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Isotopic characteristics of canopies in simulated leaf assemblages

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Abstract

The geologic history of closed-canopy forests is of great interest to paleoecologists and paleoclimatologists alike. Closed canopies have pronounced effects on local, continental and global rainfall and temperature patterns. Although evidence for canopy closure is difficult to reconstruct from the fossil record, the characteristic isotope gradients of the "canopy effect" could be preserved in leaves and proxy biomarkers. To assess this, we employed new carbon isotopic data for leaves collected in diverse light environments within a deciduous, temperate forest (Maryland, USA) and for leaves from a perennially closed canopy, moist tropical forest (Bosque Protector San Lorenzo, Panamá). In the tropical forest, leaf carbon isotope values range 10‰, with higher $\delta^{13}C_{leaf}$ values occurring both in upper reaches of the canopy, and with higher light exposure and lower humidity. Leaf fractionation (Δ_{leaf}) varied negatively with height and light and positively with humidity. Vertical ^{13}C enrichment in leaves largely reflects changes in Δ_{leaf}, and does not trend with $\delta^{13}C$ of CO_2 within the canopy. At the site in Maryland, leaves express a more modest $\delta^{13}C$ range (~6‰), with a clear trend that follows both light and leaf height. Using a model we simulate leaf assemblage isotope patterns from canopy data binned by elevation. The re-sampling (bootstrap) model determined both the mean and range of carbon isotope values for simulated leaf assemblages ranging in size from 10 to over 1000 leaves. For the tropical forest data, the canopy's isotope range is captured with 50 or more randomly sampled leaves. Thus, with a sufficient number of fossil leaves it is possible to distinguish isotopic gradients in an ancient closed canopy forest from those in an open forest. For very large leaf assemblages, mean isotopic values approximate the $\delta^{13}C$ of carbon contributed by leaves to soil and are similar to observed $\delta^{13}C_{litter}$ values at forested sites within Panamá, including the site where leaves were sampled. The model predicts a persistent ~1‰ difference in $\delta^{13}C_{litter}$ for the two sites which is consistent with higher water availability in the tropical forests. This work provides a new framework for linking contemporary ecological observations to the geochemical record using flux-weighted isotope data and lends insights to the effect of forest architecture on organic and isotopic records of ancient terrestrial ecosystems.

1. INTRODUCTION

Dense or "closed" forest canopies are of enormous climatic and ecological significance. The extent of canopy closure represents the area of a forest floor is covered by overlying vegetation. A common definition of a closed canopy is 40% or more of the floor surface covered by plant biomass (FAO, 1999). Canopy closure affects surface albedo, atmospheric circulation, surface roughness, and hydrologic cycling, which can, in turn, influence terrestrial temperature and rainfall redistribution (Bruenig, 1989; Bastable et al., 1993; van Dijk and Keenan, 2007; Boyce

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and Lee, 2010; Boyce et al., 2010). Closed canopies stabilize soils, giving them enormous above- and below-ground carbon storage potential. By preventing soil erosion and promoting soil moisture, canopy structure can affect soil carbon and the carbon cycle – both on the local and global scales (Grace et al., 1995; Buchmann et al., 1997; Koch, 1998; Giambelluca, 2002). Dense forest canopies also host a great variety of microhabitats into which much of the diversity of animal and plant life has specialized (Kohyama, 1993; Wilson, 1994; Ozanne et al., 2003), and the proliferation of canopy habitats may have been important in the evolution of arboreal primates and our hominid ancestors (Sussman, 2005).

The three-dimensional structure of a canopy – the height, density, distribution, and coverage of aerial biomass (Parker, 1995) – remains an elusive collection of traits to detect in the fossil record. Fossil assemblages seldom preserve branchless boles, or evidence of tree spacing or branch density associated with canopy density (Secord et al., 2008). While a few exceptional assemblages include large, fleshy fruits (an adaptation to dense canopy) (Tiffney, 1984; Eriksson et al., 2000; Bruun and Ten Brink, 2008; Friis et al., 2011) or *in situ* litter, logs, and stumps (Williams et al., 2003; DiMichele and Falcon-Lang, 2011), the most common macroscopic plant fossils are leaves and leaf fragments (Ellis et al., 1999). Forest architecture is inferred from leaf fossils by morphologic and taxonomic comparison to extant ecosystems (Upchurch and Wolfe, 1987; Wing et al., 1991, 2000, 2009; Wilf, 2000; Johnson and Ellis, 2002).

The origin of the closed forest canopy is generally linked with the rise of angiosperms (Morley, 2000; Burnham and Johnson, 2004) but the emergence and evolution of closed canopies are poorly understood. Indeed, even the timing of these first canopies is disputed (Upchurch and Wolfe, 1987; Davis et al., 2005). It is difficult to determine from the fossil record the character of the canopied forest habitats and how canopies responded to climate perturbations or other events (Boyce et al., 2010). Given the impact of forests on climate systems, there is great interest in identifying canopy properties of ancient ecosystems. A proxy method that captures carbon isotopic gradients could enable characterization of ancient canopy closure (Ehleringer et al., 1986; Secord et al., 2008; Cerling et al., 2011). At present, however, the specific imprint of forest architecture on isotopic and geochemical properties of preserved leaf fossils, bulk organic matter or plant biomarkers, is not well known.

A long recognized indicator of canopy conditions, the "canopy effect", is a decrease in $\delta^{13}C$ of leaves (hereafter called $\delta^{13}C_{leaf}$) from the top of the canopy to the forest floor (Vogel, 1978). Gradients in light, humidity, and the concentration and carbon isotopic composition of atmospheric CO_2 can all influence $\delta^{13}C_{leaf}$ (Aoki et al., 1978; Medina and Minchin, 1980; Madigosky, 2004; Ometto et al., 2006). Higher carbon assimilation rates in the well-lit canopy top cause leaves to draw down internal CO_2 rapidly, thus lowering discrimination against ^{13}C compared with leaves in the understory (Chazdon and Fetcher, 1984; Ehleringer et al., 1986; Zimmerman and Ehleringer, 1990; Hanba et al., 1997; Niinemets et al., 1999; Meir et al., 2002; Poorter et al., 2006; Niinemets, 2007). This is a major cause of the canopy effect (Ellsworth and Reich, 1993; Baldocchi, 1994). In addition, lower humidity in the upper canopy stimulates stomata closure to slow water loss. A smaller stomata opening restricts the supply of CO_2 to the leaf, limiting the extent to which Rubisco (the primary enzyme of carbon fixation in photosynthesis) prefers $^{12}CO_2$ (Madhavan et al., 1991; Stewart et al., 1995; Brooks et al., 1997). Finally, CO_2 lower in the canopy can be more depleted in ^{13}C compared to the atmosphere due to a higher proportion of respired CO_2 (Vogel, 1978; Medina and Minchin, 1980; Sternberg et al., 1989; van der Merwe and Medina, 1989). Because air circulation is restricted by the density of biomass CO_2 in closed forests commonly exhibits ^{13}C enrichment from the forest soil to the canopy top.

The canopy effect is potentially preserved as a large range in the stable carbon isotope composition of individual leaves from a fossil assemblage (Arens et al., 2000; Beerling and Royer, 2002; Fricke et al., 2007). Yet, data for leaves in living closed canopy forests, suggest that the canopy effect might be attenuated and difficult to detect in fossils. In tropical closed-canopy forests most leaf biomass occurs in the upper canopy. Well-lit leaves have higher photosynthetic rates and have shorter life spans compared to more shaded leaves, although this is not always observed (Parker et al., 1989; Wright and Cannon, 2001; Santiago and Wright, 2007). Reich et al. (1991) found, on a global scale, an inverse logarithmic relationship between leaf life span and net photosynthetic rate. The relationship between litter flux and sun exposure suggests leaves from the upper canopy strata are more likely to be represented in fossil litter assemblages. Further, canopy leaves typically have lower mass and exposed to higher winds making them more likely to be removed, transported and preserved within aqueous depositional environments (Dilcher, 1973; Spicer, 1980; Ferguson, 1985; Burnham et al., 1992) thereby entering the fossil record.

Here, we seek to constrain how these taphonomic pressures – the factors that influence the transition of a living organism to a fossil – affect the isotopic expression in a fossil assemblage. We employed the isotopic and flux data for leaves of two modern forests with different degrees of closure to characterize the isotopic compositions of leaf assemblages of different sizes. We used data from: (1) a seasonally closed-canopy, mature deciduous temperate forest in coastal Maryland and (2) an old-growth, perennially closed-canopy, moist evergreen tropical forest in Panamá. We used a re-sampling (bootstrap) model that simulates leaf-litter assemblages in order to answer the following questions.

- How many leaves from a litter assemblage are necessary to distinguish the isotopic gradient characteristics of canopy closure?
- Are mean $\delta^{13}C_{leaf}$ values for a litter assemblage diagnostic of a forest biome?
- Can we predict the $\delta^{13}C$ values of cumulative litter, soil organic matter, and organic carbon in sedimentary archives using litter flux and isotope patterns in canopies?

We determined the $\delta^{13}C$ range and mean for different sized assemblages of leaves sampled from data for each

forest. We re-sampled very high numbers of leaves in order to estimate the isotopic composition of cumulative carbon delivered to soils as litter, and compared these results to available data from forest soils. Modeled leaf and soil organic carbon isotope patterns in this study offer insights to how forest structure can be derived from carbon isotope measurements of fossil leaves, as well as secondary material – such as teeth, hair, paleosol carbonates, or organic soil carbon (van der Merwe and Medina, 1989; Koch, 1998; Secord et al., 2008; Levin et al., 2011).

Distinct climate and seasonal difference in the Panamá and Maryland, USA forests are reflected in their canopy isotope gradients. In the tropical forest of Panamá, leaves are produced throughout the year within a canopy that is both extensively and persistently closed (Leigh, 1975; Lowman and Wittman, 1996). In the temperate forest of Maryland leaves are produced during the spring when canopy conditions are relatively open (Korner and Basler, 2010).

2. METHODS

2.1. Collection sites

The Bosque Protector San Lorenzo forest preserve of Panamá (Fig. 1) is located at 9°17′N, 79°38′W. This evergreen, moist tropical *terre firme* forest is 130 m above sea level and 4.4 km from the Caribbean coast. The site has a tropical monsoonal climate (Köppen classification by FAO GeoNetwork). It receives, on average, 330 cm of rainfall annually, and it has a mean annual temperature of 26 °C. The 6-ha preserve contains more than 22,000 trees and 240 recorded species of trees and lianas. The canopy is co-dominated by *Manilkara* spp., and *Brosimum utile*. *Calophyllum longifolium*, and *Aspidosperma cruenta* occupy much of the mid-canopy, and *Virola sebifera* occupies mid-canopy, understory, and gaps (CTFS; http://www.ctfs.si.edu/site/Sherman). This forest exhibits a strong, persistent light gradient with less than 1% of available light (>99% light attenuation) reaching the forest floor. No logging has occurred on this site for at least 200 years and it has been an officially protected site for over a century.

In Maryland, the Smithsonian Environmental Research Center (SERC) forest (38°53′N, 76°33′W) is located on the inner coastal plain of the Chesapeake Bay (Fig. 1). Mean annual temperature at the site is 13 °C with hot, humid summers and short, cool winters. Average annual precipitation (MAP) in this area is 108 cm and the region is classified as a humid subtropical climate (Köppen classification by FAO GeoNetwork). This seasonally deciduous forest is dominated by *Liriodendron tulipifera* in the upper and mid-canopy, *Fagus grandifolia* in the lower mid-canopy and *Carpinus caroliniana* in the understory (Parker et al., 1989; Brown and Parker, 1994). Most foliage is produced during spring leaf flush when the canopy is open (Brown and Parker, 1994), although canopy closure of >99% light attenuation is reached at the height of the summer growing period. This site has been undisturbed for ~150 years.

The Maryland and Panamá leaf data sets represent different moisture regimes (MAP = 108 cm versus 330 cm). In addition, the two sites represent leaf populations produced during different canopy closure conditions (open, closed).

2.2. Sampling methods and environmental measurements

In Panamá, at the San Lorenzo forest, leaves were collected along a 47-m vertical transect using a canopy crane. The Smithsonian Tropical Research Institute (STRI) manages this crane which provides access to nearly the entire vertical forest structure in a 0.92 ha area. Bulk leaf samples were taken at 72 unique sampling sites, which were also evaluated to determine the percent available light during the time of maximum photosynthesis. Sites were not selected at random as the sampling goal was to collect leaves from the full range of light, humidity, and limb density environments found in the canopy. Leaves were clipped from trees, stored in paper bags, and dried at 70 °C the same day as collection. Atmospheric air samples were collected in 160 ml serum bottles from sites associated with leaf sampling. These bottles were allowed to equilibrate with atmosphere for 60–90 min, and then crimp-sealed with butyl rubber gas-tight septa. All samples were acquired during a two-month time window, from January to February of 2010.

The percent of available light in the Panamá forest was determined from 9 a.m. to 12 p.m., which is the portion of the day when the most photosynthesis occurs (Goulden et al., 2004). Light measurements were made using data from multiple light meters and over a range of time scales. A LiCor LI200SB pyranometer installed on the crane well above the canopy collected mean, maximum, minimum, and total solar radiation at 15-min time intervals. A second set of light measurements were made using a LiCor LI190 quantum PAR (photosynthetically active radiation) sensor, recording 5 min averages, maxima, and minima at each sampling site. Finally, a Spectrum Technologies Field Scout hand-held quantum PAR meter was utilized to characterize leaf sites during collection. Light measurements were repeated by all three devices throughout each day of the sampling expedition. Light data for the tropical forest are summarized in Fig. 2A and C (see also Supplemental Table 1). Humidity and temperature measurements were coordinated with light measurements using a Traceable digital thermo-hygrometer.

At the SERC site in Maryland leaves were collected over a 40-m vertical transect using a mobile construction crane. Again, sampling was not random as sites were specifically chosen to capture the full range of light within this canopy. Leaf samples were taken at 81 unique sites, which were characterized for their light environment. Leaves were clipped from trees, stored in paper bags and dried at 70 °C within 8 h of collection. All samples were acquired during the summer of 2001. Available light was characterized using digital hemispherical photography. PAR is calculated from these images using measurements of cover and direct, indirect, and total site factors. Light was binned into five distinct categories of estimated environmental light, the crown illumination index, using methods similar to those outline in Keeling and Phillips (2007). Light data from the Maryland forest are summarized in Fig. 2B and D.

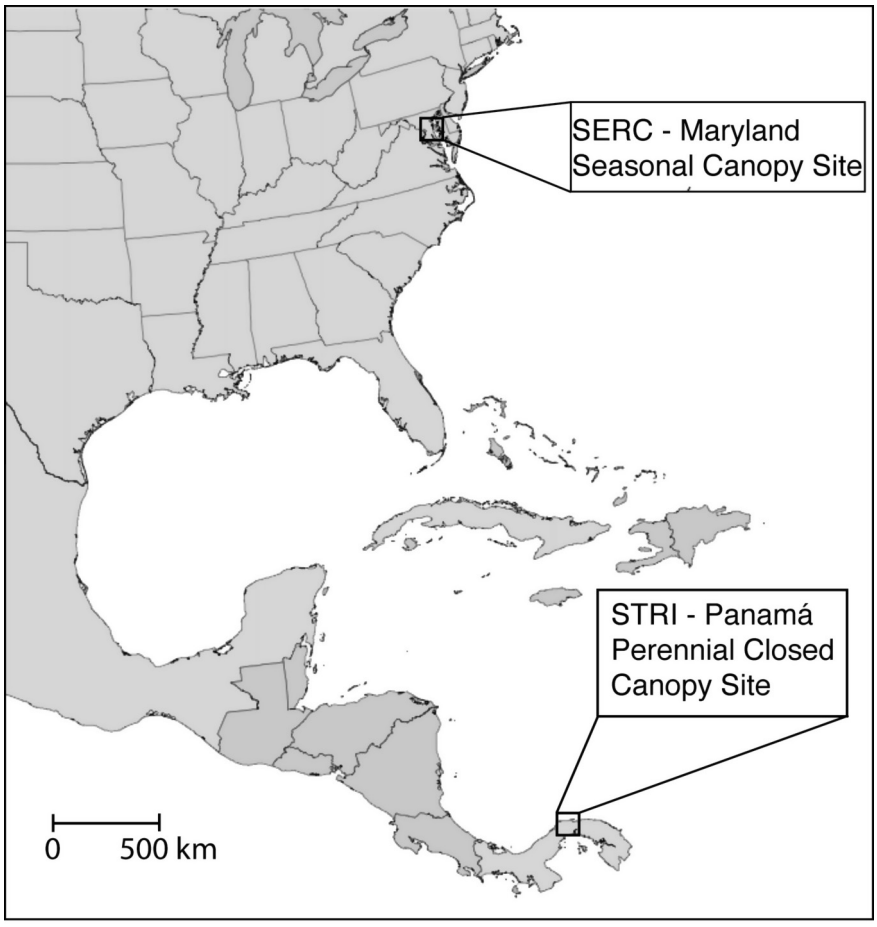

Fig. 1. Two field sites for vertical sampling of leaf material and light measurement.

2.3. Isotopic analysis methods

The carbon isotope analyses of leaves collected in Panamá were performed at Pennsylvania State University. A composite (no fewer than six and on average 18) of dried leaves from each site were ground, homogenized, and analyzed using a Costech elemental analyzer connected to a Thermo Finnigan Delta XP IRMS. Isotopic measurements were determined in duplicate, corrected for sample size and reported relative to Vienna Pee Dee Belemnite (VPDB) following recommendations by Coplen et al. (2006). Errors were evaluated using in-house standards previously calibrated against international standards (IAEA CH-7, IAEA CH-6, and USGS-24). Instrument precision was 0.07‰ ($n = 36$). Accuracy, the average difference between the measured and true $\delta^{13}C$ value, was 0.11‰ ($n = 28$).

Isotope analyses of leaves collected in Maryland were performed at the Carnegie Institution of Washington. Dried leaves were ground and homogenized before analysis using a Carlo Erba Instruments NC 2500 elemental analyzer coupled to a Thermo Scientific Delta V Plus IRMS. Isotopic data were corrected for sample size and reported relative to VPDB. Instrumental precision of (~0.25‰) was evaluated based on the reproducibility of standards repeatedly analyzed as unknowns (NBS-18, NBS-19, and Iso-Analytic R022).

Carbon dioxide was cryogenically distilled from atmospheric samples, and isotopically analyzed using a Finnigan MAT 252 dual-inlet isotope ratio mass spectrometer equipped with a microvolume inlet. Data were corrected for sample size and reported relative to VPDB. The isotopic fractionation between leaf carbon and CO_2 was computed as Δ_{leaf},

$$\Delta_{leaf} = (\delta^{13}C_{CO_2} - \delta^{13}C_{leaf})/(1 + \delta^{13}C_{leaf}/10^3) \quad (1)$$

Fractionation during carbon fixation reflects fractionation as CO_2 diffuses across plant stomata and during enzymatic fixation to sugar (Farquhar et al., 1989). The Δ_{leaf} notation for fractionation is common in ecological literature and is a close approximation of the negative of ε_{leaf}, which is more widely used in the geochemical community:

$$\varepsilon_{leaf} = 1000\frac{\delta_{leaf} + 1000}{\delta_{CO_2} + 1000} - 1 \quad (2)$$

2.4. Flux and biomass

There are no data available that represent the vertical distribution of biomass and litter within the San Lorenzo site in Panamá. Such data generally require destructive sampling methods, which would not be allowed in the protected forest. Instead, we employ published analog data

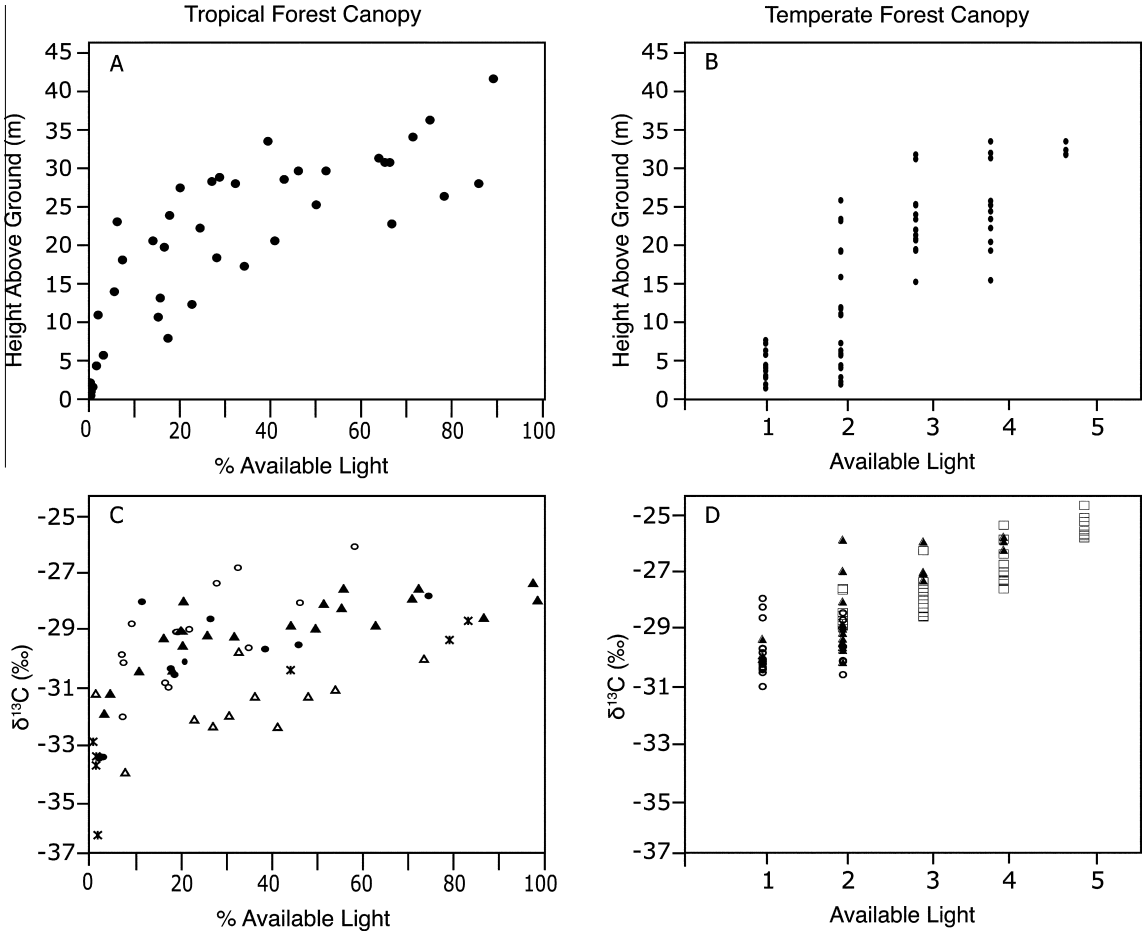

Fig. 2. Light measurements and foliar $\delta^{13}C$ for both tropical and temperate canopy leaf sampling sites. Light at the Panamá site (A) light is expressed as a percentage of available light whereas light at the Maryland site (B) is binned in order of increasing irradiance as determined by hemispheric photography. Symbols denote the five different genera collected at each site. Tropical canopy foliage (C) spans a ~10‰ range in $\delta^{13}C$ that correlates with percentage of available light. $\delta^{13}C$ of temperate canopy leaves (D) also trend with available light but express only a ~6‰ range.

from the Pasoh forest (Osada et al., 2001) to estimate standing biomass and annual leaf flux in a closed-canopy tropical forest. The Pasoh Forest Reserve in Malaysia (2°59′N, 102°18') is a well-studied site and widely used for inferring and comparing properties of lowland tropical rainforests (Manokaran and LaFrankie, 1990; Wills and Condit, 1990; Condit et al., 1996, 1999; Wright et al., 2005). The Pasoh forest is similar to the San Lorenzo forest. They are both moist, evergreen, tropical forests and have similar canopy and stand structures (Manokaran and LaFrankie, 1990). The Pasoh forest is dominated by Dipterocarpaceae, but has a high diversity among the lower abundance taxa. *Shorea* spp. dominates the upper canopy, whereas *Xerospermum noronhianeae* and *Dacryodes* spp. are prominent in the mid-canopy. Biomass refers to the total mass (tons) of leaf material per area (hectare) on the trees at the height of the growing season. Vertically resolved flux data are evaluated as the mass of leaf litter per year and per area derived from within each one-meter height interval. The authors estimated forest biomass using equations that relate leaf biomass to diameter and height of bole. These equations were derived from destructive sampling at a representative site and then extrapolated to the rest of the stand. Leaf fall flux was estimated by relating leaf life span at a particular height to the biomass at that height (Osada et al., 2001).

While the two tropical closed-canopy forests used for biomass and flux data (Pasoh Forest; Osada et al., 2001) and for isotopic data (San Lorenzo; this study) are populated by different taxa, the stand structures are very similar (Manokaran and LaFrankie, 1990; Condit et al., 1996). The data set for the Pasoh forest has sufficient vertical resolution to be compared to our data from Panamá and from the temperate Maryland forest at SERC (Parker et al., 1989). Biomass and flux data for the SERC forest were originally published in Parker et al. (1989); leaf sampling and isotopic analyses are more recent.

2.5. Bootstrap analysis

Leaf fluxes and $\delta^{13}C_{leaf}$ values were incorporated into a resampling model using the 'R' statistical package (R Development Core Team, 2008). All isotope data were binned in five-meter increments vertically, with nine height

bins for the San Lorenzo forest and seven for the SERC forest. The model performed a bootstrap analysis of binned flux and isotope data for both forests.

The height-binned data were sampled with replacement by the model algorithm. The resampling model selected a 'leaf' from vertical data bins (of height x) with the likelihood (L) for each bin determined by its relative contribution to the total flux.

$$L = \Phi_{binx}/\Phi_{\Sigma allbins} \quad (3)$$

Each selected 'leaf' was assigned a $\delta^{13}C$ value randomly selected from the data for that height bin (data are tabulated in the supplementary material). The model repeated sampling and assignment for y number of times, where y is the assemblage size – the number of 'leaves' sampled *in silico*. This process of assemblage collection is repeated 2000 times resulting in 2000 simulated collection scenarios.

The mean $\delta^{13}C_{leaf}$ value for each assemblage of y leaves ($\delta^{13}C_{y\text{-mean}}$) was calculated and recorded by the model yielding 2000 $\delta^{13}C_{y\text{-mean}}$ values. The model also calculated the isotopic range for leaves (Δ_y) in each assemblage within a collection scenario. The range is the difference between the highest and lowest $\delta^{13}C_{leaf}$ values: $\Delta_y = \delta^{13}C_{y\text{-max}} - \delta^{13}C_{y\text{-min}}$. The highest 50 and lowest 50 of the $\delta^{13}C_{y\text{-mean}}$ values were removed from the data set, and subsequent analyses employed the remaining 95% of the data, approximately two standard deviations, for a collection scenario.

3. RESULTS

3.1. Light and height measurements

Fig. 2A and B summarizes vertical light data for both forest types and both show how light increased with height in the canopy. Fig. 2A also shows a wide diversity of light environments were available to leaves in the dense mid-canopy in Panamá. The Maryland forest light data falls into three distinct light regions (0–15 m, 15–25 m, and 25–35 m, see Fig. 2B) divided by the bimodal biomass distribution divided between dense understory and mid-canopy.

3.2. Leaf carbon isotope data

$\delta^{13}C_{leaf}$ values were more enriched in the leaves from the Maryland forest (Fig. 2D) and more depleted $\delta^{13}C_{leaf}$ values were observed in leaves from the Panamá forest (Fig. 2C). Both forests exhibit a vertical isotope gradient (i.e., the canopy effect) with greater height in the canopy (see Fig. 3C and F) as well with light (Fig. 2C and D). $^{13}C_{leaf}$ values are lowest near the ground, in the in the poorly lit portion of the forest, and trend toward higher values in the well-lit upper canopy. The magnitude and character of this gradient differs between the sites. The Maryland forest has a range of 5.8‰ (Fig. 3F). The Panamá forest (Fig. 3C) $\delta^{13}C_{leaf}$ values have a wider range (9.6‰) and have a less well-behaved trend than values in the Maryland forest. This wider range is due in part to the highly ^{13}C-depleted leaves (−36.5‰) observed in understory samples collected less than 5 m above the forest floor. Isotopic variability with height is likely linked to highly variable growth conditions, particularly in the upper and mid-canopy (i.e., 20–30 m above the forest floor), where available light ranged between 10% and 90% and $\delta^{13}C_{leaf}$ values ranged over 6‰ (see Supplemental Table 1).

4. DISCUSSION

4.1. Light properties and the $\delta^{13}C$ canopy effect at San Lorenzo

4.1.1. Intra-canopy light trends and the $\delta^{13}C$ canopy effect

The canopy effect is commonly attributed to ^{13}C-depleted CO_2 that collects at the base of a forest as a result of microbial respiration in the soil (Vogel, 1978; Medina and Minchin, 1980; van der Merwe and Medina, 1989). This attribution is derived from atmospheric sampling experiments in forests that find an overall depletion in nighttime $^{13}C_{CO_2}$ when photosynthesis ceases but microbial respiration continues (see Pataki et al., 2003). Diurnal gas isotope data are not available for our sites, but sampled atmospheric gases in a subset of leaf sampling sites in the mid-canopy of the San Lorenzo forest represent the character of $\delta^{13}C_{CO_2}$ with height, humidity, and with light (as a measure of canopy density and atmospheric mixing) at the time when photosynthetic activity is highest (before 12 pm; Goulden et al., 2004) (Fig. 4A and C). Our data show no significant correlation between $\delta^{13}C_{CO_2}$ and light or height ($R^2 = 0.21$; $p = 0.10$ and $R^2 = 0.13$; $p = 0.23$; respectively) (Fig. 4A and C). This suggests that the atmosphere was well mixed at mid-day.

By pairing CO_2 isotope data and $\delta^{13}C_{leaf}$ values at a subset of leaf sampling sites we calculate Δ_{leaf}. This subset of leaf samples includes *Aspidosperma*, *Brosimum*, *Calophyllum* and *Virola*. Δ_{leaf} is plotted in Fig. 4B and D relative to leaf height and available light. Δ_{leaf}, as defined by Farquhar et al. (1989) (Eq. (1)) describes the fractionation of carbon isotopes by a leaf during the uptake and fixation of CO_2. Greater Δ_{leaf} values are observed in leaves with higher stomatal conductance. Stomatal conductance is higher where the difference between the vapor pressure of water within the leaf versus that in the air (i.e., vapor pressure deficit or VPD) is small (Madhavan et al., 1991). Higher Δ_{leaf} values are also expected under low light, which reduces rates of photosynthesis and carbon fixation. Our data revealed decreased fractionation (smaller Δ_{leaf} values) at both higher light availability and with higher leaf elevation (Fig. 4B and D). Regression analysis of the data shows that there is a significant relationship between Δ_{leaf} values and light, but not height ($R^2 = 0.39$; $p = 0.02$ and $R^2 = 0.20$; $p = 0.13$; respectively).

4.1.2. Intra-canopy humidity trends and the $\delta^{13}C$ canopy effect

VPD is low when conditions are humid, as is common in a tropical rainforest so higher stomatal conductance and greater Δ_{leaf} values are expected (see Fig. 5). Higher relative humidity under a dense forest canopy could result in increased fractionation and larger Δ_{leaf} values. The effects of humidity and light on photosynthesis tend to co-vary and are highly variable, and it is difficult to separate the relative importance

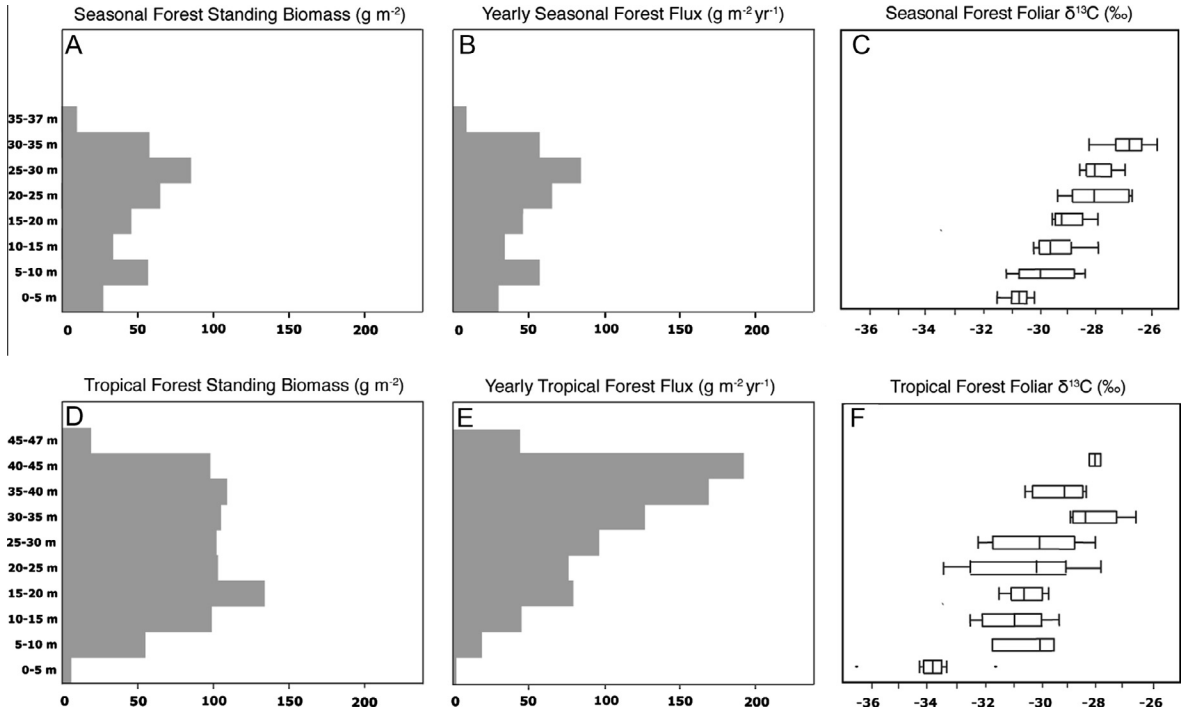

Fig. 3. Seasonal canopy standing biomass (A), annual leaf flux (B), and $\delta^{13}C_{leaf}$ data (C) for 5 m vertical bins from Maryland site. Tropical canopy biomass (D) and annual leaf flux data (E) are from Pasoh Reserve in Malaysia (Osada et al., 2001). Tropical canopy $\delta^{13}C_{leaf}$ (F) for Panamá site. Isotope ranges are expressed as box-and-whisker plots representing the median as well as lower and upper quartiles.

of their influence within the small mid-section of the canopy (Fig. 5A and B). Relative humidity did not correlate with either $\delta^{13}C_{CO_2}$ or Δ_{leaf} values in the small mid-canopy subsampling ($R^2 = 0.05$; $p = 0.42$ and $R^2 = 0.01$; $p = 0.70$; respectively) nor did relative humidity correlate with light or height ($R^2 = 0.04$; $p = 0.12$ and $R^2 = 0.05$; $p = 0.09$; respectively). Sampling from the full height of the forest (forest floor to the canopy top) did reveal a weak but significant correlation between relative humidity and both light and height measurements (see Fig. 5) ($R^2 = 0.20$; $p < 0.05$ and $R^2 = 0.40$; $p < 0.05$; respectively). This correlation is dependent on the very humid conditions in the deep understory and does not exist if these values or omitted.

4.1.3. A stepped regression model to de-convolve environmental influences on Δ_{leaf}

A stepped regression model was used to test the relative effects of light and humidity on carbon isotope fractionation at the leaf. This model uses the subset of leaf data for which $\delta^{13}C_{CO_2}$ measurements were also made. This model indicated that light is more strongly associated with the Δ_{leaf} trend ($R^2 = 0.39$; $p = 0.02$). Adding the relative humidity data only marginally improved the predictive ability of this model ($R^2 = 0.41$; $p = 0.06$). These results suggest that light level exerts a greater influence on Δ_{leaf} values than relative humidity. The lowest $\delta^{13}C_{leaf}$ values found in the lower regions of the canopy primarily reflect slow photosynthetic rates due to the deep shade, and that increased stomatal conductance associated with higher humidity was a secondary factor.

Correlation between light and the carbon isotope composition of CO_2 could potentially reflect photosynthetic carbon assimilation patterns that ultimately affect the atmosphere.

The results of a stepped regression model that evaluated the influence of light and humidity on $\delta^{13}C_{CO_2}$ values were not statistically significant. This suggests other factors not included in this analysis (such as respiration) likely account for variations in the $\delta^{13}C_{CO_2}$ within the canopy. Alternately, light varies little throughout the year in this equatorial location whereas humidity and $\delta^{13}C_{CO_2}$ may be more variable such that measurements made in a shorter time period are less representative of the long-term average.

4.1.4. Canopy isotope gradients in biomass and litter flux

Fig. 3 illustrates biomass allocation, annual litter carbon flux and carbon isotope compositions for the two forests. The Maryland forest exhibits a bimodal biomass distribution with thick understory and upper canopy layers (Fig. 3A; Parker et al., 1989). By comparison, the tropical forest data (Fig. 3D; Osada et al., 2001) place most biomass in the upper canopy and very little is located in the understory below 5 m. Many studies (Lowman and Wittman, 1996; Leigh, 1999) have confirmed that low-latitude closed-canopy forests have greater leaf biomass flux amounts than temperate canopy forests (Fig. 3E). The leaf flux amount for the Maryland forest (382.66 g/m^2; Fig. 3B) differs only slightly from the standing biomass estimate (Fig. 3A) because the trees in this forest are annually deciduous and herbivory is low at this site (Parker et al., 1989). Biomass flux in the tropical forest (829 g/m^2; Fig. 3E) illustrates the shorter lifespan and rapid turnover of leaves in the upper canopy (Osada et al., 2001). Decreased leaf life span in the upper canopy is associated with higher light

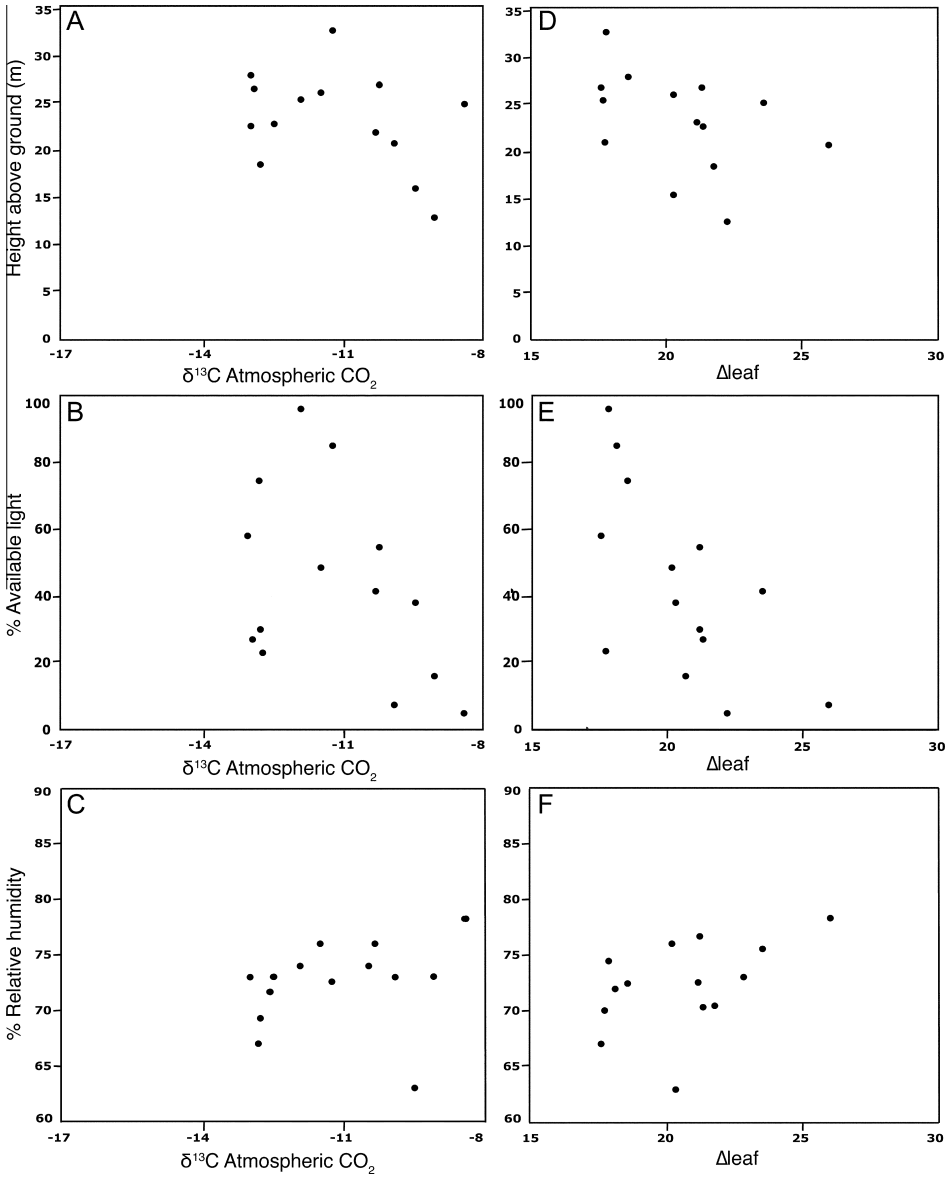

Fig. 4. $\delta^{13}C_{air}$ of isolated atmospheric CO_2 from the Panamá site as related to height within the canopy (A), % light availability (B), and relative humidity (C). Carbon isotope discrimination (Δ_{leaf}) values is calculated from $\delta^{13}C_{air}$ and $\delta^{13}C_{leaf}$ measured at each sampling site and related to canopy height (D), % light availability (E), and relative humidity (F).

levels and very high rates of photosynthesis while leaves in the humid understory have much longer lifespans and often do not senesce on an annual basis (Reich et al., 1991).

Leaves in the Panamá forest (especially in the mid-canopy bin between 20 and 30 m) exhibit a wider range of $\delta^{13}C$ values (Fig. 3F) when compared with leaves from the Maryland forest (Fig. 3C). $\delta^{13}C_{leaf}$ values for both sampling sites exhibit the increased enrichment with height that is generally described as the "canopy effect", but the relationship between height and $\delta^{13}C_{leaf}$ is weaker in the Panama forest ($R^2 = 0.54$) compared to the Maryland forest ($R^2 = 0.77$). This suggests the Maryland forest experienced greater atmospheric mixing or more even light conditions during the time of spring leaf flush, when most atmosphere-derived carbon biomass is added to leaves (Comstock and Ehleringer, 1992; Polgar and Primack, 2011).

4.2. Resampling model outcomes

4.2.1. How many leaves from a litter assemblage are necessary to distinguish the isotopic gradient characteristics of canopy closure?

The understory produces a low flux of leaves to depositional environments in tropical forests, including the San Lorenzo site in Panamá (see Fig. 3E). As a result, a relatively large number of leaves must be sampled to ensure understory leaves are included. As these leaves carry the lowest carbon isotope composition characteristic of the

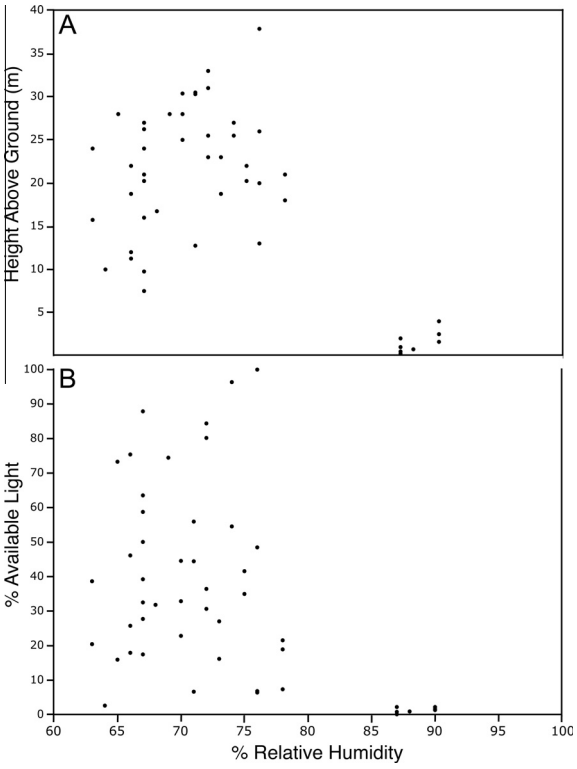

Fig. 5. Relative humidity as related to height within the Panamá site canopy (A) and % light availability (as a measure of canopy openness) (B). Relative humidity is highest in understory but highly variable throughout the mid- and upper canopy.

canopy isotope gradient, our aim was to determine the minimum sample size needed to ensure a high probability of capturing the isotopic range diagnostic of canopy closure. Based on the availability of leaves from paleoflora of interest (compiled in Wing et al., 2009), we simulated assemblages of $y = 10$, 25, 50, 100, and 250 leaves.

The mean isotopic range of leaves (Δ_y) for all 2000 iterations was determined for each of collection scenario. Δ_y values for collections of $y \leqslant 50$ overlap considerably between the two forests. For larger values of y, Δ_y consistently differs by ~1‰ between forest types (Fig. 6). For $50 < y > 100$, the Δ_y values increased with increasing sample size. Ranges for the two forests also increasingly diverged to the point where there was no overlap in Δ_y values when $y > 100$.

Overall, our modeling results indicates that it is possible to capture a diagnostic isotope range from a random sampling of at least $y > 50$ leaves and even confidently when more leaves were sampled. The percentage of simulated collection assemblages from the tropical data set that included at least one understory leaf (i.e., a leaf from the lowest bin) was found to be 5.5% for $y = 10$, 14.9% for $y = 25$, 28.8% for $y = 50$, 49.9% for $y = 100$ and 84.6% $y \geqslant 250$. These sample sizes are manageable for many paleofloral assemblages. Further, the odds increase for finding understory leaves increase when leaf selection is based on morphologic metrics of light environment (e.g., size, vein density stomatal index, etc.) of whole leaves from well-described families

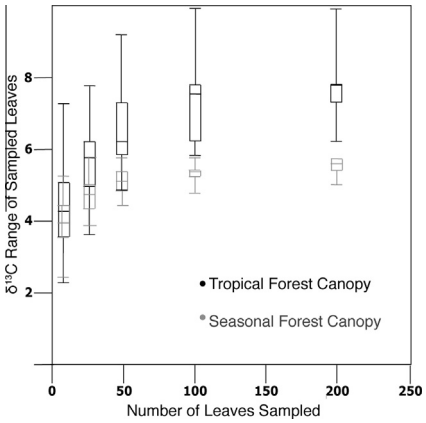

Fig. 6. Isotopic ranges (Δy) returned by 2000 iterations of the resampling model for the discrete sample size (y) specified. Box plots show the median, maximum, minimum, and upper and lower quartiles generated by the model. As sample sizes increases expressed range reaches an effective plateau for larger sample sizes and the median changes very little.

(Upchurch and Wolfe, 1987; Kubiske et al., 1996; Richards, 1996; Boyce, 2008; Dillen et al., 2008).

4.2.2. Are mean $\delta^{13}C_{leaf}$ values for a litter assemblage diagnostic of a forest biome?

The mean carbon isotope compositions of simulated leaf-litter assemblages ($\delta^{13}C_{y\text{-mean}}$) consistently reflect the dominance of upper canopy biomass in all leaf collections for which $y > 50$. As expected for smaller sample populations, when $y < 50$, $\delta^{13}C_{y\text{-mean}}$ values vary considerably (Fig. 7).

For all leaf collection scenarios ($y = 10$ to 1000), $\delta^{13}C_{y\text{-mean}}$ values for the tropical forest were consistently 1‰ lower than for the temperate forest (Fig. 7). This observation is not due to the input of the highly ^{13}C-depleted, shade leaves. Indeed, $\delta^{13}C_{y\text{-mean}}$ values for the two forests differed by 1‰, even for very small sample sizes ($y = 10$), where only 5.5% of simulated assemblages included at least one understory leaf. A similar difference of 1‰ was also observed for assemblages with large sample sizes ($y = 1000$), which always included at least one understory leaf and included up to 5.1% percent understory leaves.

The canopy between 20 and 30 m contributes 55% of leaf flux (Osada et al., 2001) and dominates the simulated leaf assemblages (Fig. 3E). This region experiences highly variable conditions that range from open gaps to highly dense foliage (Brokaw, 1982), and yields a wide range of $\delta^{13}C_{leaf}$ values (Fig. 2C) with a mean value of −30‰ and a standard deviation approaching 4‰. Further, observed $\delta^{13}C_{leaf}$ values for the canopy top in the tropical forest are 1–2‰ lower than for the leaves at the very top of the temperate forest (Fig. 3C and F). This is consistent with high humidity and greater fractionation (Δ_{leaf}) observed globally for tropical rainforest biomes (Diefendorf et al., 2010). Foliage in the mid-canopy at the Panamá site has Δ_{leaf} values that range from 18‰ to 26‰, consistent with overall high, but variable, intra-canopy humidity (Fig. 5; Diefendorf et al., 2010). The small range of $\delta^{13}C_{leaf}$ values

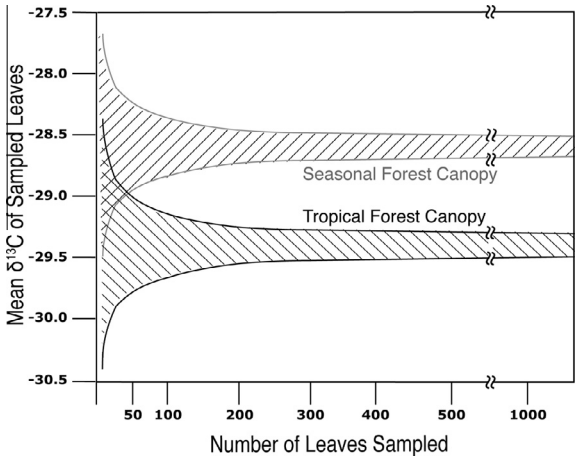

Fig. 7. Curves bound the range of foliar $\delta^{13}C_{y\text{-mean}}$ returned by model for both canopy types. $\delta^{13}C_{y\text{-mean}}$ for small sample sizes (y) can vary widely in response to rare isotope values but large sample sizes approach a mean that represents the more probable foliar carbon isotope input for leaves in litter assemblages.

and overall lower Δ_{leaf} values in the Maryland forest indicate greater air mixing and overall lower humidity in the temperate canopy, respectively (Stewart et al., 1995; Diefendorf et al., 2010).

4.2.3. Can we predict the $\delta^{13}C$ values of cumulative litter, soil organic matter, and organic carbon in sedimentary archives using litter flux and isotope patterns in canopies?

A simulated collection of many thousands of leaves represents accumulated leaf litter integrated over time, provided to soil and, ultimately, to sedimentary organic carbon stores. High numbers of leaves ($y \geqslant 1000$) converge at $\delta^{13}C_{y\text{-mean}}$ values that are characteristic of the two data sets, reflecting both climate and the architectural differences between the forest types (Diefendorf et al., 2010).

Santiago (2003) reported carbon isotopic data for litter and mineral-soil organic carbon in and near the San Lorenzo preserve. For 16 sites, $\delta^{13}C_{litter}$ values ranged from $-30.5‰$ to $-28.4‰$: for 24 samples collected at four locations, $\delta^{13}C_{soil}$ (A-horizon) values ranged from $-27.5‰$ to $-26.3‰$. The average value for litter samples ($-29.7 \pm 1‰$) is indistinguishable from the predicted value $-29.3 \pm 0.3‰$ based on our $\delta^{13}C_{leaf}$ data ($y = 1000$; Fig. 8).

In a compilation of published $\delta^{13}C_{leaf}$, $\delta^{13}C_{litter}$, and $\delta^{13}C_{soil}$ data, Bowling et al. (2008) suggest variability in the degree of decomposition in the litter collection (Wynn and Bird, 2005; Wynn et al., 2006; Wynn, 2007) but this result could also be biased by small numbers of leaves in some litter samplings (Nadelhoffer and Fry, 1988). Further, Bowling et al. (2008) found $\delta^{13}C_{soil}$ values were up to $4.5‰$ higher than $\delta^{13}C_{leaf}$. The isotopic difference between litter and soil carbon in tropical forests determined by Santiago (2003) was $+2.8‰$, within the range found by Bowling et al. (2008). Bowling et al. (2008) included only sun leaves, which are generally more enriched in ^{13}C (Ehleringer et al., 1986; see Figs. 2 and 3 of this study), and a large component of leaf flux (Fig. 3E). Nevertheless, the upper 10 m of canopy accounts for slightly less than half (~45%) of total biomass flux and does not represent mid-canopy leaves, which provide the majority of carbon to forest litter. A full accounting of canopy inputs must be considered in any estimation of soil carbon arising from leaf material. Our bootstrap analysis estimated $\delta^{13}C_{litter}$ that was weighted by flux and integrated leaf litter from the entire height of a forest. Such an approach potentially provides a more accurate way to estimate the $\delta^{13}C$ signatures contributed by leaves to soil organic carbon, and could improve studies seeking to understand the offset between litter and mineral soil (A-horizon) organic carbon. Soil organic carbon signatures reflect both an integrated accumulation of carbon inputs as well the consequences of their degradation.

Santiago (2003) collected litter and mineral soil horizons in Panamá from sites with a wide range in annual precipitation (1800–3500 mm/yr) (Fig. 8). We would expect litter from drier areas to have been produced with reduced carbon isotope discrimination (Nadelhoffer and Fry, 1988; Diefendorf et al., 2010). We estimate that Santiago's (2003) litter sampling likely included fewer than 500 leaves, and certainly many fewer than the thousands of leaves our model uses to generate an expected $\delta^{13}C_{litter}$. Further, the Santiago (2003) litter collection included woody debris, frass, and other floral and reproductive organs not included in the modeled litter value. The wide range of measured $\delta^{13}C_{litter}$ values potentially reflects contributions from plant components with different chemical and isotopic properties than leaves. In general, across forest types from the tropical to the temperate ecosystems, leaves account for 50–60% of litterfall (Odum, 1970; Jordan and Uhl, 1978; Parker et al., 1989; Leigh, 1999; Santiago, 2003). The remaining litterfall comes largely from wood, a material that is $\sim 3‰$ enriched with respect to leaf material (Gröcke, 2002).

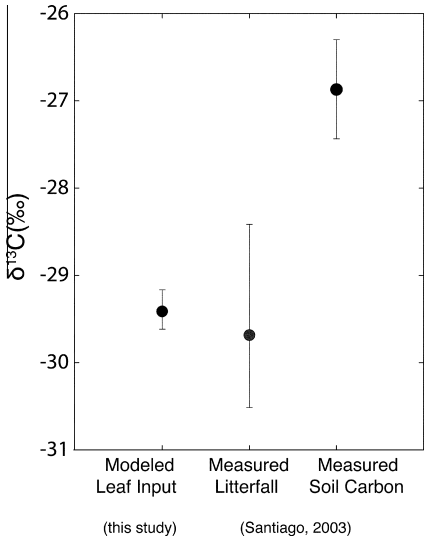

Fig. 8. Predicted $\delta^{13}C_{litter}$ returned by resampling as well as for four litterfall collection sites and six soil samples from a transect of Panamanian forest that includes the study site from this study. Values depicted are the mean and standard error for computational and observational measurements.

Most studies of soil organic carbon fail to recognize the contribution of carbon from roots. Yet, some authors suggest root carbon is equal to, or even greater than, the amount of carbon derived from leaves (Fahey et al., 2005; Rasse et al., 2005). Further study is necessary to determine the relative input of root carbon compared to leaves and woody biomass and the preservation mechanisms for these different carbon pools (Ehleringer et al., 2000).

4.3. From leaves to soil organic carbon

Soil organic matter is a complex mixture of inputs from many sources and it is subject to significant chemical change during decomposition. Plant biochemical components (e.g., leaf lipids, lignin, etc.), which preserve on geological timescales, are also subject to the influence of flux and isotopic gradients associated with canopy coverage. Canopy structure in forests generates a range of light and humidity conditions that are reflected in the isotopic composition of leaves, which transmit this heterogeneity to soils as litter flux. Our approach, which considers the height-resolved biomass flux as well as isotopic composition, offers a framework for understanding the influence of canopy on leaf molecular constituents, provided their abundances are characterized by canopy position. For example, lipid abundance distribution as well as isotopic fractionation differ between sun and shade leaves (Collister et al., 1994; Lockheart et al., 1997; Diefendorf et al., 2011).

The modeling approach outlined in this study can be applied to other forests to predict the isotopic composition of the carbon in soils that arises from foliar material. Leaf carbon isotope composition is also affected by taxanomic composition (see symbols for Fig. 2C and D), regional precipitation regimes (Diefendorf et al., 2010), and canopy coverage (Ehleringer et al., 2000; Cerling et al., 2011). Spatial changes in moisture, plant composition and canopy (which generally co-vary) can be captured in soil organic carbon (SOC) at the level of landscape patches (i.e., paleoecotones; Nadelhoffer and Fry, 1988; Mariotti and Peterschmitt, 1994; McClaren and McPherson, 1995; Bilings, 2006; Cerling et al., 2011; Magill et al., 2013).

Diagenetic enrichment of ^{13}C in soil organic carbon is rapid (sub-decadal: Bird et al., 1995; Wynn, 2007) and the temporal resolution of the isotopic composition is driven by the amount of organic input as well as the mobility of the organic matter (McClaren and McPherson, 1995; Boutton et al., 1999; see Wynn, 2007). Not only is our understanding of soil carbon diagenesis confounded by the many environmental factors that affect diagenesis but it is also hampered by an incomplete understanding of initial organic inputs. The computational approach illustrated with the Panamá and Maryland forest data can potentially improve characterization of diagenetic fractionation by providing a better estimate of the initial organic matter based on both biotic (plant type) and physical (moisture, canopy structure, litter flux) factors in a forest.

5. CONCLUSIONS

Our collection efforts produced a unique dataset combining $\delta^{13}C_{leaf}$, $\delta^{13}C_{CO_2}$, and humidity measurements within the context of canopy elevation and available light for five major genera of a tropical closed-canopy forest in Panamá. By combining these data with biomass flux measurements for an analogous closed canopy forest, we evaluated the isotopic expression within the forest and for simulated leaf assemblages. These results for samples from Panamá, were compared to those for a similarly sampled deciduous, temperate forest in Maryland. The range of $\delta^{13}C_{leaf}$ values is particular to each and reflects canopy type (open, Maryland forest = ~6‰; closed, Panamá forest = ~10‰). Our simulated leaf collections predicted isotopic range for leaf assemblages for each forest, and these exhibited with as few as $\geqslant 50$ randomly sampled leaves. For very large simulated collections from both forests, a distinct 1‰ difference in the integrated foliar ^{13}C content between forests types was evident. This difference has been observed in previous studies at leaf, biome, and global scales and often associated with moisture conditions. The lower $\delta^{13}C_{leaf}$ and larger Δ_{leaf} values at the Panamá site relative to those from the Maryland site are consistent with differences in rainfall and humidity data for the two biomes. Despite the importance of humidity differences between the temperate and tropical sites, within the canopy of the Panamá forest, Δ_{leaf} values were most strongly influenced by light intensity, and somewhat less influenced by humidity.

Foliar material can account for over half the carbon flux to soils and has an important influence on the components and isotopic character of soil organic carbon. Soil organic carbon, which can be preserved on very long time-scales, is subject to diagenetic influences that impact $\delta^{13}C$ values in ways that are not fully understood. Modeling efforts, such as this study, can aid our understanding of these effects by providing constraints on the $\delta^{13}C$ values of leaf litter in the context of forest canopy structure and the resulting differences in environmental drivers such as light, humidity, and source atmospheric CO_2.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2014.08.032.

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