correspondence in the isotopic composition of water vapor in the ecosystem boundary layer (Fig. 5). The vapor turbulence in the vegetation layer was higher than in the rest of the canopy, likely due to a very humid environment. Furthermore, low wind speeds were recorded within the lower profile (0.1 and 0.5 m) during the collection periods. Consequently, the relative contributions to the ET flux from each stratum vary according to the energy and moisture available for each functional type and the soil. Under these settings, Keeling plots, generated with vapor collected in a low profile, partitioned the understory flux into its components.

The understory ET flux during 22nd September was about equally divided between evaporation from the soil surface and transpiration from the herbaceous layer (Fig. 5a). In order to generate these estimates, the Keeling plots were produced under the assumption of poor mixing conditions between the understory and the rest of the canopy. During the collection periods in September, the vapor concentrations within the lower profile (0.1 and 0.5 m) were consistently higher than in the rest of the profile (data not shown). Furthermore, low wind speeds were recorded within the canopy during this period (Fig. 2). Our results suggest that transpiration was the dominant source of water vapor in the ecosystem boundary layer (Fig. 5). Although transpiration was high after a large precipitation event on 25th July (Fig. 2), the isotopic composition of the ET flux was very similar to the background atmosphere (Fig. 3). Furthermore, we observed little correspondence in the y-intercepts between the δ18O and δD Keeling plots for this wet period. Therefore, the fractional contribution of transpiration during July was unresolved. Two conditions may account for these spurious results: (1) a lack of vapor concentration gradients in the canopy boundary layer due to a very humid environment (Fig. 2) and (2) the time intervals (6 min) used to determine the vapor concentration at each height during the collection periods lagged behind the frequency required to capture the vapor turbulence in the vegetation layer. The contrast between July and September demonstrated that our Keeling plot based approach to partition the total ET flux is suitable when appropriate sampling strategies (i.e. precise measurements of ambient moisture) and stable environmental conditions are met.

Flux dynamics underneath canopies have been commonly investigated using eddy covariance systems, lysimeters and gas exchange chambers positioned in the understory (Baldocchi, 1997; Tuzet et al., 1997; Wilson et al., 2001; Kostner, 2001; Scott et al., 2003). Patch heterogeneity limits the application of these approaches for scaling to the ecosystem level. As a result, understory estimations are usually not measured as a separate component of the total ecosystem flux measured above the upper canopy. Keeling plots integrate the fluxes at a spatial scale that varies as a function of the heights where samples are collected (Flanagan and Ehleringer, 1998; Dawson et al., 2002). In the heterogeneous understory environment, formulation of Keeling plots with vapor collected at low height profiles is an innovative technique to characterize the nature of the understory flux separated from the upper canopy. In our case, the understory and the upper canopy were dominated by a perennial C4 grass and a woody leguminous tree, respectively. These plant functional types are exposed to different microclimates (Breshears et al., 1998) and have contrasting water sources (Table 1, see also Scott et al., 2000; Snyder and Williams, 2000). Consequently, the relative contributions to the ET flux from each stratum vary according to the energy and moisture available for each functional type and the soil. Under these settings, Keeling plots, generated with vapor collected in a low profile, partitioned the understory flux into its components.
from the total ET, the authors calculated that, on this day, the trees transpired 2.5 mm per day (71% of the total flux). Using the above values of total ecosystem fluxes, we multiplied the ET fractions estimated from the isotopic analysis (Fig. 5a) to further partition ET into all its components. In the case of δ18O, we multiplied 0.88 × 3.5 mm per day and 0.6 × 1.0 mm per day to obtain the transpiration from bulk vegetation (∼3 mm per day) and the transpiration from the understory vegetation only (∼0.6 mm per day). We then subtracted the understory transpiration from the bulk transpiration to obtain the tree transpiration (∼2.4 mm per day) and the remaining fraction corresponded to soil evaporation (∼0.5 mm per day) (Fig. 5b). The results from our isotopic partitioning were remarkably similar to those from eddy covariance estimations (Fig. 7 in Scott et al., 2003).

Our partitioning estimates depend on the formula used to calculate the average δT (Table 3). To assess this potential source of error, we conducted an isotopic partitioning (Eq. (4)) for 22nd September assuming that δT was (0.5δTm + 0.5δTu) or (0.9δTm + 0.1δTu) (i.e. increasing or decreasing the proportion of tree transpiration). However, since the δTm and δTu were remarkably similar during this period (Table 2), these changes only altered our estimates for tree transpiration vs. soil evaporation to the total ET by less than 2%. This had a net effect of just 0.1 mm per day, and was certainly within the 95% confidence interval (C.I.) reported in Fig. 5b.

The ability to partition the ET flux relies on the contrasting isotopic composition of the evaporation and transpiration fluxes (Table 2; Wang and Yakir, 2000). The differences between these two fluxes are, however, not consistent over short periods during the course of the day since the isotopic composition of the transpired vapor deviates around isotopic steady state (Harwood et al., 1999). In laboratory experiments with broadleaved species a gradual approach to isotopic steady state was reported within 1 and 3 h after drastic changes in ambient conditions (Flanagan et al., 1991; Wang and Yakir, 1995). We do not have direct estimates of the isotopic composition of water vapor transpired from the vegetation and therefore we did not verify the assumption of isotopic steady state. However, we conducted our vapor collection under environmental and physiological conditions that likely promoted a rapid approach to ISS. We started the water vapor collection 3 h after sunrise, and proceeded during periods of high transpiration rates and relatively stable VPD and radiation (Fig. 2). Additionally, mesquite trees have small leaves (43±1.15 mm²).

These features should promote a rapid progression to isotopic steady state due to a fast turnover time of leaf water (Flanagan et al., 1991). Therefore, we believe that we allowed enough time to achieve a near isotopic steady state during our collection periods.

The potential short-term deviations from isotopic steady state of transpired vapor is usually in the order of 1–3% for δ18O under stable conditions (i.e. VPD) (Flanagan et al., 1991; Wang and Yakir, 1995; Harwood et al., 1998) this variation should have minimal impact on partitioning the evapotranspiration flux in our study because the magnitude of the variation of the transpired vapor is small compared to the highly fractionated evaporation flux from soil (Table 2; Gat, 1996; Wang and Yakir, 2000). A deviation of 1.5% δ18O in δTm would alter the tree transpiration and soil evaporation fractions by only ±0.2 mm per day. A deviation from isotopic steady state of 3% in δ18O (extreme for the microphyllous mesquite) would represent a 10% change in the ratio of tree transpiration to soil evaporation (or 0.4 mm per day), in the first case this variation falls within our current 95% confidence intervals. The close agreement with independent measurements of ecosystem water fluxes based on the eddy covariance technique also suggests that mesquite was transpiring at ISS (dotted line in Fig. 5b).

Describing the water relations of different ecosystem components contributes to a more refined understanding of ecosystem function in semiarid regions. Our results from the post-monsoon period indicate that the vegetation used the available moisture efficiently since transpiration appeared to dominate ET. The large contribution of tree transpiration in September may be explained by the ability of mesquite trees to avoid moisture limitations by using deep sources of water not available to the grass layer (Snyder and Williams, 2000; Scott et al., 2003), a mechanism that is commonly found in savanna ecosystems (Walter, 1971; Weltzin and McPherson, 1997). This adaptation can strongly influence the ecosystem functioning by decoupling major ecosystem processes (i.e. tree transpiration and productivity) from precipitation inputs. Precipitation should, however, influence the magnitude of ET and CO2 fluxes from the understory by
wetting the upper layers of the soil and stimulating microbial respiration and herbaceous plant activity (Scott et al., 2000). The low understory contribution to ET in our case may reflect post-monsoon low moisture availability in the upper layers of the soil and the senescence of grasses and herbs. This highlights the need of defining the phenological stages of the different vegetation components when accounting or modeling ecosystem fluxes.

The upper San Pedro River basin is the focus of a large scale, multidisciplinary effort that aims to describe the water balance at the watershed level (Goodrich et al., 2000; Scott et al., 2003). The incorporation and expansion of the stable isotope techniques proposed here will help to resolve the biophysical controls on ET at the regional level. Understanding the use and circulation of water by the vegetation in ecosystems where the relative abundances of different functional types varies (i.e. changes in shrub/grass ratios), also contributes to management of such ecosystems in the face of climate and land use change.

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