Oecologia (2008) 157:197–210
DOI 10.1007/s00442-008-1071-5

PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Life form-specific variations in leaf water oxygen-18 enrichment in Amazonian vegetation

Chun-Ta Lai · Jean P. H. B. Ometto · Joseph A. Berry · Luiz A. Martinelli · Tomas F. Domingues · James R. Ehleringer

Received: 21 November 2006 / Accepted: 14 May 2008 / Published online: 10 June 2008

Abstract Leaf water ^{18}O enrichment (Δ_o) influences the isotopic composition of both gas exchange and organic matter, with Δ_o values responding to changes in atmospheric parameters. In order to examine possible influences of plant parameters on Δ_o dynamics, we measured oxygen isotope ratios ($\delta^{18}O$) of leaf and stem water on plant species representing different life forms in Amazonia forest and pasture ecosystems. We conducted two field experiments: one in March (wet season) and another in September (dry season) 2004. In each experiment, leaf and stem samples were collected at 2-h intervals at night and hourly during the day for 50 h from eight species including upper-canopy forest trees, upper-canopy forest lianas, and lower-canopy forest trees, a C_4 pasture grass and a C_3 pasture shrub. Significant life form-related differences were detected in ^{18}O leaf water values. Initial modeling efforts to explain these observations over-predicted nighttime Δ_o values by as much as 10‰. Across all species, errors associated with measured values of the $\delta^{18}O$ of atmospheric water vapor (δ_v) appeared to be largely responsible for the over-predictions of nighttime Δ_o observations. We could not eliminate collection or storage of water vapor samples as a possible error and therefore developed an alternative, plant-based method for estimating the daily average δ_v value in the absence of direct (reliable) measurements. This approach differs from the common assumption that isotopic equilibrium exists between water vapor and precipitation water, by including transpiration-based contributions from local vegetation through ^{18}O measurements of bulk leaf water. Inclusion of both modified δ_v and non-steady state features resulted in model predictions that more reliably predicted both the magnitude and temporal patterns observed in the data. The influence of life form-specific patterns of Δ_o was incorporated through changes in the effective path length, an important but little known parameter associated with the Péclet effect.

Keywords Craig–Gordon model · Water vapor · Isotopic non-steady state · Transpiration · Tropics

Communicated by Todd Dawson.

C.-T. Lai (✉)
Department of Biology, San Diego State University,
5500 Campanile Drive, San Diego, CA 92182, USA
e-mail: lai@sciences.sdsu.edu

J. P. H. B. Ometto · L. A. Martinelli
Centro de Energia Nuclear na Agricultura, Av. Centenário 303,
Piracicaba, SP Cep 13416-000, Brazil

J. A. Berry
Department of Global Ecology, Carnegie Institution
of Washington, Stanford, CA, USA

T. F. Domingues
Institute of Geography, School of GeoSciences,
University of Edinburgh, Drummond Street,
Edinburgh EH8 9XP, Scotland, UK

J. R. Ehleringer
Department of Biology, University of Utah, 257S 1400E,
Salt Lake City, UT 84112-0840, USA

Introduction

Quantifying temporal variations of the oxygen isotope ratios ($\delta^{18}O$) of leaf water is of ecological interest because of the role this parameter plays in its influences on the isotopic composition of CO_2 and H_2O fluxes in gas exchange and on organic matter (Farquhar and Lloyd 1993; Yakir 1998; Helliker and Ehleringer 2000; Dawson et al. 2002; Ometto et al. 2005; Lai et al. 2006a, b; West et al. 2006; Barbour 2007). Evaporation, driven by vapor pressure deficit of the air, discriminates against $H_2^{18}O$ fluxes. Leaf water thereby becomes ^{18}O-enriched relative to the source water as rates of transpiration increases during the day. Meanwhile, CO_2 that diffuses into leaf intercellular space and later out of stomata become labeled by this enriched ^{18}O signature. Improving our understanding of leaf water ^{18}O enrichment would allow for the development of better process-based models to investigate biosphere–atmosphere water and CO_2 exchange processes spanning from ecosystem to global scales (Farquhar et al. 1993; Flanagan et al. 1997; Wang and Yakir 2000; Cuntz et al. 2003; Lai et al. 2006a, b; Welp et al. 2006). Leaf water ^{18}O enrichment, a labile signal, is also an important determinant of the ultimate oxygen isotope composition recorded in plant cellulose (Epstein et al. 1977; Roden and Ehleringer 1999a, b; Barbour 2007), providing a valuable tool for environmental reconstructions (Epstein et al. 1977; Anderson et al. 1998; Roden et al. 2000; Roden and Ehleringer 2000).

Diurnal patterns of leaf water ^{18}O enrichment above source water (Δ_o), are primarily driven by climatic variables, such as relative humidity (RH), temperature, and the $\delta^{18}O$ values of atmospheric vapor (Craig and Gordon 1965). The Craig–Gordon model, assuming Δ_o values are dependant on instantaneous environmental conditions (i.e., steady state), simulates the diurnal Δ_o pattern, but often over-predicts the magnitude of Δ_o in field conditions (Dongmann et al. 1974; Bariac et al. 1989; Flanagan and Ehleringer 1991; Yakir 1992; Roden and Ehleringer 1999a). This modeling discrepancy has been investigated with a focus on two aspects: evaluation of the steady-state assumption in contrasting environmental conditions (Roden and Ehleringer 1999a; Cernusak et al. 2002; Lai et al. 2006b; Seibt et al. 2006; Barnard et al. 2007), and quantification of the spatial heterogeneity of Δ_o within a leaf (Farquhar and Lloyd 1993; Farquhar and Gan 2003; Gan et al. 2002, 2003; Barbour et al. 2004). New models that consider the combined effect of non-steady state (NSS) and progressive enrichment within a leaf (Helliker and Ehleringer 2000; Gan et al. 2002, 2003) have been developed and tested (Farquhar and Cernusak 2005; Ogée et al. 2007).

Despite these recent advances, two critical aspects concerning NSS influences on variations in Δ_o are still not well understood: the influence of life form through its impacts of leaf and hydraulic properties and the influence of water vapor. First, $\delta^{18}O$ values of water vapor (δ_v) are infrequently measured on short time scales because of the time needed to acquire a sample of sufficient size. Investigations associated with δ_v variability and its interactions with the isotope composition of leaf water have long been limited by the scarcity of measurements, except for steady state measurements (e.g., Roden and Ehleringer 1999a). Recent advances in spectroscopic technique are likely to somewhat alleviate this difficulty (Lee et al. 2007). When direct observations are not available, a first approximation for δ_v values is to assume that precipitation and water vapor are in isotopic equilibrium. This δ_v estimate is then assumed invariable over the course of a day for modeling Δ_o on hourly time scales. The assumption that an isotopic equilibrium exists between water vapor and precipitation water and its influences on Δ_o calculation has not been adequately evaluated. Second, little is known about potential life form-specific variations of leaf water ^{18}O enrichment from plants grown in a common environment. The Wang et al. (1998) survey of 90 plant species grown in a common garden represents the only study that examined non-climatic effects on the evaporative enrichment of bulk leaf water.

Our objectives in this study are twofold: to elucidate the variability in the observed Δ_o values among plant life form that cannot be explained by climatic variables alone, and to investigate a plant-based method for estimating the daily average δ_v in the absence of direct (reliable) measurements.

We conducted two intensive field experiments during the rainy and dry seasons in pasture and forest ecosystems of central Amazonia. We selected eight species associated with the six functional groups described by Domingues (2005) at the top, the middle and bottom portion of the canopy; each represented a distinct microclimate. Contrasts in biotic factors are often related to life form-specific differences in physiological properties such as stomatal conductance (g_s) and leaf anatomy. The latter affects the pathway of water movement and turnover within leaves (Gan et al. 2002). In addition to the collection of leaf and plant source waters for stable isotope analyses, we also measured air temperature (T_a), RH, δ_v, g_s and leaf water contents. These measurements were used to model ^{18}O enrichment of leaf water, from which we gained additional information to assess life form-specific patterns.

Materials and methods

Prediction of leaf water enrichment

A modified Craig–Gordon formulation (Craig and Gordon 1965) was used to predict isotopic enrichment of leaf water. Steady-state leaf water enrichment at the sites of evaporation (Δ_{es}) was modeled according to Farquhar et al. (1989), given by:

$$\Delta_{es} = \varepsilon^* + \varepsilon_k + (\Delta_v - \varepsilon_k)\frac{e_a}{e_i} \quad (1)$$

where ε^* is the temperature-dependent equilibrium fractionation factor between liquid and vapor (Majoube 1971) and ε_k is the kinetic fractionation factor that occurs during diffusion through the stomatal pore. The latter can be calculated for oxygen isotopes as $\varepsilon_k = (32r_s + 21r_b)/(r_s + r_b)$, where r_s and r_b are the stomatal and boundary layer resistances, respectively (Barbour et al. 2004). Δ_v is the isotopic discrimination between water vapor and the source water ($\Delta_v = R_v/R_s - 1$, where R represents the molar ratio of heavy to light isotopes, and subscripts v and s represent vapor and source water, respectively). e_a/e_i is the ratio of vapor pressure in the air to that of the leaf intercellular space.

A modified Craig–Gordon model (Eq. 1) often overestimates observed leaf water enrichment (Dongmann et al. 1974; Bariac et al. 1989; Flanagan and Ehleringer 1991; Yakir 1992). Farquhar and Lloyd (1993) suggested that this discrepancy may be partly explained by the Péclet effect. The Péclet effect describes the convection of non-fractionated water to the site of evaporation opposed by the diffusion of the enriched water. According to Farquhar and Lloyd (1993), the average lamina water enrichment above source water at steady state (Δ_{Ls}) can be calculated by incorporating the Péclet "correction," and is given by:

$$\Delta_{Ls} = \frac{(1 - e^{-P})}{P}\Delta_{es} \tag{2}$$

where P is the Péclet number, defined by $P = LE/CD$, where E is leaf transpiration rate (mol m^{-2} s^{-1}), C is the molar concentration of water (5.55×10^4 mol m^{-3}), D is the diffusivity of $H_2{}^{18}O$ in water (2.66×10^{-9} m^2 s^{-1}), and L is an effective path length (m). L is a species-specific parameter that may vary widely due to the tortuous nature of water movement within a leaf (Barbour and Farquhar 2004). To our knowledge, L has never been directly measured. The uncertainty associated with L and its impact on the calculation of Δ_{Ls} has not been frequently tested. Few studies have estimated L, with values varying from 0.004 to 0.166 (Flanagan et al. 1993, 1994; Wang et al. 1998). These authors used a common approach to calculate L by matching predicted Δ_{Ls} with observed values. In this study, we use a similar approach to estimate L.

NSS models have been proposed to simulate leaf water enrichment (Dongmann et al. 1974; White 1983; Cernusak et al. 2002; Farquhar and Cernusak 2005). Dongmann et al. (1974) proposed a transient model that considers NSS leaf water enrichment, given by:

$$\Delta_L^t = \Delta_{Ls} + (\Delta_L^{t-1} - \Delta_{Ls}) \cdot e^{-dt/\tau} \tag{3}$$

where Δ_L^t is the NSS leaf water enrichment at time t, Δ_{Ls} is predicted steady-state leaf water enrichment, Δ_L^{t-1} is the NSS leaf water enrichment at time t-1, dt is time interval (s), and τ represents the turnover time of leaf water, calculated by:

$$\tau = \frac{W\alpha_k\alpha^*}{gw_i} \tag{4}$$

where W is the lamina leaf water concentration (mol m^{-2}), $\alpha_k = 1 + \varepsilon_k$, $\alpha^* = 1 + \varepsilon^*$, and $\alpha_k\alpha^+ \approx 1$. g is the total conductance to water vapor of stomata plus boundary layer (mol m^{-2} s^{-1}) and w_i is mole fraction of water vapor in the leaf intercellular air spaces (mol mol^{-1}).

Study sites and precipitation patterns

The study sites were located south of Santarém, Pará, Brazil at primary sites associated with the Large-Scale Biosphere-Atmosphere Experiment Amazonia (LBA)-Ecology Project (ECO). The forest site was located at a primary forest in the Tapajós National Forest (2.85°S, 54.05°W) with a mean canopy height of 35 m and emergent trees reaching up to 50 m. The pasture was ~40 km distant (2.77°S, 54.58°W) and was chosen because these sites represent the major land-use change impacting the primary forests in Amazonia; pastures are dominated by a non-native C_4 grass (*Brachiaria* spp.). Information on soil characteristics in the region can be found in Telles et al. (2003). Briefly, soils are deeply weathered oxisols (Hapludox) with high clay content (60–80%), low pH (4.0–4.3) and low nutrient contents. Additional details are available online at http://beija-flor.ornl.gov/lba/. The precipitation regime in the region defines two distinct seasons: a rainy season from December up to and including July and a dry season (<100 mm precipitation per month) from August to November. The two 3-day experiments occurred between 12–14 March and 17–19 September 2004, when leaf and stem samples were collected 10–15 times per day for stable isotope ratio analyses.

Water sample collection and isotope analysis

Leaf and stem water samples were collected at 2-h intervals at night and hourly during the day for a total of 50 h during each experiment. In the forest, samples were taken from vegetation adjacent to a 45-m tower where two overstory trees (*Manilkara huberi* and *Copaifera duckei*) and two lianas (*Prionostemma aspera* and *Abuta rufescens*) were sampled at 38 m, a mid-canopy species (*Derris amazonica*) was sampled at 14 m, and an understory tree (*Inga* sp.) was sampled between 0.5 and 1 m. In the pasture two species, a C_4 grass (*Brachiaria brizantha*) and a C_3 shrub (*Vismia* sp.), were sampled at a height of 1 m. For each species, we collected and pooled three to five stem samples from non-green tissues and then three to five leaves from these same branches. After collection, leaf and stem samples were immediately placed inside separate individual glass vials sealed with a screw cap and the vial-cap junction was wrapped with Parafilm. Samples were kept cold (0–5°C) in the field and later frozen in the laboratory. Water was then extracted from these plant samples using cryogenic vacuum distillation for δ^{18}O analyses of stem (δ_s) and leaf (δ_L) waters (West et al. 2006). We express the measured ^{18}O enrichment of leaf water (Δ_o) relative to stem water, calculated as: $\Delta_o = (\delta_L - \delta_s)/(1 + \delta_s/1000)$. Hydrogen and oxygen isotope ratios of all water samples were determined using an online thermal conversion elemental analyzer–isotope ratio mass spectrometer process (Finnigan MAT, Bremen, Germany).

Atmospheric water vapor was cryogenically captured by pumping air through dry ice–ethanol cold traps at a flow rate of 1 ml s^{-1}. This flow rate was at the lower end of the reported range when sampling apparatus was developed in the laboratory (Helliker et al. 2002). Water vapor samples were collected from three heights within the forest and two heights in the pasture. Sample collection time usually ranges between 10 and 12 min.

We report δ^{18}O measurements with an overall precision of ±0.2‰. All observations are reported in the δ notation on the Vienna standard mean ocean water scale (Coplen 1996). We only present δ^{18}O measurements in this study. Hydrogen isotope ratio data can be found at the NASA LBA-ECO website (http://beija-flor.ornl.gov/lba/).

Meteorological and physiological measurements

A hand-held probe sensor was used to measure T_a and RH at heights where plant tissue samples were collected. Values of e_a/e_i were estimated from T_a and RH measurements by assuming leaf temperature equals T_a. Total leaf conductance to water vapor (stomatal plus boundary-layer conductance, g) was measured every 2 h from 0700 to 1700 hours for all the species using a Li-Cor 6400 photosynthesis system (Li-Cor, Lincoln, Neb.) (Domingues 2005). The boundary-layer conductance was set to a constant (1 mol m^{-2} s^{-1}) in these measurements. We did not measure g at night. The midday leaf water content (W) was determined gravimetrically by measuring the difference between leaf fresh and dry weights, usually on several leaves combined per species. This way, we obtained one W value for each species but no within-species variability can be determined. Measurements of g and W were used to evaluate leaf water enrichment with modeling.

Results

Environmental conditions in the forest and the pasture

The total monthly rainfall in March and September 2004 was 158 and 48 mm, respectively. This difference in precipitation was typical for the wet and dry seasons in Amazonia. Figure 1 compares T_a and RH measured at two heights in the forest and one height in the pasture for the two study periods. There was a considerable vertical gradient of T_a and RH within the forest. RH near the forest floor was considerably lower in the dry than in the wet season, a result of decreased surface soil moisture. Diurnal temperature fluctuations were greater in the pasture than the forest ecosystem, with the pasture having higher daytime T_a in March and lower nighttime T_a in September when compared to the forest. RH was consistently lower in the pasture during the day than in the forest. Nighttime RH values approached 100% in both ecosystems. These patterns of T_a and RH were consistent with observations from micrometeorological towers at these sites.

g and W

g and W values, expressed on a per leaf area basis, were season dependent (Fig. 2a–h; Table 1). The higher g and W values measured in all species during the wet season was anticipated, as were the reductions in both parameters during the dry season. Large life form-specific variations of g and W were observed during both seasons. Upper-canopy trees showed higher g values at midday compared to upper-canopy lianas and understory trees in the forest. Highest g values were also observed in *Vismia* sp., a pasture shrub, during the wet season (Fig. 2d). In contrast to *Vismia* sp., the C_4 grass, *B. brizantha*, had lower g values. As water became relatively more limiting, leaves of *Vismia* sp. reduced g values by nearly fivefold while leaves of *B. brizantha* reduced g values by less than twofold (Fig. 2h), suggesting that C_3 shrubs were more drought-sensitive than C_4 grasses in this pasture ecosystem.

W values varied from 4.4 to 13.3 mol m^{-2} in the wet season, and from 1.2 to 10.2 mol m^{-2} in the dry season (Table 1). Among the contrasting life forms, the understory tree *Inga* sp. had lowest W values, which was expected since this species appeared to have the thinnest leaves of all the species measured in the study. All species had higher midday W values during the wet season, but the magnitude of changes differed among species. All species maintained higher midday W values even though conductances were higher in the wet season than in the dry season (Fig. 2). These physiological adjustments, along with changes in environmental conditions, were likely to have contributed to individual and seasonal variations of ^{18}O enrichment in bulk leaf water, because leaf water will reflect both turnover- and flux-related components.

Measured δ^{18}O of plant source waters

The δ^{18}O values of stem water varied from −5.8 ± 0.2‰ (*B. brizantha*) to −1.9 ± 0.4‰ (*Inga* sp.) in March and from −3.3 ± 0.3‰ (*Vismia* sp.) to 1.1 ± 0.1‰ (*Inga* sp.)

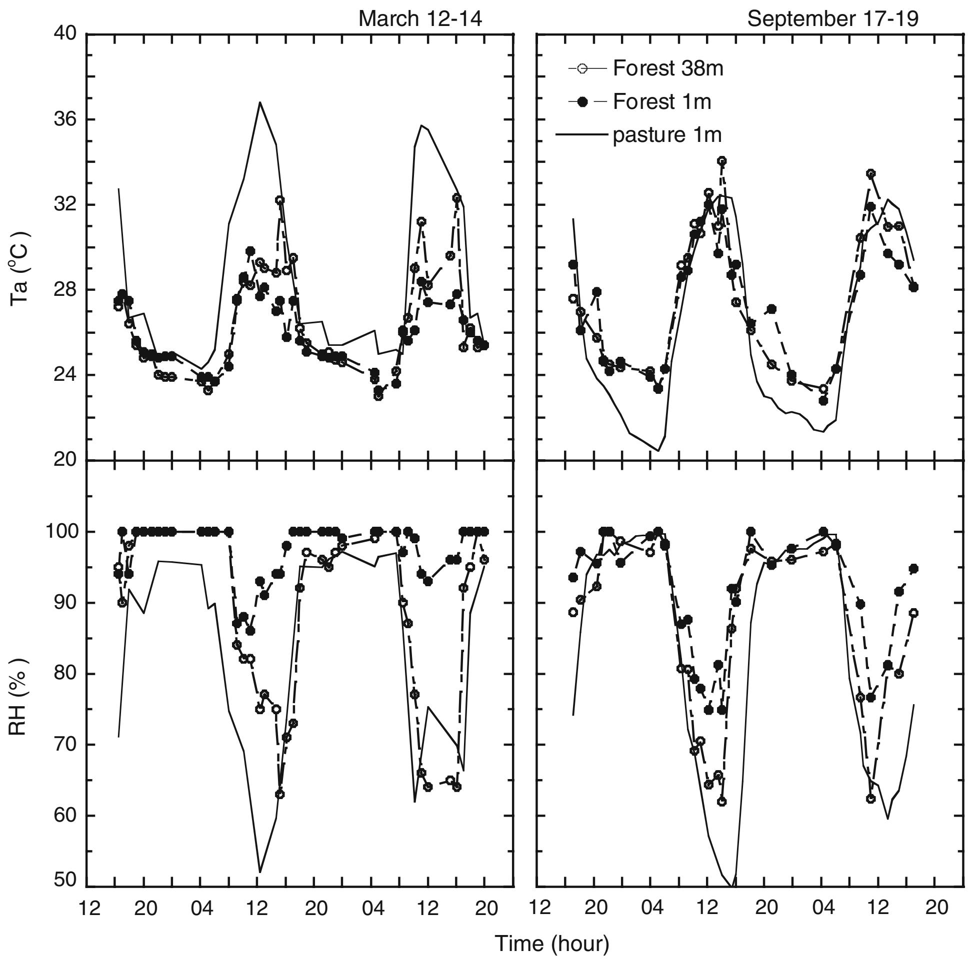

Fig. 1 Air temperature (T_a) and relative humidity (*RH*) measured within the forest and in the pasture for two study periods in March (wet season) and September (dry season) 2004

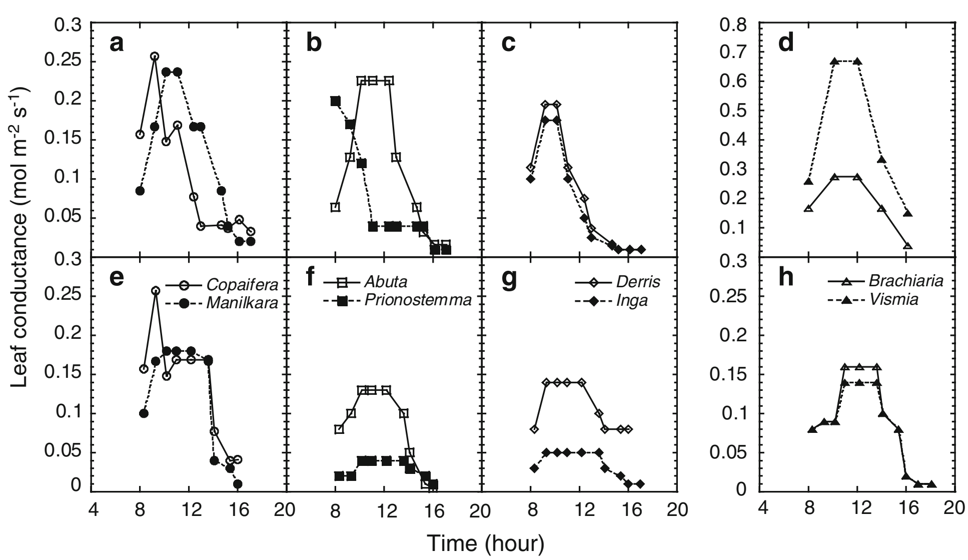

Fig. 2 Measured leaf conductance for the eight study species on 13 March (**a**–**d**) and 18 September (**e**–**h**) 2004

in September (Table 1). Overall, the average δ^{18}O value of the plant source water was 3.0‰ more enriched in the dry season than in the wet season. The International Atomic Energy Agency–Global Network of Isotopes in Precipitation (IAEA-GNIP) has reported long-term, precipitation-weighted δ^{18}O values for Manaus (3.12°S, 60.02°W) of −6.5‰ in March and −2.0‰ in September (source: http://www-naweb.iaea.org/napc/ih/GNIP/IHS_GNIP.html). This seasonal difference of 4.5‰ is similar in magnitude to the δ^{18}O difference in plant sources. Within each season,

Table 1 Average leaf water content and oxygen isotope ratios ($\delta^{18}O$) of stem water measured for the eight study species

Species	Functional group	Average leaf water content[a] (mol m^{-2} leaf)		Stem water $\delta^{18}O$ average (SE) (‰)	
		March	September	March	September
Copaifera duckei	Overstory tree	9.6	7.8	−4.07 (0.31)	−2.04 (0.28)
Manilkara huberi	Overstory tree	12.7	10.2	−5.14 (0.30)	−2.90 (0.20)
Abuta rufescens	Overstory liana	11.8	6.8	−3.32 (0.38)	0.27 (0.38)
Prionostemma aspera	Overstory liana	13.3	2.4	−4.24 (0.39)	−0.74 (0.17)
Derris amazonica	Mid-canopy liana	12.8	4.6	−2.98 (0.31)	0.88 (0.13)
Inga sp.	Understory tree	4.4	1.2	−1.92 (0.42)	1.10 (0.06)
Brachiaria brizantha	Pasture grass	9.0	4.0	−5.78 (0.23)	−1.36 (0.25)
Vismia sp.	Pasture shrub	10	7.5	−4.86 (0.16)	−3.29 (0.28)

[a] No within-species variability was determined for leaf water content (see text)

xylem sap of upper-canopy trees had the lowest source water $\delta^{18}O$ values, followed by upper-canopy lianas and lastly understory species. The pasture grass had lower source water $\delta^{18}O$ values than the shrub in the wet period and higher values in the dry period.

Measured ^{18}O enrichment in leaf waters

Figures 3 and 4 show diel patterns of Δ_o observed in the wet and dry seasons, respectively. Midday leaf water enrichment was observed for all the species except *Inga* sp., an understory tree in the dense forest. These midday Δ_o values were higher in the dry season, which can partly be explained by the lower RH encountered during the measurement period (Fig. 1). Forest species, except one liana *A. rufescens*, exhibited pre-dawn Δ_o values close to zero in the wet season. In contrast, forest species had pre-dawn Δ_o values that were elevated by 2–6‰ above zero in the dry season. The two pasture species showed diel Δ_o patterns similar to the forest species in both seasons, although the magnitudes of the patterns differed.

We observed large differences in the diel pattern of Δ_o among upper-canopy forest species. Considerable Δ_o differences were noted between liana and overstory tree functional groups. Because leaf samples were collected from co-located branches from the same height, little/no changes occurred in general microclimatic conditions that surround these leaves. Instead these observations suggest biotic, life form-specific parameters were likely to have contributed to differences of Δ_o among these Amazonian forest species. Leaf water in the understory tree (*Inga* sp.) showed no or little Δ_o enrichment (Fig. 3c). These Δ_o patterns were consistent with previous observations in the Amazonian forest (Ometto et al. 2005). In contrast to the forest species, the pasture species showed nearly identical Δ_o patterns in both wet and dry seasons.

Measured δ_v

Atmospheric water samples collected throughout each of the field campaign periods were at the minimal size necessary for stable isotope analyses. Measured δ_v values ranged from −11.7 to −7.7‰ in the forest and from −10.0 to −3.0‰ in the pasture in March, and from −7.9 to −3.6‰ in the forest and from −8.8 to −3.7‰ in the pasture in September. These measured δ_v values showed considerable hour-to-hour variation (data not shown). These apparent large changes contrasted with previous δ_v measurements in the Amazonia forests, which were based on larger sample volumes (Moreira et al. 1997). From a theoretical perspective, the δ_v values seemed too high and possibly reflected contaminated or partially enriched samples. The IAEA/GNIP Manaus station reported weighted $\delta^{18}O$ precipitation values as −6.5‰ in March and −2.0‰ in September. Using equilibrium fractionation factors (ε_k) of 9.3‰ in March and 9.2‰ in September, corresponding with average T_a values of 26.0 and 27.7°C respectively for the 2 months, provided the basis of the expected δ_v values. If we assumed that water vapor was in equilibrium with precipitation, the $\delta^{18}O$ value of water vapor ($\delta_{v,equil}$) should have been ca. −15.8‰ in March and −11.2‰ in September, respectively. Our measured δ_v values were considerably more enriched than predicted $\delta_{v,equil}$ values. To examine how the rapid transition of δ_v values interacted with leaf water ^{18}O enrichment at steady state, we initially used the measured δ_v values to model ^{18}O enrichment of bulk leaf water.

Modeling leaf water ^{18}O enrichment with measured δ_v

To model steady-state leaf water ^{18}O enrichment at the sites of evaporation (Δ_{es}), we assumed nighttime values of $g = 0.01$ mol m^{-2} s^{-1} for all C_3 species and

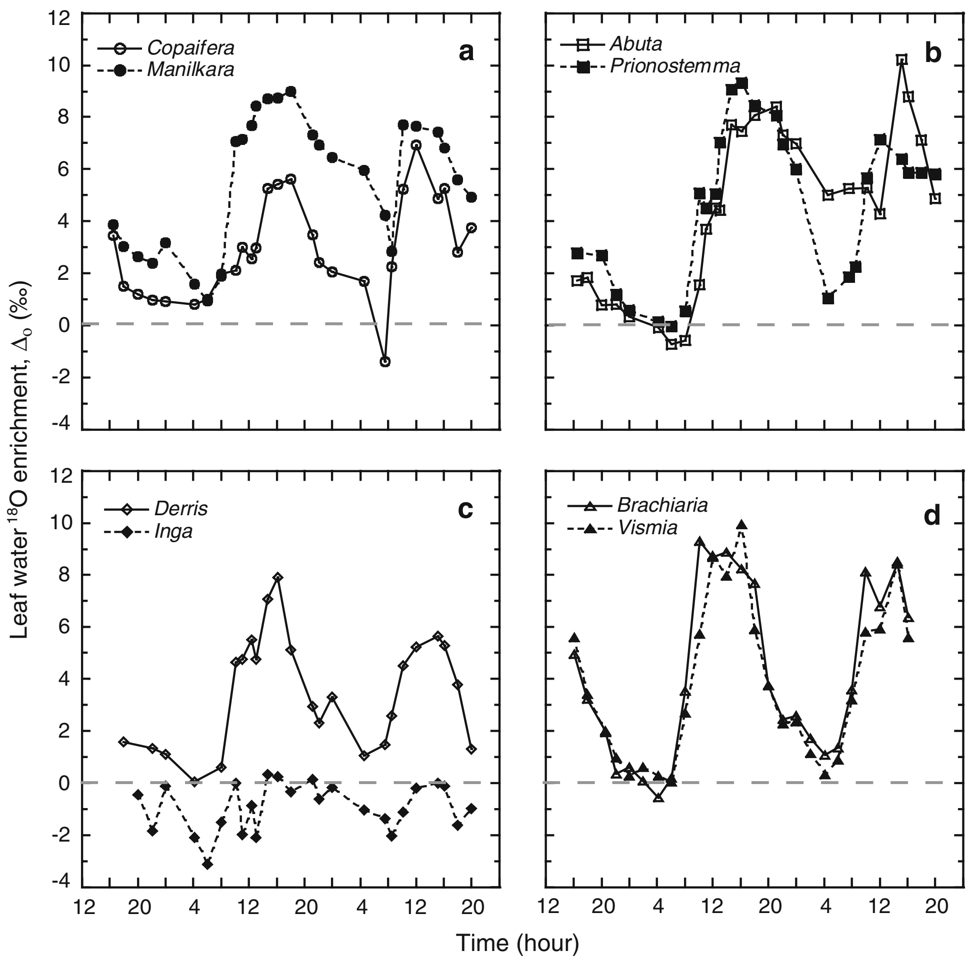

Fig. 3 Measured leaf water δ^{18}O enrichment in the wet season for **a** upper-canopy trees, **b** upper-canopy lianas, and **c** mid-canopy and understory species in the forest, and **d** a C_4 grass and a C_3 shrub in the pasture. For clarity, only average values of leaf water ^{18}O enrichment are shown

0.1 mol m^{-2} s^{-1} for the C_4 grass when observations were not available. These nighttime g values were in close agreement with literature values assumed for C_3 and C_4 plants (Sellers et al. 1996). For each species, we calculated Δ_{es} to compare with observed Δ_o values (Fig. 5).

Our calculations over-predicted observed Δ_o in all instances; differences were most pronounced in the wet season, especially in the pasture ecosystem where the discrepancy reached 10‰ at night and was >10‰ at midday (Fig. 5). The Péclet effect has been suggested as a mechanism to partially explain differences in predicted versus observed Δ_o values during the day (Farquhar and Lloyd 1993), but its effect is expected to be negligible at night because of low transpiration rates. Errors associated with RH measurements could potentially introduce large discrepancies in the prediction of Δ_{es} (Roden et al. 2000; Barbour et al. 2004). However, this was not the case here because the probe-based RH measurements were confirmed by tower-based observations. We assumed that δ^{18}O values of leaf and stem water were correct, because replicates yielded similar values and because these values were similar to previously published observations (Ometto et al. 2005). Thus, the measured δ_v values were then reconsidered.

In humid environments, errors associated with measured δ_v values have largest impacts on Δ_{es}. Figure 6 shows the sensitivity of Δ_{es} to changes of δ_v under various RH conditions. In this example, when RH = 0.1 (10%), a δ_v change from −5 to −15‰ results in a 1‰ decrease in the value of Δ_{es}. When RH = 0.9 (90%), the same δ_v change results in a decrease of the Δ_{es} value by nearly 9‰. Hence, we further examined whether potential errors associated with measured δ_v could lead to the discrepancy in predicted versus modeled values shown in Fig. 5.

Estimating δ_v

We estimated δ_v values using a plant-based approach, considering the equilibrium between leaf water and atmospheric water vapor. The ^{18}O abundance in the vapor is usually less than that in the leaf water, because ^{18}O has a lower saturation vapor pressure than ^{16}O. As RH approaches unity in the late afternoon, the equilibrium process becomes dominant, which consequently decreases

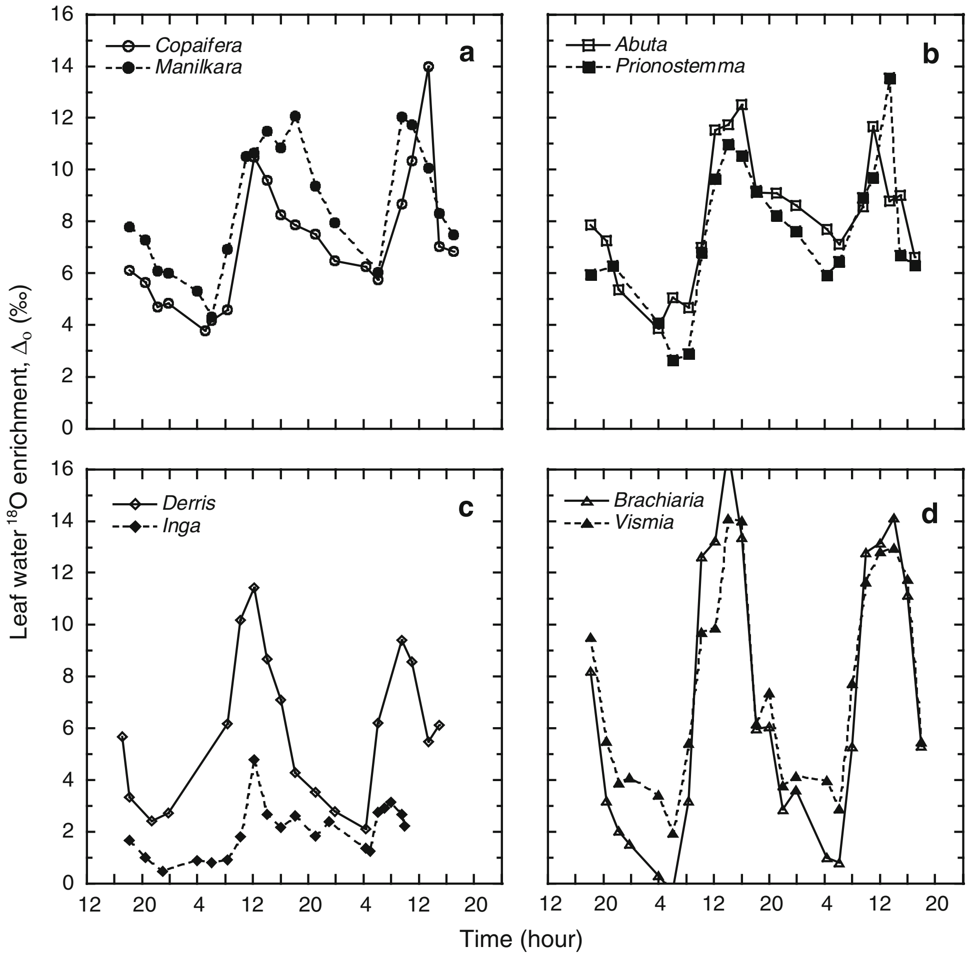

Fig. 4 Measured leaf water δ^{18}O enrichment in the dry season for the same species as shown in Fig. 3

the ^{18}O content of leaf water. This exchange process can continue throughout the night if stomata remain partially open.

On this basis, leaf water and atmospheric vapor may eventually approach a complete equilibrium sometime during the night. This equilibrium process can conveniently be explained by considering Eq. 1. When RH equals 100%, the ratio $e_a/e_i = 1$, which reduces Eq. 1 to

$$\Delta_{es} = \varepsilon^* + \Delta_v. \quad (5)$$

Using Eq. 5, one can calculate Δ_v if Δ_{es} is known. When preferred, δ_v can be calculated instead of Δ_v using the following relationship:

$$\delta_v = \delta_L - (\delta_s + 1) \cdot \varepsilon^*. \quad (6)$$

From a modeling perspective, Δ_{es} represent steady-state leaf water enrichment at sites of evaporation. From a measurement point of view, this variable is rarely directly measured. What was normally measured in the field is the enrichment of bulk leaf water (Δ_o).

A plant-based approach to estimate δ_v relies on measured δ_L and δ_s values, two quantities that are relatively easy to measure in field experiments. The question is whether bulk leaf δ_L values can be used as a reasonable proxy for the estimates of Δ_{es}. This requires that δ_L be measured at a time when leaf water enrichment is at steady-state and that the Péclet effect is negligible. The latter criterion implies low rates of leaf transpiration, most likely to be met during nighttime conditions.

We assumed measured Δ_o values would be closest to a complete equilibrium at pre-dawn (0400–0600 hours), providing a reasonable proxy for Δ_{es}. We then calculated the expected δ_v value at this time using Eqs. 5 and 6. We obtained average δ_v values of -13.4 ± 0.8‰ in the forest and -14.9 ± 1.1‰ in the pasture for the wet season; -7.1 ± 1.1‰ in the forest and -11.2 ± 0.2‰ in the pasture for the dry season. These estimates were similar to the $\delta_{v,equil}$ values (-15.8 and -11.2‰ for the two periods, respectively), assuming that atmospheric water vapor was in equilibrium with precipitation. The estimated forest δ_v value was more positive than $\delta_{v,equil}$ in the dry season (-7.1 vs. -11.2‰). Interestingly, this estimate was similar to measured δ_v values (-6.7 ± 1.5‰, $n = 9$).

Using estimated δ_v and assuming that δ_v remained constant throughout each study period, we re-calculated Δ_{es} and compared it to observed Δ_o in Fig. 5. These revised

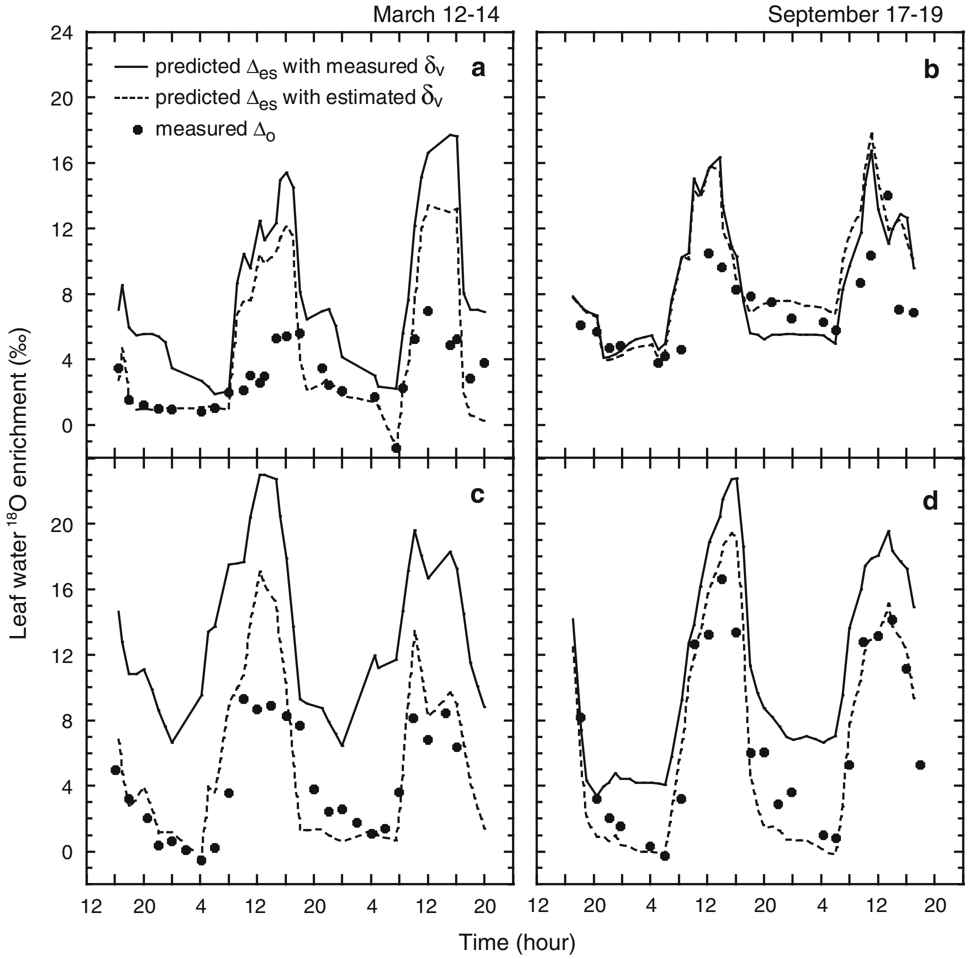

Fig. 5 Comparisons between modeled and measured leaf water δ^{18}O enrichment for a forest species *Copaifera duckei* (**a**, **b**) and a pasture species *Brachiaria brizantha* (**c**, **d**). The modified Craig–Gordon model (Eq. 1) was used here to demonstrate the influence of errors associated with measured δ^{18}O of water vapor (δ_v) on model predictions. Δ_{es} ^{18}O enrichment at sites of evaporation

modeled Δ_{es} values showed greater agreement with the observed nighttime measurements, suggesting that errors associated with measured δ_v may have been the basis for why model and observation did not initially agree. However, the re-calculations still over-predicted daytime Δ_o values, suggesting a second factor not incorporated that was responsible for the midday enrichment (the Péclet effect). Hereafter, we use estimated δ_v and include the Péclet effect in the model to examine biotic influences on daytime patterns of Δ_o.

Biotic effects on leaf water ^{18}O enrichment

To evaluate life form-specific variations of and biotic effects on leaf water enrichment, we consider the Péclet effect when predicting Δ_{Ls} and Δ_L for each species. To contrast individual differences, we focused on two comparisons among life forms: steady-state versus NSS patterns of leaf water enrichment, and differences in the *L*. We used different *L* values for each species when calculating Δ_{Ls} and Δ_L (discussed later).

Two distinct groupings of enrichment patterns can be seen when measured Δ_o values are compared to modeled Δ_{Ls} and modeled Δ_L values for the two growing seasons (Figs. 7, 8). The NSS model appeared to explain observed Δ_o values better than the steady state model for *M. huberi*, *A. rufescens*, and *P. aspera*, while the steady state model explained observed Δ_o values better than the NSS model for *D. amazonica, Vismia* sp., *C. duckei* and *B. brizantha* (see comparisons in Fig. 5 for the latter two species). Both models predicted relatively small ^{18}O enrichment in leaves of *Inga* sp., consistent with observed Δ_o values. We calculated *L* values for all the forest and pasture species except *Inga* sp., which showed no/little enrichment during our experiment. The value of *L* was determined by minimizing root mean square errors between predicted and observed Δ_o at midday (1200–1600 hours).

Discussion

Life form-specific variations of leaf water ^{18}O enrichment

Biotic influences on leaf water ^{18}O enrichment depend on life form-specific factors such as *L* and leaf-water turnover

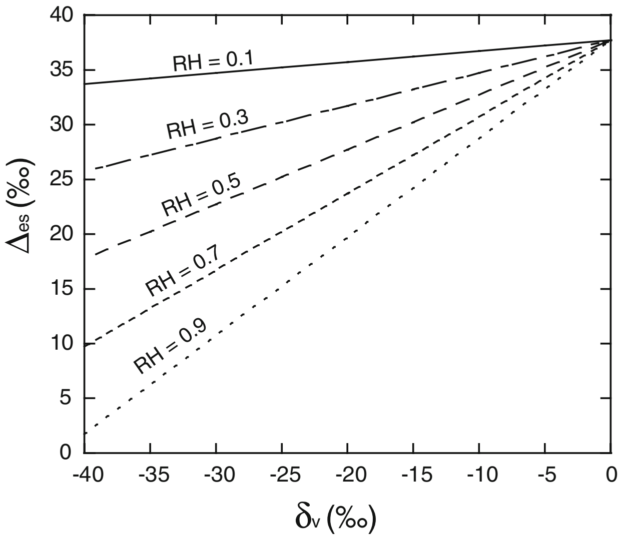

Fig. 6 The sensitivity of Craig–Gordon modeled Δ_{es} to changing δ_v in different RH conditions. In this example, we kept $\Delta_{es} = 37.74$‰ at $\delta_v = 0$‰ by using a value of 0.0316 for the kinetic fractionation factor (ε_k) and a value of 0.0093 for the equilibrium fractionation factor between liquid and vapor (ε^*) when RH = 10%. These fractionation factors were average values representing of the conditions during our experiments. Values of ε^* were allowed to increase by 0.1 ε_k for every 10% increase of RH to keep a constant Δ_{es} at $\delta_v = 0$‰. For other abbreviations, see Figs. 5 and 1

rate (a function of leaf g_s and leaf water content). Together, leaf g_s and L are determinants of the Péclet effect.

NSS leaf water enrichment has been shown to explain under-estimates of predicted nighttime Δ_o values in leaves of *Lupinus angustifolius* (Cernusak et al. 2002; Farquhar and Cernusak 2005) and over-predictions of daytime Δ_o values in needles of *Pseudotsuga menziesii* (Douglas-fir) under water-stress conditions (Lai et al. 2006b). Based on model comparisons (Figs. 5, 7, 8), we found that: (1) the steady state model was suitable to explain Δ_o observations made in the pasture ecosystem, likely because of the high rates of water turnover in leaves; (2) the NSS model was suitable to explain Δ_o observations made in the two lianas which had relatively lower leaf-level water turnover rates; and (3) the two overstory forest trees had contrasting patterns. The model predictions also suggest that leaf water enrichment at midday was at an approximate steady state in all the species. The difference between modeled Δ_{Ls} and Δ_L values became relatively indistinguishable at midday, when leaf water turns over more quickly.

The NSS influence was most pronounced at night when leaf conductances were reduced; inclusion of NSS effects explained nighttime differences in the observed Δ_o values among the studied species. We cannot attribute the nighttime difference to the Péclet effect because we assume nighttime values of $g = 0.01$ mol m^{-2} s^{-1} for all C_3 species in the NSS calculation. This is obviously an over-simplified parameterization as modeled Δ_L only partly explains nighttime Δ_o. Dawson et al. (2007) showed that nighttime transpiration generally occurred when nighttime atmospheric pressure deficit (VPD) exceeded ~0.2 kPa for plants inhabiting ecosystem types without soil water limitation. During our two study periods, nighttime VPD never exceeded 0.2 kPa in these moist tropical ecosystems. The absence of a strong VPD likely prohibits significant nighttime transpiration in the stands studied, assuming that leaf temperature approximately equals T_a.

The calculated L values should be interpreted as initial estimates, because of the assumption of a constant δ_v value. Given the uncertainty in these estimates, only *C. duckei* and *D. amazonica* showed significant differences of L between wet and dry periods. The high L value for *C. duckei* in the wet period was perhaps the calculation with lowest confidence, with uncertainties 1 order of magnitude greater than other estimates. Excluding L values for these two species, average L values fall within a small range (0.04–0.15 m) for forest species. The average L values for the pasture species were similar in both seasons, ranging from 0.01 to 0.04 m. These results fall within the range reported for 90 different plant species grown in a common garden by Wang et al. (1998).

We used 1 SD of the daily-average midday Δ_o values, ranging from 1 to 3‰ depending on species, to estimate uncertainties with respect to the calculated L values. This range of uncertainty is equivalent to a 2–5‰ change in the δ_v value, if diurnal variations were to be accounted for. In other words, the uncertainty estimates shown in Table 2 also address the sensitivity of L calculation to the assumption of a constant δ_v value in the model.

An alternative approach to estimate water vapor $\delta^{18}O$

We assume that an equilibrium establishes at night between leaf water and atmospheric water vapor. This assumption may be supported by a growing number of studies indicating that in many woody plants stomata remain partially open at night (Donovan et al. 1999; Burgess and Dawson 2004; Barbour et al. 2005; Dawson et al. 2007). Dawson et al. (2007) used sap flow and deuterium tracer techniques to measure nighttime g_s and transpiration from woody plants grown in an array of environments, including Amazonia species. They concluded that nighttime stomatal conductance to water vapor is widespread among woody plant species that inhibit a broad range of environments.

Although we did not directly measure nighttime g_s in the current study, flask measurements made at 21 and 45 m in the forest showed enriched $\delta C^{18}OO$ values when compared to those near the ground (J. Berry, unpublished data). These enriched $\delta C^{18}OO$ values are most likely a result of an equilibration process at night between CO_2 molecules and

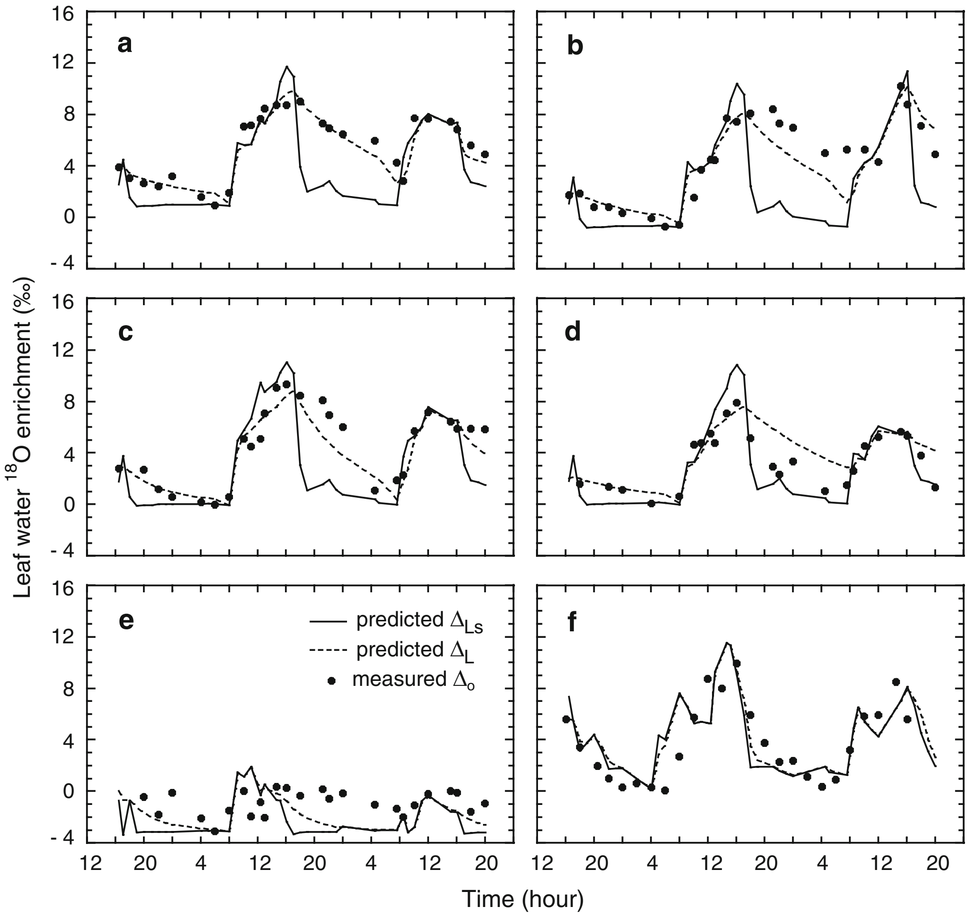

Fig. 7 Comparisons between modeled and measured leaf water δ^{18}O enrichment of six study species: **a** *Manilkara huberi*, **b** *Abuta rufescens*, **c** *Prionostemma aspera*, **d** *Derris amazonica*, **e** *Inga* sp., and **f** *Vismia* sp. in the March period. Average leaf water ^{18}O enrichment was evaluated using a steady state model (Δ_{Ls}) and a non-steady state (NSS) model (Δ_{Ln})

enriched leaf water (Cernusak et al. 2004; Barbour et al. 2005; Seibt et al. 2007). Flask measurements in the pasture showed a similar $\delta C^{18}OO$ gradient. These profile measurements provide indirect evidence to support the notion that it is very likely some of the species in our study had leaves with stomata that were partially open at night.

Bidirectional ^{18}O exchange between water vapor and leaf water can still occur even without detectable nocturnal transpiration as long as stomata remain partially open. Observations from *Inga* sp. leaves suggest the dominant effect of water vapor on leaf water ^{18}O contents under extremely moist conditions at the bottom of a tropical rainforest during the rainy season. At high RH values, equilibrium between water vapor and leaf water is more likely to occur than in an evaporative environment, explaining the diel pattern of negative enrichment observed in the understory of tropical forests. At mid- and upper-canopy heights, evaporative enrichment resumes as the dominant influence.

We assume pre-dawn Δ_o observations a reasonable proxy for Δ_{es}. This proxy selection was supported by nighttime leaf water enrichment in *Lupinus angustifolius* measured under field conditions (Cernusak et al. 2002). Their study provides perhaps the most detailed comparison between observed Δ_o values and predictions from steady-state and NSS models. These authors showed that modified Craig–Gordon model underestimated nighttime Δ_o observations during the majority of the experiment. At pre-dawn hours, however, calculations from both steady state and NSS models converge to agree with observed Δ_o values. We were encouraged by the similar diel Δ_o pattern observed in all but the understory species in the current study. Future surveys on plants grown in controlled environments are needed to validate this assumption.

Transpirational ^{18}O-isoflux contributes positively to the δ^{18}O of water vapor in forest canopies (Lai et al. 2006b). That is, local vegetation contributes to increasing ^{18}O content of atmospheric vapor via transpiration. Estimated δ_v values in the forest were more positive than $\delta_{v,equil}$ values by 2.4 and 4.1‰ in the wet and dry period, respectively. The larger difference implies that local vegetation contributes a greater fraction to the canopy water vapor in the dry season. This ^{18}O enrichment in the vapor would be most significant in areas with large transpirational fluxes, such as tropical forests. Our approach differs from the common assumption that water vapor is likely in equilibrium with precipitation. We incorporate contributions from local vegetation through direct measurements of

Fig. 8 Comparisons between modeled and measured leaf water $\delta^{18}O$ enrichment for the same six species in the September period

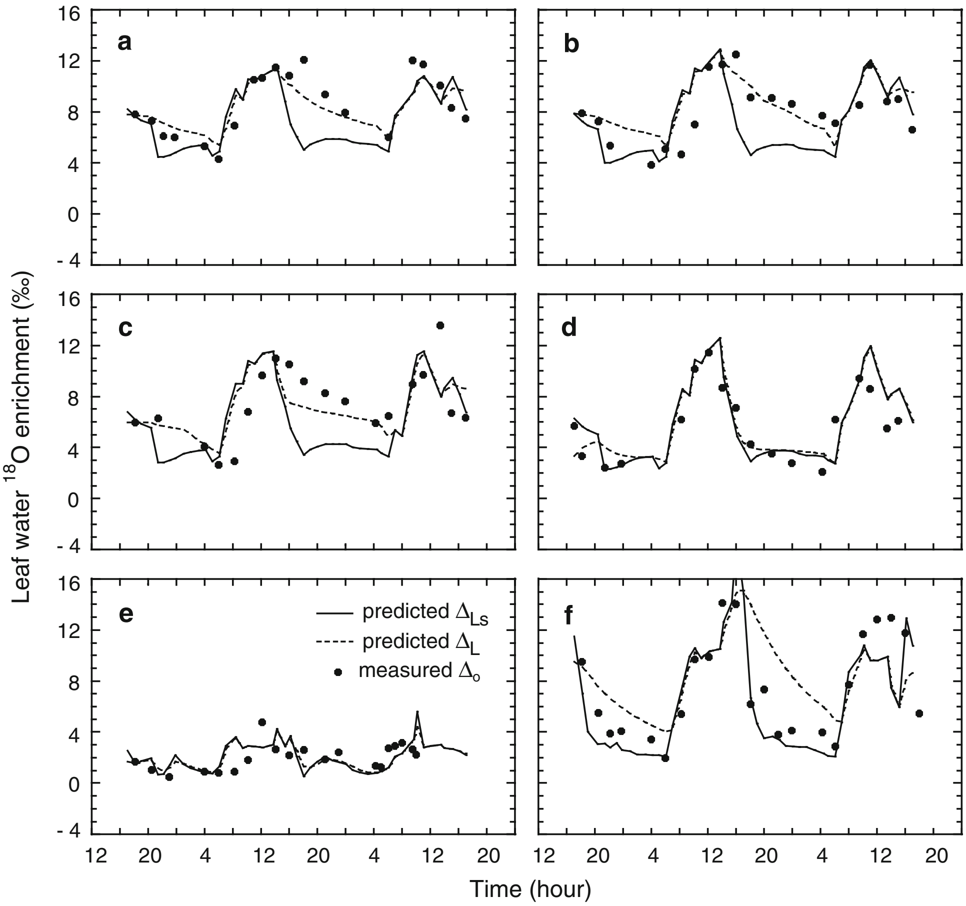

Table 2 Estimated effective path length[a] (L; m) for seven studied species

Species	March	September
C. duckei	0.57 (0.37–0.93)	0.04 (0.01–0.07)
M. huberi	0.05 (0.04–0.06)	0.04 (0.02–0.05)
A. rufescens	0.11 (0.04–0.26)	0.04 (0.02–0.07)
P. aspera	0.07 (0.04–0.12)	0.15 (0.06–0.29)
D. amazonica	0.22 (0.15–0.34)	0.06 (0.03–0.12)
B. brizantha	0.03 (0.02–0.05)	0.01 (0.001–0.03)
Vismia sp.	0.01 (0.007–0.04)	0.04 (0.02–0.06)

[a] Values of L were calculated by fitting modeled enrichment to measurements at midday (1200–1600 hours) by minimizing root mean square errors. The range *in parentheses* represents 1 SD

leaf water enrichment. We hypothesize that this approach has implications, at least qualitatively, for assessing contributions of local vegetation to water vapor contents in regions with distinct seasonal water inputs.

Our approach allows for estimates of a daily average δ_v using bulk leaf water measurements from woody species. Helliker and Griffiths (2007) demonstrated that leaf organic material of tropical CAM epiphytes can potentially be used to reconstruct $\delta^{18}O$ of water vapor over decadal time scales. Our approach differs from that of Helliker and Griffiths (2007) in two fundamental ways. First, we focus on daily δ_v estimates. Second, we use leaf waters from common woody plant species grown in moist environments. Our approach relies on the assumption that in woody plant species stomata remained partially open at night, whereas that is not the case in the approach used by Helliker and Griffiths (2007).

Acknowledgements This study was supported by a grant from NASA to project CD-02 in the LBA Terrestrial Ecology Program. C.-T. Lai was supported, in part, through the Terrestrial Carbon Processes program by the Office of Science (BER), USA Department of Energy under grant no. DE-FG03-00ER63012, and the National Institute for Climatic Change Research program, USA Department of Energy under grant no. DE-FC02-06ER64156.

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