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OXYGEN ISOTOPE RATIOS OF WATERS AND RESPIRED CO_2 IN AMAZONIAN FOREST AND PASTURE ECOSYSTEMS

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Abstract. The oxygen isotope ratio ($\delta^{18}O$, SMOW) of atmospheric CO_2 is a powerful indicator of large-scale CO_2 exchange on land. Oxygen isotopic exchange between CO_2 and water in leaves and soils controls the $\delta^{18}O$ of atmospheric CO_2. Currently there is little empirical information on the spatial and temporal variation in the $\delta^{18}O$ of leaf and stem water in tropical ecosystems. We measured the seasonal dynamics of $\delta^{18}O$ in atmospheric CO_2 and water in different ecosystem compartments in both primary forest and pasture ecosystems in three different regions of the Amazonian Basin of Brazil (Ji-Paraná, Manaus, and Santarém). Within regions, the source (stem) water $\delta^{18}O$ values for primary forests and pastures were similar; neither vegetation type exhibited distinct wet–dry season patterns. Daytime leaf water isotope ratios were strongly correlated with predictions of the Craig-Gordon model. The $\delta^{18}O$ value of leaf water was positively correlated with leaf height above ground because of associated variation in vapor pressure deficit and the $\delta^{18}O$ of atmospheric water vapor within forest canopies. Consistent with these observations, the $\delta^{18}O$ value of leaf cellulose was positively correlated with forest height. Leaf water from pasture grasses was more ^{18}O enriched than leaf water from forest vegetation. There was a tendency for daytime leaf water to be more enriched in ^{18}O during the dry season, reflecting generally lower humidity conditions during the dry season. Nighttime measurements of the oxygen isotope ratio of ecosystem respired CO_2 in both forest and pasture vegetation were not consistent with values expected for CO_2 in equilibrium with stem (soil) water, despite nighttime vapor pressure deficits close to zero. Apparently, the $\delta^{18}O$ of leaf water lagged and did not attain isotopic equilibrium at night. Thus, the deviation of nighttime $\delta^{18}O$ values of ecosystem respiration from that expected from a CO_2 efflux in equilibrium with soil (stem) water increased as $\delta^{18}O$ values of ecosystem respiration became ^{18}O enriched. Discrimination against CO_2 containing ^{18}O ($\Delta C^{18}OO$) during photosynthesis was calculated based on measured leaf water $\delta^{18}O$ values. Forests had consistently higher modeled $\Delta C^{18}OO$ values than pastures. The daytime isotope effects we calculate for photosynthesis and respiration were consistent with previous model predictions of a strong depletion of ^{18}O in atmospheric CO_2 over the Amazon Basin of Brazil.

Key words: *carbon cycle; carbon dioxide (CO_2); oxygen isotope ratio; photosynthesis; respiration; stable isotope ratio; tropical forests.*

Introduction

The annual increase in atmospheric carbon dioxide concentration varies substantially from year to year, despite relatively constant anthropogenic CO_2 emissions from fossil fuel burning (Marland et al. 1999). This has led to the hypothesis that environmentally induced variation in carbon dioxide exchange in terrestrial ecosystems is largely responsible for the observed interannual variation in the rise of atmospheric CO_2 (Fung 2000). Measurement and analysis of the stable carbon isotope composition of atmospheric CO_2 also support large yearly variation in terrestrial net ecosystem carbon uptake (Tans and White 1998). The mechanisms responsible for annual changes in terrestrial ecosystem CO_2 exchange are not well understood, but El Niño/La Niña events contribute substantially to yearly variation in temperature and precipitation with likely significant effects on ecosystem function in tropical regions (Tian et al. 1998, Behrenfeld et al. 2001). Strong interest has been expressed in using additional analyses of the concentration and stable isotope ratio of atmospheric carbon dioxide to infer changes in large-scale biosphere activity and to increase understanding of the controlling processes (Canadell et al. 2000).

The oxygen isotope ratio ($\delta^{18}O$) of atmospheric CO_2 is a powerful indicator of large-scale CO_2 exchange on land (Farquhar et al. 1993, Ciais et al. 1997*a*, *b*, Gillon

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and Yakir 2001, Ehleringer et al. 2002). The primary factor influencing the ^{18}O content of atmospheric CO_2 is an equilibrium isotope effect that occurs between oxygen in water and oxygen in CO_2 dissolved in the water. During photosynthesis, CO_2–H_2O exchange in the chloroplast tends to enrich the atmosphere in ^{18}O because chloroplast water has a higher ^{18}O:^{16}O ratio than water in the soil. Isotopic fractionation during transpiration causes the water pool remaining in leaves to become enriched in ^{18}O relative to water taken up from the soil (Helliker and Ehleringer 2000, Yakir and Sternberg 2000). As a consequence, soil respiration releases CO_2 to the atmosphere that is relatively ^{18}O depleted, in contrast to the normal ^{18}O enrichment associated with CO_2 exchange during photosynthesis.

Seasonal changes in the $\delta^{18}O$ value of atmospheric CO_2 at both regional and global scales are largely determined by gross terrestrial fluxes of photosynthesis and respiration (Ciais and Meijer 1998). Francey and Tans (1987) and Friedli et al. (1987) first proposed that isotopic exchange involving soil and leaf water compartments in terrestrial ecosystems were the driving forces behind the observed latitudinal gradient in $\delta^{18}O$ of CO_2. Subsequently, mechanistic models have been developed for the major isotopic fractionation processes contributing to variations in the $\delta^{18}O$ value of atmospheric CO_2 (Farquhar et al. 1993, Ciais et al. 1997*a*, *b*, Peylin et al. 1999, Riley et al. 2002). However, the full application of these models is difficult because of the complexity and interacting nature of the many controlling factors. In addition, there are limited field data available to validate major parameters in the models, and there is no information on seasonal variation and the effect of El Niño/La Niña cycles in tropical areas. Ciais and Meijer (1998) have specifically emphasized that the following information is necessary to reduce uncertainties in the application of $\delta^{18}O$ in global carbon budget models. First, we need further assessment of the validity of the Craig-Gordon (1965) model (Flanagan et al. 1991) for calculating leaf water isotopic enrichment under field conditions. Second, better characterization is needed of the isotope ratio of ecosystem respired CO_2 and its controlling factors. Despite the fact that the largest photosynthetic and respiratory fluxes are found in tropical ecosystems (Cramer et al. 1999, 2001), only a limited number of data sets exist that can be used to test model predictions in these ecosystems.

Our objective in this study was to measure seasonal and interannual variation in the $\delta^{18}O$ value of leaf and stem (soil) water, and to compare these to measurements of the oxygen isotope ratio of ecosystem respired CO_2. We made measurements in primary forests and pastures in the Amazon Basin of Brazil in order to evaluate temporal changes in environmental conditions and shifts in land use for their effects on the oxygen isotope ratio of atmospheric CO_2. This research was part of a larger study, the Large Scale Biosphere Atmosphere Experiment in Amazonia (LBA), which aims to improve our understanding of the interactions between the atmosphere and ecosystems of the Amazon Basin, a globally important biome.

Oxygen isotope ratio of ecosystem H_2O and CO_2

Throughout this paper we use the conventional "delta" notation, which expresses the isotopic composition of a material relative to that of a standard:

$$\delta = \left(\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1\right) \tag{1}$$

where δ is the isotope ratio and R is the molar ratio of heavy to light isotopes. The δ values are conveniently expressed in parts per thousand (‰). The international standard for oxygen in water, cellulose, and gaseous forms is Standard Mean Ocean Water (SMOW). R_{SMOW} (^{18}O:^{16}O) = 2.0052×10^{-3}.

In order to determine the impact of ecosystem gas exchange on the $\delta^{18}O$ of atmospheric CO_2, we measured and/or calculated (1) the $\delta^{18}O$ of leaf water over a diurnal cycle, (2) photosynthetic discrimination against CO_2 molecules containing ^{18}O ($\Delta C^{18}OO$), and (3) the isotope ratio of CO_2 respired at night from the entire ecosystem ($\delta^{18}O_R$) and from the soil ($\delta^{18}O_{R\text{-soil}}$). All $\delta^{18}O_R$ values were calculated as the intercept from a Keeling plot, two-end-member mixing model (Pataki et al. 2003). Data for these analyses were from flask collections during brief nighttime periods (2–3 h following sunset) and are only presented when the statistical significance of the linear regression was $P < 0.05$.

The $\delta^{18}O$ value of water in the roots and stems ($\delta^{18}O_{\text{Stem}}$) remains the same as the source soil water until reaching the leaves, where evaporative enrichment occurs. Leaf water (R_{Leaf} or $\delta^{18}O_{\text{Leaf}}$) can be calculated using a Craig-Gordon (1965) model as modified by Flanagan et al. (1991):

$$R_{\text{Leaf}} = \alpha^*\left[\alpha_k R_{\text{Stem}}\left(\frac{e_i - e_a}{e_i}\right) + R_{\text{Atm}}\left(\frac{e_a}{e_i}\right)\right] \tag{2}$$

$$\delta^{18}O_{\text{Leaf}} = \left(\frac{R_{\text{Leaf}}}{R_{\text{SMOW}}} - 1\right) \tag{3}$$

where R is the molar ratio of heavy to light isotopes (subscripts Atm, Leaf, and Stem refer to atmospheric water vapor, leaf water, and stem water, respectively) and e is the vapor pressure (subscripts a and i refer to air outside of a leaf and leaf intercellular air spaces, respectively). The liquid–vapor equilibrium fractionation factor is represented by α^* ($\alpha^* = 1.009$ at 25°C) and varies with temperature according to the equations of Majoube (1971); α_k is the kinetic fractionation associated with H_2O diffusion in air ($\alpha_k = 1.0285$).

During photosynthetic gas exchange, discrimination against CO_2 molecules containing ^{18}O is influenced by diffusion and by oxygen isotope exchange with leaf

water. A portion of the CO_2 that enters a leaf during photosynthesis diffuses back to the atmosphere with an altered oxygen isotope ratio, after equilibration with leaf (chloroplast) water. The oxygen isotope exchange process is facilitated by the enzyme carbon anhydrase (CA), which rapidly catalyses the hydration of CO_2 and dehydration of HCO_3^- in the chloroplast. In C_3 shrubs and trees CA activity is high, while CA activity is low in C_4 grasses (Gillon and Yakir 2001). Discrimination against $C^{18}OO$ during photosynthetic gas exchange ($\Delta C^{18}OO$) also depends on the gradient in concentration of CO_2 between the air and the chloroplast surface. These processes are all included in the model developed by Farquhar et al. (1993) and Farquhar and Lloyd (1993):

$$\Delta C^{18}OO = \bar{a} + \left(\frac{c_c}{c_a - c_c}\right) \times \left[\theta(\delta_c - \delta_a) - \left(\frac{\bar{a}}{\frac{c_c}{c_a - c_c} + 1}\right)(1 - \theta)\right] \quad (4)$$

$$\delta_c = \delta^{18}O_{Leaf} + \varepsilon \quad (5)$$

where c_c and c_a are the chloroplast and ambient air concentrations of CO_2; δ_c and δ_a are $\delta^{18}O$ values of CO_2 in the chloroplast and ambient air, respectively; θ is the proportional extent of isotopic equilibrium between oxygen in CO_2 and oxygen in chloroplast water (θ = 0.93 for C_3 trees and shrubs and θ = 0.3 for C_4 grasses (Gillon and Yakir 2001)); ε is the equilibrium fractionation between oxygen in CO_2 and oxygen in water (41.2‰ at 25°C; oxygen in CO_2 becomes enriched in ^{18}O relative to the oxygen in the water pool); and $\bar{a}$ is the weighted mean fractionation during diffusion of CO_2 between the atmosphere and the chloroplast surface (Farquhar et al. 1993). We used measurements of leaf carbon isotope composition in the C_3 forest vegetation to estimate c_c based on c_a values of 370 μmol of CO_2 per mol of air. The average $\delta^{13}C$ value of "sun" leaves was approximately −30‰ in these forests (Ometto et al. 2002), which translates into a c_c value of 282 μmol/mol (Farquhar et al. 1989). For C_4 plants we assumed a c_c value of 180 μmol/mol (Farquhar et al. 1989). The δ_a was assumed to be 41‰ for the model calculations.

The $\delta^{18}O$ value of CO_2 respired from soils is controlled by isotopic fractionation during equilibration of soil CO_2 with water in the soil, and fractionation that occurs during diffusion of CO_2 out of the soil. Therefore, we calculate the $\delta^{18}O$ of soil CO_2 efflux ($\delta^{18}O_{R\text{-}soil}$) as

$$\delta^{18}O_{R\text{-}soil} = \delta^{18}O_{Stem} + \varepsilon - a \quad (6)$$

where a is assumed to have a value of 8.8‰ (Tans 1998).

Materials and Methods

Study sites and precipitation patterns

The sites were located within the Amazon Basin near Santarém, Manaus, and Ji-Paraná, Brazil. In each region, measurements were collected at forest and pasture sites. Each of the forest sites were primary evergreen forests ("terra firme") with a mean canopy height of 30–35 m, although some emergent trees reached 45–50 m. The major land use change replacing the primary forests in Amazonia is pastures, dominated by *Brachiaria* spp., a non-native C_4 grass widespread throughout the region.

The primary forest site near Santarém was located 67 km south of the city, in an area between the Tapajós River and the highway BR 163 (2.85° S, 54.05° W). The forest covers an area of ~600 000 ha and was established as a national forest in 1974 (FLONA Tapajós). The Santarém pasture site was located 77 km south of Santarém (3.02° S, 54.06° W). The Manaus forest site was located 70 km from the city (2.69° S, 60.11° W), in a forest reserve controlled by the Instituto Nacional de Pesquisas Amazonicas (INPA). The Manaus pasture site was located about 60 km from the forest site, along a secondary road (ZF-3). The grass at this site had less vigorous growth than the Santarém pasture grass. The soils at the Manaus and Santarém sites were deeply weathered oxisols (Hapludox) with high clay content (60–80%), low pH (4.0–4.3), and low nutrient content. The Ji-Paraná primary forest site (Rebio Jaru, 10.08° S, 61.92° W) was a reserve controlled by the Brazilian Environment Protection Agency (IBAMA), located north of Ji-Paraná (~80 km), by the Machado River (120 m above sea level). The pasture site (Fazenda Nossa Senhora da Aparecida; 10.75° S, 62.37° W) was a cattle ranch 50 km northeast from Ji-Paraná. The soil in both Ji-Paraná sites has been classified by Hodnett et al. (1996) as an orthic acrisol, with 85%, or more, of sand at the surface layer. All sites, except the Manaus pasture, were official LBA-Ecology study areas. Field measurements were made during several field campaigns from March 1999 through to March 2001 at the Santarém and Manaus sites. Less frequent sampling occurred in Ji-Paraná.

At each of our study sites, there was a clear dry season (<100 mm precipitation per month), although the length and timing of the dry season was different among sites (Fig. 1). The transition from wet season to dry season (and vice versa) was not abrupt and did show some interannual variation. The monthly wet season precipitation was ~250–400 mm (Fig. 1).

Water collection and analysis

Water samples were collected to characterize the stable isotope ratios of different water compartments in pasture and forest ecosystems. Xylem water samples were collected from stems ($\delta^{18}O_{Stem}$) to determine plant water sources in the soil. Leaves were sampled for their leaf water ($\delta^{18}O_{Leaf}$) and atmospheric water vapor ($\delta^{18}O_{Atm}$) was trapped so that $\delta^{18}O_{Leaf}$ values could be modeled. The leaves and stems were collected from several heights within the forest canopy, placed immediately inside individual glass vials sealed with a

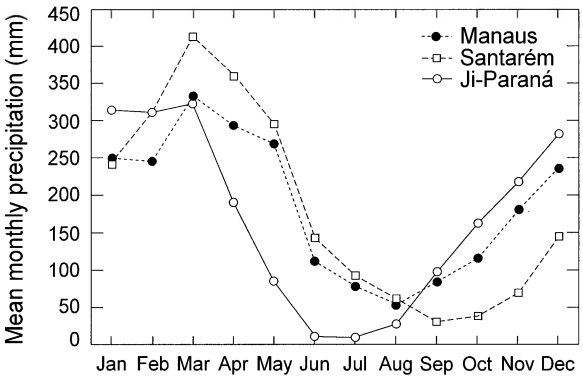

FIG. 1. Mean monthly precipitation for Manaus, Santarém, and Ji-Paraná. The means are for the years 1968–1998 at Santarém, 1961–1990 at Manaus, and 1977–1997 at Ji-Paraná.

rubber stopper and wrapped with Parafilm. The samples were kept cold (0–5°C) in the field and later frozen in the lab until water was extracted using cryogenic vacuum distillation (Ehleringer et al. 2001). Atmospheric water vapor was collected by separately pumping air from different heights within the canopy (three in the forest and two in the pasture) through cold traps (dry ice–ethanol ice slurry) at a flow rate of 1 mL/s (Helliker et al. 2002). Oxygen isotope ratios for all water samples were determined by equilibration of water with CO_2 (Socki et al. 1999). Measurements of the oxygen isotope composition of the equilibrated CO_2 were performed by online injection of CO_2 into a coupled gas chromatograph and isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany), operating in a continuous flow mode (Fessenden et al. 2002).

Air sample collection and analysis

Air samples from within the forest canopy and pasture sites were collected using Dekoron tubing (6 mm outside diameter) attached to a canopy access tower at different heights in the forest (42–31, 21, 7, 0.2 m with a variable inlet at the highest level dependent on the site) and attached to a pole at pasture (0.7, 0.2 m; Ometto et al. 2002). Air was pulled through the tubing using a battery operated pump (Capex V2X, Charles Austin, West Byfleet, Surrey, UK) at a rate of ~20 mL/s. As air flowed through the tubing, it was first dried by passing through a desiccant tube containing magnesium perchlorate and then through a 100-mL glass flask with Teflon stopcocks at either end; the pump was located at the end of this line (Ehleringer and Cook 1998). Flasks were flushed for 7–10 min before stopcocks on the flask were closed.

We sampled the CO_2 respired from soils using a closed cuvette system (Flanagan et al. 1999). A 50 × 40 × 40 cm cuvette was sealed to the soil surface by insertion 5 cm into the soil. A small fan operating at a low flow rate was used to mix air within this cuvette. A long narrow tube was connected between the cuvette and the outside atmosphere to limit pressure differences from developing between inside and outside the cuvette. Air was moved slowly from the cuvette by a separate closed loop through an infrared gas analyzer (LiCor Gas Hound, LI-800, Lincoln, Nebraska, USA) to monitor CO_2 concentration within the cuvette. Attached to the side of the cuvette was a coupled desiccant trap and evacuated 100-mL flask. The CO_2 level was allowed to rise within the cuvette in response to soil respiration and at prescribed CO_2 intervals cuvette air was sampled using the attached flasks.

Stable isotope ratios of CO_2 in air samples collected in 100-mL glass flasks were analyzed using a coupled gas preconcentrator (PreCon) and isotope ratio mass spectrometer (Model 252, Finnigan MAT, Bremen, Germany) operating in a continuous flow mode. A 400-μL subsample of air from the flask was injected into a PreCon system, which allowed isolation of the CO_2 from all other gases before entering into the mass spectrometer. The long-term $\delta^{18}O$ measurement precision of this technique has been ±0.23‰. After stable isotope ratio analysis of the flask had been completed, the CO_2 concentration was determined using a coupled bellows–infrared gas analyzer system (Bowling et al. 2001). The long-term [CO_2] measurement precision has been ±0.3 ppm.

We used a mixing model developed by Keeling (1958), and applied extensively in studies of ecosystem-scale gas exchange (Buchmann et al. 1997, Flanagan and Ehleringer 1998, Bowling et al. 2002, Fessenden and Ehleringer 2002, Ometto et al. 2002, Pataki et al. 2003), to calculate the isotope ratio of CO_2 respired by an entire ecosystem ($\delta^{18}O_R$) and that released from the soil surface ($\delta^{18}O_{R\text{-}soil}$). Estimates for $\delta^{18}O_R$ were obtained from the *y*-intercept of a linear regression between δ_a and c_a^{-1} values measured on air samples collected at different heights within a vegetation canopy at night when photosynthesis was not active. This analysis was also applied to the air samples collected from the soil respiration chamber. The $\delta^{18}O_R$ values presented were all statistically significant ($P < 0.05$). It is important to recognize that the isotope ratio of soil respired CO_2 ($\delta^{18}O_{R\text{-}soil}$), as calculated by Eq. 6, is only one component of the isotope ratio of CO_2 respired by an entire ecosystem (represented by $\delta^{18}O_R$).

The original data have been archived within Ecological Archives as a Supplement and additionally at NASA LBA-ECO and CPTEC LBA.[5,6]

RESULTS

Stem and leaf water $\delta^{18}O$ values

Over a 24-mo period, stem water $\delta^{18}O$ values varied from −9‰ to −3‰ across the three study locations (Fig. 2). Within a region, the $\delta^{18}O_{Stem}$ values of plants

[5] ⟨http://beija-flor.ornl.gov/lba/⟩
[6] ⟨http://lba.cptec.inpe.br⟩

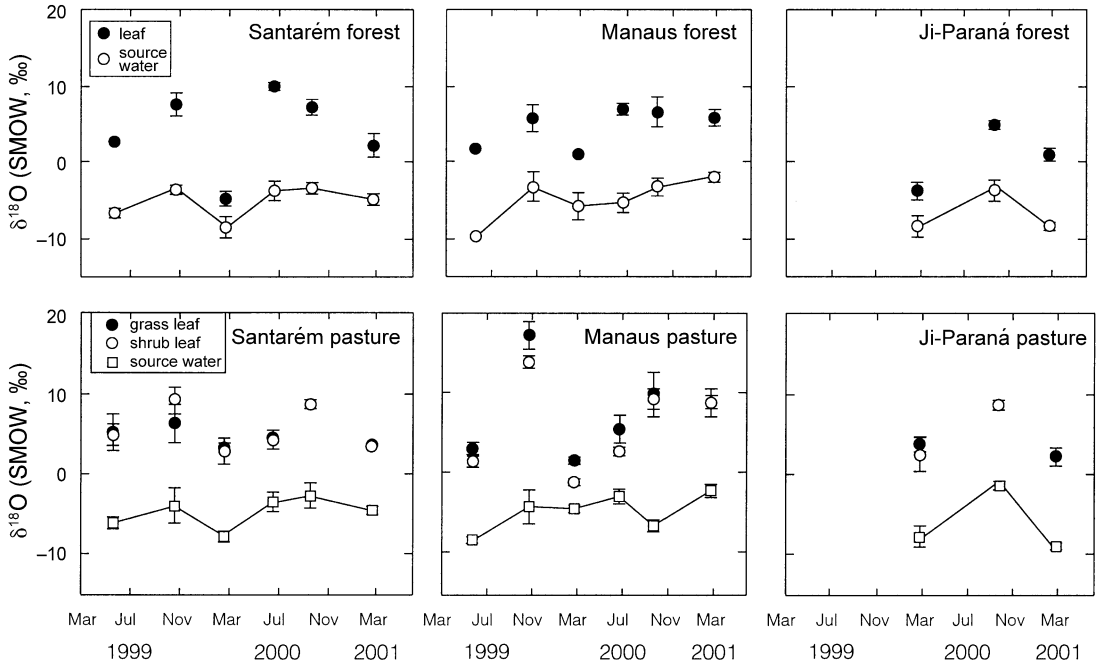

FIG. 2. Temporal variation (mean ± 1 SE) in the oxygen isotope ratio ($\delta^{18}O$, ‰) of stem and leaf water in forest and pasture sites in Brazil.

at forest and pasture sites varied in concert and were very similar on any given sampling date. There was no clear indication of any seasonal (wet vs. dry season) pattern in $\delta^{18}O_{Stem}$ values within a site at any of the six different locations. At the Manaus and Santarém sites where data collections were most extensive (including both pasture and forest), both the minimum and maximum $\delta^{18}O_{Stem}$ values occurred during the wet season period.

While the $\delta^{18}O$ value of stem water remained relatively constant, the $\delta^{18}O_{Leaf}$ values were enriched in ^{18}O relative to stem water and showed much larger temporal and spatial variation (Figs. 2, 3). We used air temperature and humidity data, along with measurements of $\delta^{18}O_{Stem}$ and $\delta^{18}O_{Atm}$ values to model the expected variation in the oxygen isotope ratio of leaf water. At the start of our study, continuous measurements of air temperature and humidity were only available at one study location, the Manaus primary forest site. We observed a strong agreement ($r^2 = 0.83$, $P < 0.01$) between modeled and observed leaf water $\delta^{18}O$ values at the Manaus forest site (Fig. 3). Since $\delta^{18}O_{Stem}$ values were relatively constant, the Craig-Gordon (1965) model (Eq. 2) predicts that leaf water should vary in response to changes in vapor pressure deficit (VPD) and $\delta^{18}O_{Atm}$ (Flanagan et al. 1991). Variation in VPD, in particular, might be expected to result in differences in the $\delta^{18}O$ value of leaf water (1) along a height gradient within a forest canopy, (2) between wet and dry seasons, and (3) in a comparison of forest and pasture sites. We used our measurements and model calculations to examine the influence of these three factors on the oxygen isotope ratio of leaf water.

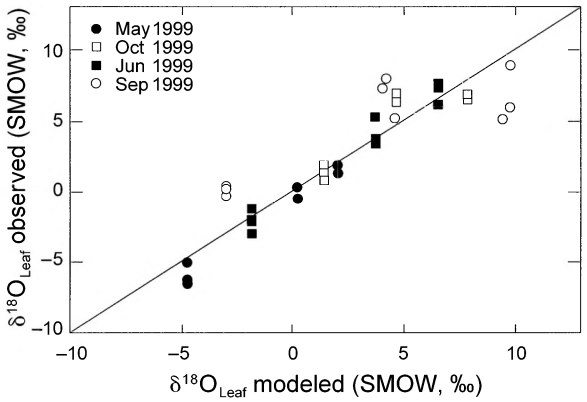

FIG. 3. The relationship between measured and modeled oxygen isotope ratio ($\delta^{18}O$, ‰) of leaf water in the Manaus ZF2 forest in Brazil.

There was a consistent vertical gradient in the midday $\delta^{18}O_{Leaf}$ values in the forest canopies (Fig. 4). Upper canopy leaves from forest sites had water that was more enriched in ^{18}O by 4–6‰ than leaves from lower positions in the canopy. This vertical gradient in leaf water isotope ratio was likely caused by changes in both $\delta^{18}O_{Atm}$ values and VPD throughout the forest canopy. At the Santarém forest site, there was also a strong vertical gradient for the $\delta^{18}O$ value of cellulose purified from leaves (Fig. 5). The oxygen isotope composition of leaf cellulose represents a temporal integration of the $\delta^{18}O$ of leaf water present during cellulose synthesis (Roden et al. 2000).

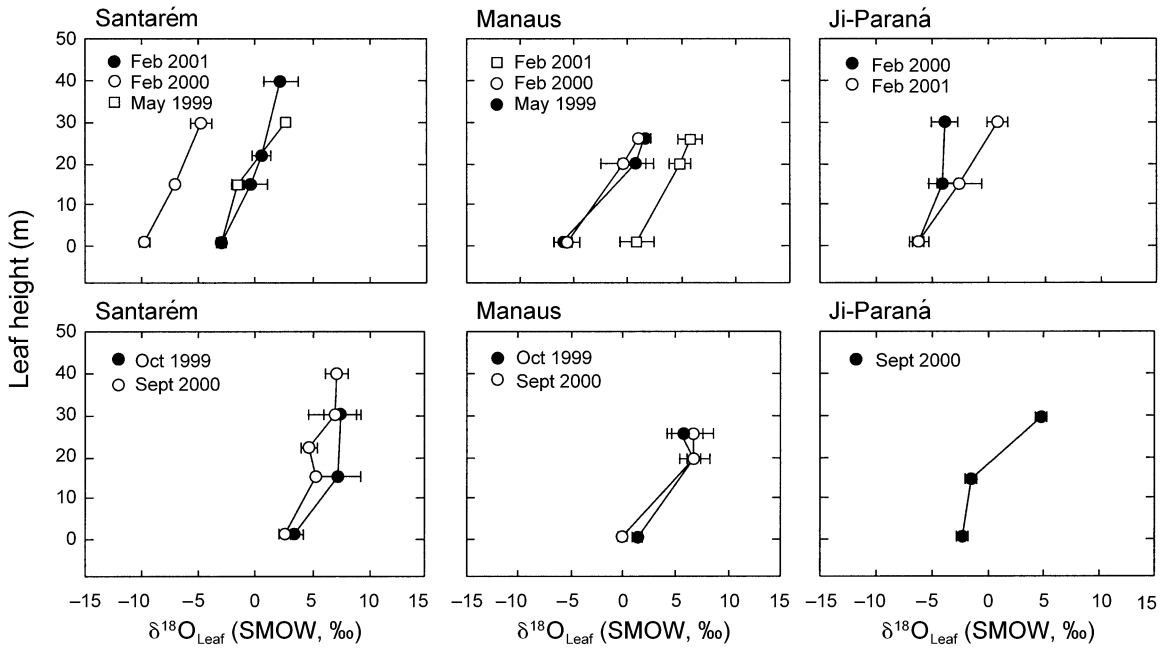

FIG. 4. The relationship between leaf height and the oxygen isotope ratio of leaf water (mean ± 1 SE) in forest sites in Brazil. Data from the wet season (upper panels: >100 mm precipitation/mo) and dry season (lower panels: <100 mm precipitation/mo) are shown in separate panels for each site.

Forest leaf water $\delta^{18}O$ values tended to be more enriched in ^{18}O during the dry season than the wet season (Fig. 4). Our sampling was too limited to rigorously test for dry vs. wet season differences in pasture ecosystems.

Leaf water from pasture grasses was generally more enriched in ^{18}O during the day than leaf water from forest vegetation, with $\delta^{18}O$ values ranging from 1.3‰ to 15.2‰ in Manaus pasture, and similar values occurring in pastures from other regions (Fig. 2). Model calculations, for a comparison of leaf water $\delta^{18}O$ values in forest and pasture sites in Santarém during September 2000, showed that greater enrichment of ^{18}O in leaf water was expected in the pasture site (Fig. 6). This difference in modeled $\delta^{18}O_{Leaf}$ was most likely a result of the higher VPD in the pasture site, because measurements of $\delta^{18}O_{Atm}$ and $\delta^{18}O_{Stem}$ were very similar in the forest and pasture sites (Fig. 6).

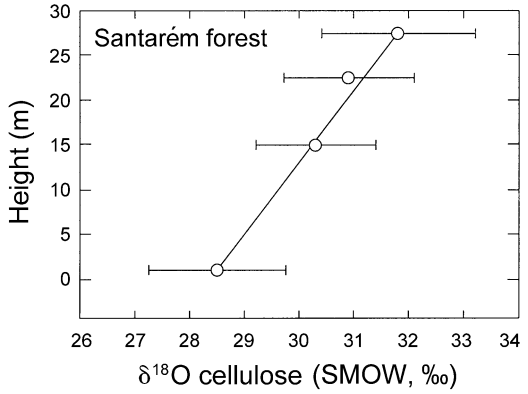

FIG. 5. The relationship between leaf height and the oxygen isotope ratio of leaf cellulose (mean ± 1 SE) in the Santarém primary forest site in Brazil.

$\delta^{18}O$ of respired CO_2 in forest and pasture ecosystems

Highly significant linear relationships occurred between the inverse of CO_2 concentration and the $\delta^{18}O$ values of CO_2 measured on air samples collected within a vegetation canopy at night ($r^2 > 0.85$ typically; see Supplement) and in the soil respiration chamber (Fig. 7). In March 2001, the $\delta^{18}O_{Stem}$ value for trees in the Santarém forest was −7‰. Therefore, we predicted (from Eq. 6) that $\delta^{18}O_{R\text{-}soil}$ should be ~25‰, which is what was observed (Fig. 7). We repeated these $\delta^{18}O_{R\text{-}soil}$ measurements on several occasions and each time the observed $\delta^{18}O_{R\text{-}soil}$ values are within 1–2‰ of that predicted from measured $\delta^{18}O_{Stem}$ values and Eq. 6.

At the whole ecosystem level, the oxygen isotope composition of nighttime respiration ($\delta^{18}O_R$) should reflect the integrated inputs of CO_2 respired by both aboveground vegetation and soil components. We observed temporal variation in $\delta^{18}O_R$ values in both forest and pasture sites (Fig. 8). Forest ecosystem $\delta^{18}O_R$ values ranged between 19.8‰ and 33.1‰, whereas $\delta^{18}O_R$ values from pasture sites ranged between 22.8‰ and 39.2‰. In general, $\delta^{18}O_R$ values were more ^{18}O enriched during the dry season than during the wet season at both forest and pasture sites (Fig. 8). Some of the temporal variation in $\delta^{18}O_R$ values was correlated with

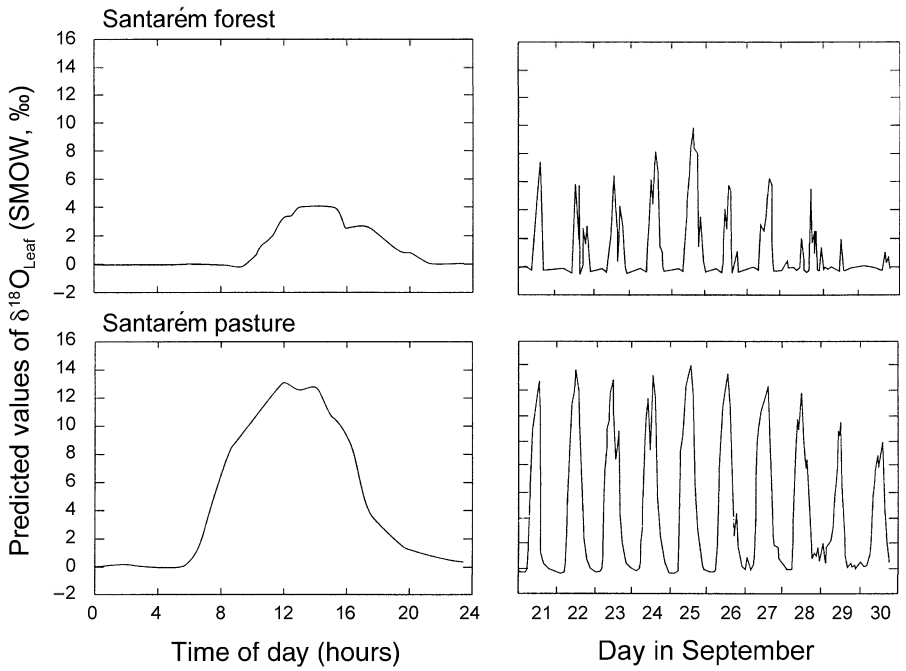

FIG. 6. Comparison of modeled oxygen isotope ratio of leaf water in Santarém forest and pasture sites during September 2000. The left-hand panels represent the mean diurnal pattern for the 10-d period shown in the right-hand panels. The calculations used air temperature and humidity data collected by D. Fitzgerald at the LBA pasture site, and similar data collected by M. Goulden at the forest site. Measurements of the following values were also used in the calculations: $\delta^{18}O_{Stem} = -3.50‰$ (forest) and $-2.81‰$ (pasture); $\delta^{18}O_{Atm} = -9.69‰$ (forest) and $-8.51‰$ (pasture).

changes in the isotope ratio of source water as reflected in the $\delta^{18}O_{Stem}$ values (Fig. 8).

We can interpret some of the variation in $\delta^{18}O_R$ values, although we are restricted from a full interpretation because nighttime $\delta^{18}O_{Leaf}$ values were not measured in this study. In these tropical ecosystems, nighttime humidity levels generally approached 100% shortly after sundown. For an initial interpretation of $\delta^{18}O_R$ values, let us assume that the $\delta^{18}O$ of leaf and soil water should be similar at night. If so, then $\delta^{18}O_{Leaf}$ values should approach that of $\delta^{18}O_{Stem}$, which we expect to be equivalent to $\delta^{18}O$ values of the soil. Here we also assume that the $\delta^{18}O$ values of soil water in the upper soil layers and stem water are identical. This is likely to hold in most cases, since these sites are extremely moist and the high precipitation inputs and well-shaded forest floor are likely not to have experienced much evaporative ^{18}O enrichment. When interpreting $\delta^{18}O_R$ values, Bowling et al. (2003) caution against analyzing data with low r^2 values and so we considered only $\delta^{18}O_R$ values with $r^2 > 0.8$ (resulting in the elimination of 5 of the 22 observations). For both forest and pasture sites, we then calculated the $\delta^{18}O_R$ values expected for a CO_2 efflux in equilibrium with stem (soil) water (calculated using Eq. 6).

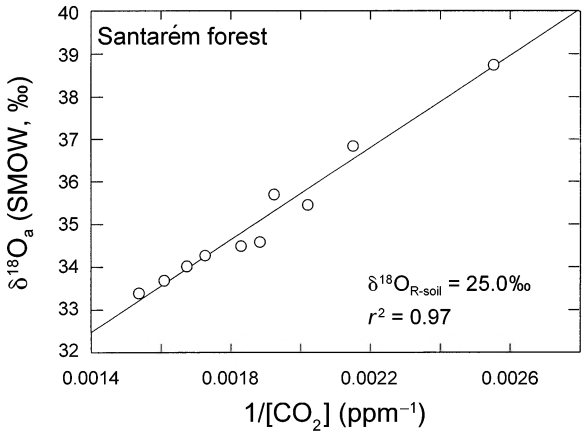

FIG. 7. Relationship between the oxygen isotope ratio of CO_2 in air samples and 1/[CO_2] (ppm^{-1}) (Keeling plot) collected from the head space of a soil respiration chamber in the Santarém primary forest site in Brazil during March 2001.

There was a weak correlation between the observed $\delta^{18}O_R$ and that predicted for CO_2 in equilibrium with soil water only (as predicted by Eq. 6), although the correlation was generally stronger for the wet season than the dry season (Fig. 9). By contrast there was a strong positive correlation between the observed $\delta^{18}O_R$ values and the difference between observed $\delta^{18}O_R$ and that predicted by Eq. 6 (Fig. 10). In general the differences between predicted and observed $\delta^{18}O_R$ values were greater in the dry season at both forest and pasture sites. This observation suggests that leaf water remains enriched above that of soil (and stem water) at night in these tropical ecosystems and that leaf respiration

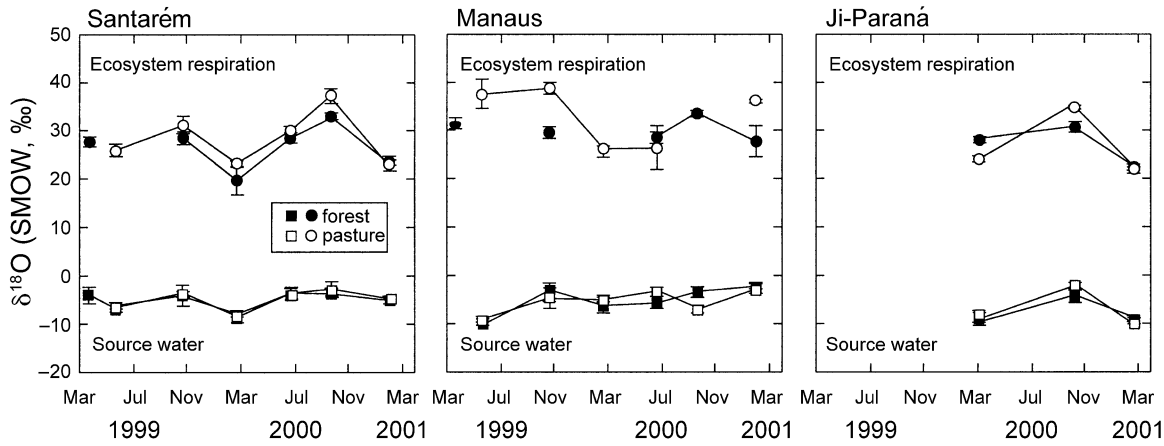

FIG. 8. Comparison of the temporal variation in the oxygen isotope ratio of stem water and the isotope ratio of CO_2 respired at night by the entire ecosystem in forest and pasture sites in Brazil. Data are means ± 1 SE.

contributes a large proportion of total ecosystem respired CO_2. In addition, the relative magnitudes of leaf and soil respiration may change between wet and dry seasons, so that the influence of leaf respiration on the oxygen isotope ratio of total ecosystem respiration increases during the dry season.

Modeling $\Delta C^{18}OO$ in forest and pasture ecosystems

Measurements of $\delta^{18}O_{Leaf}$ values were used in Eqs. 4 and 5 to make calculations of the midday values for discrimination against $C^{18}OO$ during photosynthetic gas exchange ($\Delta C^{18}OO$). Forests had consistently higher modeled $\Delta C^{18}OO$ values than pastures (Fig. 11). This resulted from similar $\delta^{18}O_{Leaf}$ values, but lower c_c and much lower θ values associated with reduced carbon anhydrase (CA) activity in C_4 grasses (Gillon and Yakir 2001). The temporal variation in Fig. 11 was caused completely by changes in $\delta^{18}O_{Leaf}$ values.

Modeled $\Delta C^{18}OO$ values were also used to compare forest and pasture sites in Santarém during September 2000, based on the calculated variation in $\delta^{18}O_{Leaf}$ values shown in Fig. 6. These data indicated that average midday $\Delta C^{18}OO$ values were ~20‰ in the forest, significantly higher than the ~9‰ calculated for the C_4 grasses of the pasture site (Fig. 12).

DISCUSSION

Our study represents the first long-term, integrated set of isotopic measurements of water and CO_2 for tropical rain forest and pasture ecosystems. These data provide insight into the factors driving changes in the $\delta^{18}O$ values of atmospheric CO_2.

We observed good agreement between measured and predicted $\delta^{18}O_{Leaf}$ values in the Manaus forest canopy (Fig. 3). Consistent with the Craig-Gordon (1965) model predictions as modified by Flanagan et al. (1991),

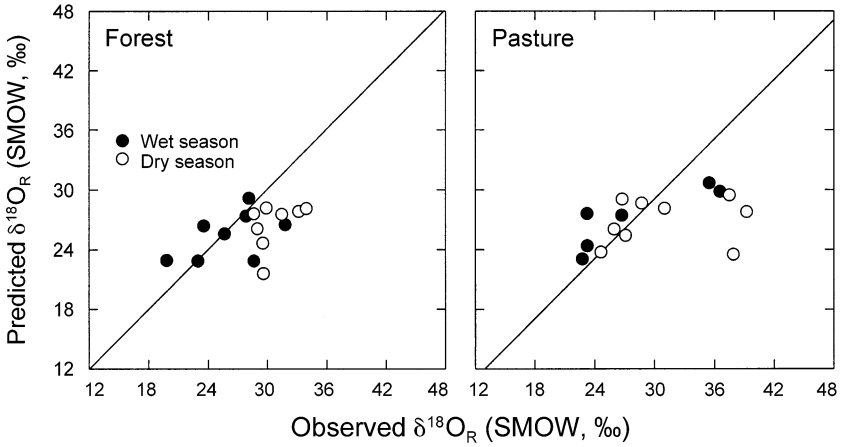

FIG. 9. Comparison between the observed oxygen isotope ratio of CO_2 respired at night by the entire ecosystem and the predicted isotope ratio of respired CO_2 calculated using Eq. 6. The calculations done using Eq. 6 assume that all respired CO_2 has equilibrated with water that has the same $\delta^{18}O$ value as that measured for stem (soil) water at a site. Values are shown separately for the wet and dry seasons for all forest and pasture sites sampled in Brazil.

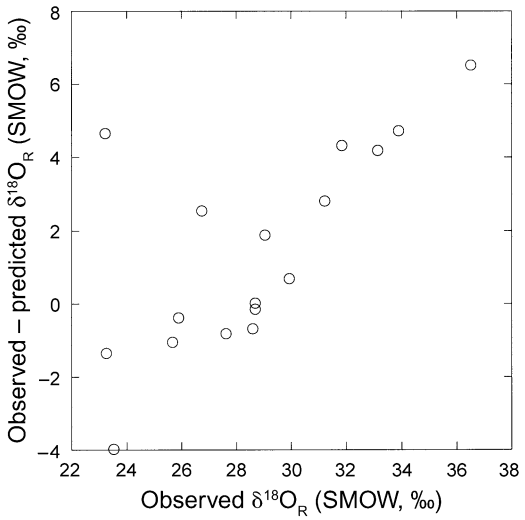

FIG. 10. Comparison of the difference between the oxygen isotope ratio ($\delta^{18}O$, ‰ SMOW) expected of respired CO_2 if all water sources were in equilibrium with soil (stem) water CO_2 and nighttime field observations as a function of the oxygen isotope ratio of ecosystem respiration. The calculations were performed using Eq. 6 and assume that all respired CO_2 has fully equilibrated with water that has the same $\delta^{18}O$ value as that measured for stem (soil) water at a site. Values represent all observations from forest and pasture sites during both the wet and dry seasons where $r^2 > 0.8$.

there were two main factors that contributed to variation in forest leaf water $\delta^{18}O$ values, height variation within a forest canopy and wet vs. dry season differences. Both of these factors were primarily controlled by changes in vapor pressure deficit (VPD), although from one sample date to the next there were changes in the isotope composition of source water (Flanagan et al. 1991, Roden and Ehleringer 1999). Our leaf water $\delta^{18}O$ observations agree with and extend previous shorter term observations for tropical forest regions. Zundel et al. (1978) reported diurnal $\delta^{18}O_{Leaf}$ values for two trees in a tropical rain forest near Itabuna (14° S, 39° W); maximum $\delta^{18}O_{Leaf}$ values of 6–10‰ were observed (with a source water value of −2‰). Interestingly, Zundel et al. (1978) also reported that $\delta^{18}O_{Leaf}$ values from semiarid caatinga forests to the south reached +14‰ at midday (with a source water value of −4‰). Moreira et al. (1997) and Sternberg et al. (1998) reported $\delta^{18}O_{Leaf}$ values from primary forest leaves at the Ducke Reserve near Manaus, Brazil, that were isotopically lighter than those observed in this study. In all likelihood, those differences may have been associated with lower VPD values during the sampling periods. Sternberg et al. (1989) also found significant height gradients in the $\delta^{18}O$ of leaf cellulose at Barro Colorado Island, Panama. The height gradients in cellulose $\delta^{18}O$ were associated with humidity gradients within the Panama tropical moist forest canopy.

In our study, leaf water ^{18}O enrichment in pastures typically exceeded that of adjacent primary forests, largely because of a greater VPD in pasture than in forest sites. The $\delta^{18}O$ values of source waters reflect precipitation inputs; these values in pastures and in adjacent forests were not different from each other and, therefore, it was primarily variation in leaf water enrichment between sites that contributed to the different leaf water $\delta^{18}O$ values observed through time. In addition to environmental variation (VPD) between pasture and forest sites, leaf water isotopic composition in the C_4 pasture grasses is affected by progressive enrichment along parallel veins (Helliker and Ehleringer 2000). Grass $\delta^{18}O_{Leaf}$ values can often be significantly higher in ^{18}O relative to predictions of the Craig-Gordon (1965) model due to this progressive enrichment. Because of the limited availability of meteorological data at the remote study sites during this investigation, we were unable to calculate predicted $\delta^{18}O_{Leaf}$ values in pasture sites for most of our sampling trips. Thus, we were unable to document the extent of the deviation between Craig-Gordon (1965) model predictions and observed leaf water $\delta^{18}O$ values under field conditions in the tropical pastures.

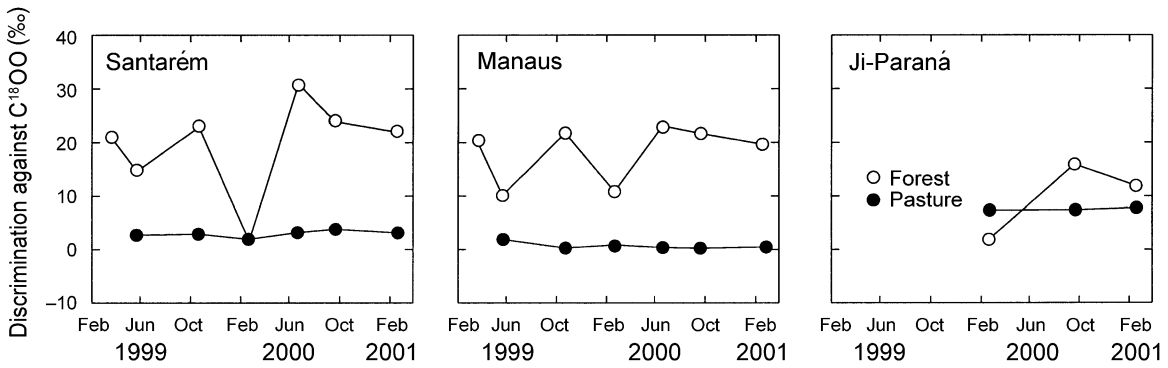

FIG. 11. Temporal variation in discrimination against CO_2 molecules containing ^{18}O ($\Delta C^{18}OO$, ‰ SMOW) at midday in forest and pasture sites in the Amazon Basin. These data represent calculations involving Eq. 4 and using measured midday values of $\delta^{18}O$ of leaf water and the following values: c_c = 282 μmol/mol for C_3 trees, c_c = 180 μmol/mol for C_4 grasses, and c_a = 370 μmol/mol, where c is the CO_2 concentration in the air (a) and sites of carboxylation (c). We assume an equilibration value of θ = 0.93 for C_3 trees and shrubs and θ = 0.3 for C_4 grasses.

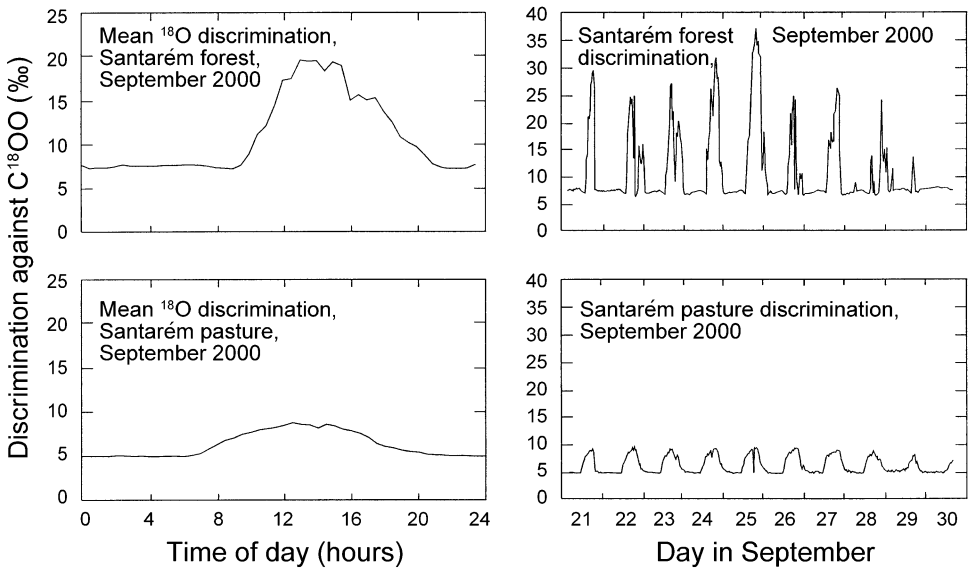

FIG. 12. Comparison of modeled discrimination against CO_2 molecules containing ^{18}O ($\Delta C^{18}OO$, ‰) in the Santarém forest and pasture sites during September 2000. The left-hand panels represent the mean diurnal pattern for the 10-d period shown in the right-hand panels. The calculations were done using Eq. 4 and the modeled leaf water $\delta^{18}O$ values shown in Fig. 6; c_c = 282 μmol/mol for C_3 trees, c_c = 180 μmol/mol for C_4 grasses, and c_a = 370 μmol/mol. We assume that θ = 0.93 for C_3 trees and shrubs and θ = 0.3 for C_4 grasses.

Additional errors can occur in the application of a steady-state model of leaf water evaporative enrichment under field conditions. During the day, the ^{18}O content of leaf water may lag steady-state values by as little as 1–2 h (Flanagan et al. 1991, Roden and Ehleringer 1999), but that time lag is greatly increased at night when water flow through the leaf is substantially reduced (Cernusak et al. 2002). The assumption of isotopic steady state at night will result in underestimation of the $\delta^{18}O_{Leaf}$ values and this has implications for interpreting Keeling plot estimates of the oxygen isotope ratio of ecosystem respired CO_2 (Flanagan et al. 1997, 1999).

We compared our observed $\delta^{18}O_R$ values with those predicted based on CO_2 in equilibrium with soil (stem) water. The predicted $\delta^{18}O_R$ values also assumed a fractionation factor (8.8‰) during diffusion of CO_2 (Tans 1998). A number of factors will contribute to variation between Keeling plot estimates and simple predictions of the oxygen isotope ratio of respired CO_2. For example, Tans (1998) has shown that invasion flux of atmospheric CO_2 into the soil can influence the equilibration between soil CO_2 and water, a result that causes curvature in the $\delta^{18}O$ and $1/[CO_2]$ relationship (at very high $[CO_2]$). Therefore, the linear regression technique (Keeling plot) should result in $\delta^{18}O_R$ values that are slightly more negative than actual $\delta^{18}O_R$ values (Tans 1998). Application of a lower value (7.3‰ rather than 8.8‰) for the effective fractionation during diffusion of CO_2 out of the soil can help deal with this problem (Miller et al. 1999, Stern et al. 1999, Yakir and Sternberg 2000). Leaf respiration at night will also affect the oxygen isotope ratio of total ecosystem respired carbon dioxide, and leaf water $\delta^{18}O$ values can be significantly different than those of the soil water. The Flanagan et al. (1991) leaf water model predicted lower $\delta^{18}O_{Leaf}$ values at night, which were often not consistent with observed $\delta^{18}O_R$ values. The difference may be explained by non steady-state conditions and the time lags necessary for the daytime ^{18}O enrichment signal to be "washed" out of the leaf by input of unenriched xylem water (Cernusak et al. 2002).

During the dry season, aboveground vegetation appeared to contribute more to the oxygen isotope ratio of ecosystem respiration than soil respiration. We base this conclusion on Keeling plot intercept observations, assuming that CO_2 fluxes were derived primarily from soil and leaf sources (Figs. 8, 9). Leaf water isotopic enrichment was generally greater in the dry season than in the wet season. In this case $\delta^{18}O_{Leaf}$ values may also remain more enriched at night and affect the Keeling plot $\delta^{18}O_R$ values to a greater extent in the dry season. Alternatively, there may be wet vs. dry season changes in the relative magnitude of leaf and soil respiration in these tropical forests. Preliminary eddy covariance and chamber data from the Santarém primary forest suggests that soil respiration is significantly reduced during the dry season and this contributes to higher net ecosystem CO_2 uptake because of only minor seasonal variation in gross photosynthesis (Saleska et al. 2003, da Rocha et al. 2004).

Cernusak et al. (2002) provided strong experimental evidence that nighttime $\delta^{18}O_{Leaf}$ values did not ever reach the expected steady-state values with their en-

vironment, most likely because the time to isotopic equilibration lengthened when stomata closed at night. The observed–modeled differences shown in Fig. 10 are consistent with the calculation not accounting for an ^{18}O enriched source of respiratory CO_2 in the forest and pasture ecosystems. Additionally, the increased deviations in expected and observed $\delta^{18}O_R$ values with increasing $\delta^{18}O_R$ values are again consistent with not accounting for CO_2 efflux from enriched $\delta^{18}O_{Leaf}$ values. This is because we would expect that when daytime humidity was low the ^{18}O enrichment of leaf water above soil water would be greater and result in an even larger deviation from assumed steady-state values at night (Eq. 2). However, as no nighttime $\delta^{18}O_{Leaf}$ values were available, we are unable to fully resolve this issue. It is also likely that the relative magnitudes of leaf and soil respiration may change between the wet and dry seasons, such that the influence of leaf respiration on the oxygen isotope ratio of total ecosystem respiration increases during the dry season in the forest sites. Saleska et al. (2003) and da Rocha et al. (2004) and provide evidence indicating that the contribution of soil CO_2 efflux to the total ecosystem respiratory flux declines during the dry season. Thus, it seems inappropriate to conclude that the soil water ^{18}O value alone dominates the $\delta^{18}O_R$ values in these forest and pasture ecosystems, but rather it is likely that the seasonal variations are influenced by both aboveground and soil components as well as by possible changes in the partitioning of the relative fractions of CO_2 derived from these distinct sources. Our focus here was not to evaluate different predictions from the multiple models now available in the literature to predict the $\delta^{18}O$ values of soil CO_2 efflux. Instead our intent was to point out that a simple assumed equilibrium calculation was unable to capture much of the observed seasonal isotopic variation.

Forests had consistently higher modeled $\Delta C^{18}OO$ values than pastures. This resulted from similar $\delta^{18}O_{Leaf}$ values, but lower c_c and much lower θ values associated with reduced carbon anhydrase (CA) activity in C_4 grasses (Gillon and Yakir 2001). With an increasing extent of forest to pasture conversion occurring in the Amazon Basin, it is possible that the reduced $\Delta C^{18}OO$ associated with pasture vegetation could contribute to the decreasing global trend in the $\delta^{18}O$ value of atmospheric CO_2 as described by Gillon and Yakir (2001).

Model calculations by Ciais et al. (1997*a*, *b*) suggested a strong depletion of ^{18}O in atmospheric CO_2 for tropical regions covered by evergreen vegetation, in both Africa and South America (Amazon Basin). The $\delta^{18}O$ values of atmospheric CO_2 predicted for the continental tropical regions with high productivity were even lower than the values calculated for the very high northern latitudes. This suggests that the oxygen isotope ratio of ecosystem respired CO_2 must be significantly depleted in ^{18}O compared to the enrichment of ^{18}O in CO_2 caused by discrimination during photosynthesis in these regions. Our model calculations, which utilize observed leaf water values, predict that $\Delta C^{18}OO$ values for pasture and forest should vary between ~8–20‰ (Fig. 12). These discrimination values are slightly lower than the average $\Delta C^{18}OO$ values of ~21‰ observed during the growing season months in the boreal forest regions of Canada (Flanagan et al. 1997).

Low VPD values generally limit the extent of leaf water isotopic enrichment in tropical forests compared to boreal regions and so $\Delta C^{18}OO$ values are low despite the high leaf c_c values indicated by leaf $\delta^{13}C$ values (Ometto et al. 2002). In addition, the average forest $\delta^{18}O_R$ value observed in this study was ~28‰ on the SMOW scale (Fig. 8), which is equivalent to a $\delta^{18}O_R$ of −13‰ on the PDB-CO_2 scale. This is quite close to the average $\delta^{18}O_R$ value of −14.1‰ (PDB-CO_2) determined by Flanagan et al. (1997) for Canadian boreal forest vegetation. Given the similar values for the isotope effects during photosynthesis and respiration in tropical and boreal regions, and the much higher CO_2 fluxes in tropical evergreen forests, it is expected that atmospheric CO_2 should be depleted in ^{18}O over the Amazon Basin region as predicted by Ciais et al. (1997*a*, *b*). Aircraft sampling of the $\delta^{18}O$ values of atmospheric CO_2 over the Amazon Basin is required to further test these ideas.

Long-term, integrated sets of isotopic measurements of water and CO_2 from terrestrial ecosystems are essential for constraining our interpretations of global and regional carbon cycles. Here we have presented the first multiseasonal data sets for tropical rain forest and pasture ecosystems. These data sets are consistent with predicted patterns proposed by Ciais et al. (1997*a*, *b*) and provide insight into the biological factors driving changes in the $\delta^{18}O$ values of atmospheric CO_2.

Acknowledgments

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SUPPLEMENT

Files of data collected at, or relevant to, different field sites that were used to construct the Keeling plot intercept figures and ^{18}O discrimination figures published in the text are available in ESA's Electronic Data Archive: *Ecological Archives* A015-003-S1.