

Carbon stable isotopic composition of soluble sugars in *Tillandsia* epiphytes varies in response to shifts in habitat

Laurel K. Goode · Erik B. Erhardt ·
Louis S. Santiago · Michael F. Allen

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Abstract We studied C stable isotopic composition ($\delta^{13}\text{C}$) of bulk leaf tissue and extracted sugars of four epiphytic *Tillandsia* species to investigate flexibility in the use of crassulacean acid metabolism (CAM) and C_3 photosynthetic pathways. Plants growing in two seasonally dry tropical forest reserves in Mexico that differ in annual precipitation were measured during wet and dry seasons, and among secondary, mature, and wetland forest types within each site. Dry season sugars were more enriched in ^{13}C than wet season sugars, but there was no seasonal difference in bulk tissues. Bulk tissue $\delta^{13}\text{C}$ differed by species and by forest type, with values from open-canopied wetlands more enriched in ^{13}C than mature or secondary forest types. The shifts within forest habitat were related to temporal and spatial changes in vapor pressure deficits (VPD). Modeling results estimate a possible 4% increase in the proportional contribution of the C_3 pathway during the wet season, emphasizing that any seasonal or habitat-

mediated variation in photosynthetic pathway appears to be quite moderate and within the range of isotopic effects caused by variation in stomatal conductance during assimilation through the C_3 pathway and environmental variation in VPD. C isotopic analysis of sugars together with bulk leaf tissue offers a useful approach for incorporating short- and long-term measurements of C isotope discrimination during photosynthesis.

Keywords Crassulacean acid metabolism · Mexico · Seasonally dry tropical forest · Stable isotope

Introduction

Crassulacean acid metabolism (CAM), a water-conserving mode of photosynthesis whereby plants take up CO_2 at night, has been under investigation since its discovery over 200 years ago (Winter and Smith 1996). It has evolved multiple times in nearly 30 plant families, both terrestrial and aquatic (Crayn et al. 2004; Lüttge 2004), and is found in at least 6% of all vascular plant species (Ehleringer and Monson 1993). There are four phases of CAM: (1) nighttime CO_2 uptake and fixation by phosphoenolpyruvate carboxylase (PEPC), (2) early morning CO_2 uptake and fixation by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), (3) daytime decarboxylation and re-fixation of CO_2 under closed stomates, and (4) late afternoon CO_2 uptake and fixation by Rubisco (Griffiths et al. 2002; Lüttge 2004). Although CAM is typically defined as nighttime CO_2 uptake, in many CAM species, daytime CO_2 fixation by Rubisco raises questions as to whether the amount of CO_2 fixed through the C_3 pathway varies with environmental conditions (e.g., Nobel et al. 1992; Winter and Holtum 2002; Winter et al. 2008). The significance of

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L. K. Goode (✉)
Biology Department, University of California,
Riverside, CA 92521, USA
e-mail: lsalz001@ucr.edu

L. K. Goode · L. S. Santiago · M. F. Allen
Center for Conservation Biology,
University of California, Riverside, CA 92521, USA

E. B. Erhardt
Department of Mathematics and Statistics,
University of New Mexico, Albuquerque,
NM 87131-0001, USA

L. S. Santiago
Department of Botany and Plant Sciences,
University of California, Riverside, CA 92521, USA

variation in activity of Rubisco in strong CAM plants has not yet been fully incorporated into an ecological framework.

The C stable isotopic composition ($\delta^{13}\text{C}$) of plant material can be used as an indicator of the relative proportion of CO_2 assimilated by CAM and C_3 photosynthetic pathways because of differential discrimination by the two carboxylating enzymes, PEPC and Rubisco. If only Rubisco is used, as occurs during light CO_2 fixation, the $\delta^{13}\text{C}$ of bulk plant material will be close to -27‰ . In contrast, if only PEPC is used, as occurs during dark CO_2 fixation, the $\delta^{13}\text{C}$ of plant material will be near -13‰ . Foliar $\delta^{13}\text{C}$ values therefore reflect the weighting of these two processes (Farquhar et al. 1989). A mixing model using plant $\delta^{13}\text{C}$ values in combination with CAM and C_3 can provide information on the proportional use of the two photosynthetic pathways. Moreover, taking into consideration variation in C_3 and CAM activity can allow a more accurate description of the contribution of each photosynthetic pathway (Winter and Holtum 2002).

It has been estimated that about half of all species exhibiting CAM are epiphytes (Winter and Smith 1996; Zotz 2004). In a study conducted by Griffiths and Smith (1983), seven out of 17 obligate epiphyte species in the genus *Tillandsia* exhibited obligate C_3 photosynthesis, as determined by $\delta^{13}\text{C}$ values between -23 and -35‰ . The other ten *Tillandsia* species were deemed obligate CAM, with $\delta^{13}\text{C}$ values of -10 to -21‰ . Values more negative than -14‰ , however, indicate the potential for daytime CO_2 uptake and fixation by Rubisco during CAM phases 1 and 2. Other published $\delta^{13}\text{C}$ values for species of *Tillandsia* show that this genus spans the range of values expected for CAM and C_3 plants, including a number of potentially intermediate species (Medina 1974; Medina and Troughton 1974; Griffiths and Smith 1983).

The isotopic composition of bulk leaf tissue has commonly been used to determine photosynthetic pathways (Winter et al. 1983; Silvera et al. 2005). The $\delta^{13}\text{C}$ of bulk leaf tissue is a time-integrated measure, describing relative photosynthetic pathway utilization and CO_2 concentration at the site of carboxylation over relatively long time scales. The $\delta^{13}\text{C}$ of leaf sugars, however, provides an indication of

pathways used for C fixation on the day the leaf was collected (Brugnoli et al. 1988). We utilized these long- and short-term measures of C fixation to investigate photosynthetic variation between seasons and among forest types with contrasting microclimate. We expected that the mode of C assimilation is flexible, leading to: (1) less negative $\delta^{13}\text{C}$ of bulk leaf tissues in drier habitats, indicating a greater relative proportion of CO_2 fixation through the CAM pathway; and (2) less negative $\delta^{13}\text{C}$ of sugars in the dry season than in the wet season, indicating greater utilization of CAM.

Materials and methods

Study sites

The study was conducted in two forests in the Yucatan Peninsula, Mexico. El Edén Ecological Reserve contains semi-deciduous lowland tropical forest (Gómez-Pompa et al. 2003), located on the northern Yucatan Peninsula, 45 km northwest of Cancun in Quintana Roo. The reserve encompasses 1,500 ha of mostly flat landscape, with shallow soils over limestone bedrock, and annual rainfall of 1,500–2,500 mm (Allen et al. 2005).

El Zapotal is a 2,358-ha reserve in the state of Yucatan, just south of Ria Lagartos, located less than 50 km northwest of El Edén and contains vegetation types comparable to El Edén with annual precipitation from 700 to 1,100 mm and median annual temperature of 26°C (Faller and Serralde 2005). Both reserves are considered seasonally dry tropical forests, characterized by a pronounced dry season with little rainfall. Within each ecological reserve, measurements were conducted in three locations classified as mature forest, secondary forest, or wetland area forest types (Table 1). Canopy openness is comparable between mature and secondary forest types and is greater in the wetland area. The mature forest comprises moderate to tall trees (to 20 m) with a leaf area index (LAI) of $2.2\text{ m}^2\text{ m}^{-2}$. The secondary forest is shorter (8 m) but contains a high density of stems and a slightly greater LAI of $2.5\text{ m}^2\text{ m}^{-2}$. The wetlands comprise grasses, sedges, and short-statured

Table 1 Six sampling locations in seasonally dry tropical forests: these include three forest types within each of two ecological reserves in the northern Yucatan peninsula, Mexico. Abiotic measurements were made and leaf samples were collected in each location

Reserve	Annual precipitation (mm)	Forest type; leaf area index ($\text{m}^2\text{ m}^{-2}$)	Approximate age vegetation (years)
El Edén	1,500–2,500	Secondary; 2.5	~ 16
		Mature; 2.2	>100
		Wetland; <1	>100
El Zapotal	700–1,100	Secondary	~ 31
		Mature	>100
		Wetland	>100

trees and shrubs (2–6 m) with a LAI $<1 \text{ m}^2 \text{ m}^{-2}$ (Vargas et al. 2008; Hasselquist et al., unpublished data).

Microclimate

Three temperature/relative humidity (RH) sensors (Hygrochron DS1923; Maxim Integrated Products, Sunnyvale, CA, USA) were placed in each forest type in each reserve, 1.2 m above the ground on the south-facing sides of trees. Hourly recordings were taken and data were averaged for each forest type in each reserve. Within the mature forest at El Edén, four sensors were used to capture a height gradient, placed at 50, 100, 175, and 250 cm above the ground. Vapor pressure deficit (VPD) of the bulk air was calculated from temperature and humidity based on equations from Jones (1992).

Study species and leaf collection

Four *Tillandsia* species were tested for isotopic composition: *Tillandsia balbisiana* Schult., *Tillandsia brachycaulos* Schldl., *Tillandsia dasyliirifolia* Baker, and *Tillandsia fasciculata* Sw. Leaves were collected from three to five individuals per species per forest type in each reserve, if present, during two sampling periods, October 2007 (rainy season) and April 2008 (dry season). All leaves were collected between ground level and 2.5-m height (where these species commonly occur), and height was recorded for each individual. For each individual plant, three leaves were collected and homogenized to make one sample.

Sample preparation and analysis

To determine bulk leaf C isotopic composition, leaves were dried at 70°C for 48 h and ground to a fine powder. Sub-samples of 7 mg were processed with a continuous flow isotope ratio mass spectrometer. Leaf sugar C isotopic composition was determined after sugars were extracted following the protocol described by Brugnoli et al. (1988) and modified by S. Mambelli at the University of California, Berkeley. This process involved centrifugation of leaf material mixed with de-ionized water to extract leaf sugars. Sugars were purified using ion-exchange resins and immediately freeze-dried before isotope analysis. All samples were analyzed at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley. C stable isotope ratios ($\delta^{13}\text{C}$) were expressed relative to the Vienna Pee Dee belemnite (VPDB) limestone standard:

$$\delta^{13}\text{C}_{\text{‰}} = \left(\left[\frac{^{13}\text{C}}{^{12}\text{C}} \right]_{\text{sample}} / \left[\frac{^{13}\text{C}}{^{12}\text{C}} \right]_{\text{standard}} - 1 \right) \times 1,000.$$

Statistical analyses

We performed both univariate and multivariate analyses to explain measured $\delta^{13}\text{C}$ values. ANOVA was used to determine differences between groups (e.g., epiphyte species, forest types). Means were compared using a Tukey–Kramer honest significant difference test. These were run with JMP software (SAS Institute 2001). We also used a multivariate analysis of covariance (ANCOVA) model to predict the proportional contribution of C_3 photosynthesis (prop_{C_3}) to our measured CAM plants, given by the mixing model: $\text{prop}_{\text{C}_3} = (\delta^{13}\text{C}_{\text{obs}} - \delta^{13}\text{C}_{\text{CAM}}) / (\delta^{13}\text{C}_{\text{C}_3} - \delta^{13}\text{C}_{\text{CAM}})$. Because the proportional contribution of C_3 is a linear combination of the observed $\delta^{13}\text{C}$ and the endpoint $\delta^{13}\text{C}$ values, inference about prop_{C_3} also applies to observed $\delta^{13}\text{C}$, so that a significant increase in prop_{C_3} is equivalent to a significant decrease in $\delta^{13}\text{C}$. The predictor variables included species, material (bulk vs. sugar), reserve, forest type, season, height, RH, temperature, and night/day VPD. For factor variables (e.g., species) one group was considered the baseline, the effect for which was incorporated in the model intercept term, and the effect of each was considered relative to the baseline. The baselines for our factor variables were species, *T. balbisiana*; material, bulk; location, El Edén; forest type, mature forest; and season, dry. We tested the interactions of all covariates, looked for outliers and multi colinearity, and checked the residuals for violations of model assumptions. The full model was first reduced by a backward stepwise procedure using the Akaike information criterion (AIC) and then further reduced manually by dropping the largest variable with *P*-value at least 0.05. Because the linear model assumes that the mean structure is correct (i.e., the response is linearly related to the coefficients and the residuals are normally distributed), we used a non-parametric bootstrap with 10,000 iterations to check that assumptions were not violated and to validate our results (Davison and Hinkley 1997).

We evaluated published $\delta^{13}\text{C}$ values for plants in the genus *Tillandsia* and assigned likelihood for use of either CAM or C_3 photosynthetic pathway, based on the discrimination inherent to each. $\delta^{13}\text{C}$ values greater than -15‰ were considered mostly or all CAM, values less than -23‰ and lower were considered mostly or all C_3 , and values between -23 and -15‰ were considered to be a mixture of CAM and C_3 . We used a bootstrap analysis on this range of endpoints to incorporate the variability in endpoint CAM and C_3 values, in order to estimate the relative proportion of C_3 photosynthesis in CAM plants with intermediate $\delta^{13}\text{C}$ values for ANCOVA model validation. These analyses were performed with R 2.8.1 (R Development Core Team 2008).

Results

Average RH measured along the low canopy vertical gradient within the mature forest was highest at 50 cm height (~97%) and decreased to 92% at 250 cm height. RH was ~95% for both intermediate heights (100 and 175 cm). Average daytime VPD in the wet season was highest in the wetland areas (0.66 ± 0.02 kPa) and lowest in the mature forests (0.33 ± 0.02 kPa), with secondary forests exhibiting intermediate values (0.40 ± 0.01 kPa). Average daytime VPD in the dry season showed a significant increase in the mature and secondary forests (to 0.60 ± 0.02 and 0.69 ± 0.01 kPa, respectively), but changed little in the wetland area (to 0.55 ± 0.02 kPa). Dry season values are based on forest types in El Edén only, as we were not able to visit El Zapotal during the dry season.

Based on bulk $\delta^{13}\text{C}$, the four *Tillandsia* species we tested are CAM species, and all fall into the intermediate range, with values less than -15‰ and greater than -23‰ (Table 2). The mean for each species, when averaged across a range of habitats, was between -17.39 and -16.12‰ (Table 2).

One-way ANOVAs revealed that bulk $\delta^{13}\text{C}$ did not differ significantly with season ($F_{1,95} = 0.920$; $P > 0.340$), whereas sugar $\delta^{13}\text{C}$ showed significant seasonal variation ($F_{1,59} = 9.905$; $P < 0.003$; Fig. 1; Table 3), with a dry season mean ($-15.73 \pm 0.14\text{‰}$) greater than the wet season mean ($-16.42 \pm 0.16\text{‰}$). Bulk $\delta^{13}\text{C}$ differed among species ($F_{3,93} = 7.767$; $P < 0.0001$; Table 2). The only differences in pairwise comparisons between species were that mean bulk $\delta^{13}\text{C}$ of *T. brachycaulos* was significantly lower than those of *T. dasyliriifolia* and *T. balbisiana*. Bulk $\delta^{13}\text{C}$ was statistically indistinguishable between reserves ($F_{1,95} = 0.747$; $P > 0.39$; Fig. 2). Bulk $\delta^{13}\text{C}$ differed between forest types ($F_{2,93} = 22.534$; $P < 0.0001$; Fig. 3a). All forests differed significantly from each other in bulk $\delta^{13}\text{C}$ with the wetland having the highest values ($-15.84 \pm 0.17\text{‰}$), secondary forests having the lowest values ($-17.72 \pm 0.15\text{‰}$), and mature forests

Table 2 Bulk C contents and C stable isotope ratios ($\delta^{13}\text{C}$) measured from four commonly occurring *Tillandsia* species within seasonally dry tropical forests of Mexico

Species	C (%) tw	$\delta^{13}\text{C}$ (‰) versus VPDB
<i>Tillandsia balbisiana</i>	46.35 ± 0.28	-16.39 ± 0.19
<i>Tillandsia brachycaulos</i>	45.01 ± 0.23	-17.39 ± 0.16
<i>Tillandsia dasyliriifolia</i>	44.97 ± 0.45	-16.12 ± 0.31
<i>Tillandsia fasciculata</i>	45.90 ± 0.40	-16.67 ± 0.28

Values represent means for each species across the range of forest types (secondary, mature, wetland), $\pm 1\text{SE}$. VPDB Vienna Pee Dee belemnite

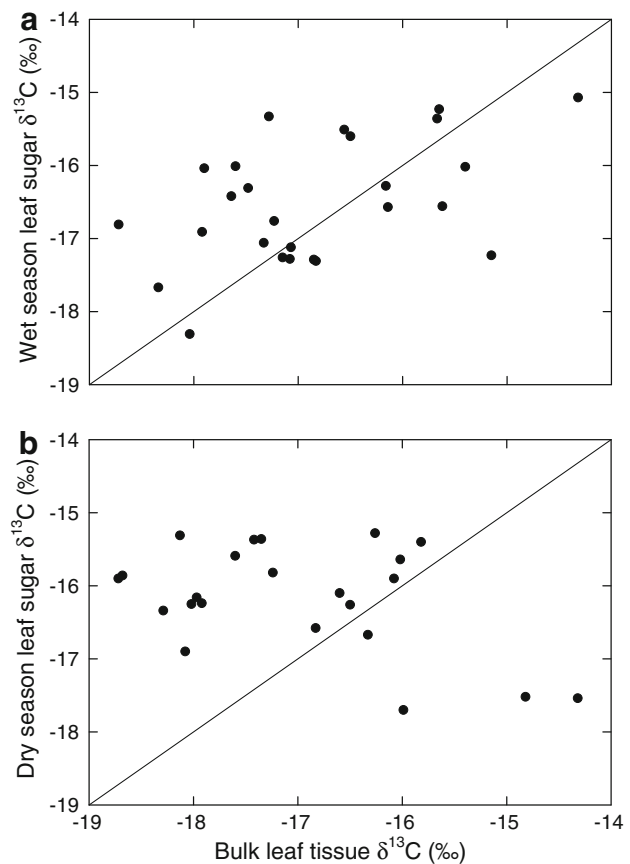


Fig. 1a, b C stable isotopic composition ($\delta^{13}\text{C}$) of leaf sugar versus bulk leaf $\delta^{13}\text{C}$ for four *Tillandsia* species sampled in seasonally dry tropical forests of the Yucatan peninsula, Mexico. Lines represent 1:1 relationship. **a** Wet season sugars; values fall on 1:1 line. **b** Dry season sugars; values are heavier than for bulk leaf tissue

showing intermediate values ($-17.00 \pm 0.14\text{‰}$; Figs. 2, 3). A two-way ANOVA using reserve, forest type, and a reserve by forest type interaction was significant ($F = 16.382$; $P < 0.0001$), with all forest types in El Edén different at the $\alpha = 0.05$ level and the wetland forest of El Edén significantly more enriched in ^{13}C than all forest types of El Zapotal. Sugar $\delta^{13}\text{C}$ did not vary with height for either season ($F_{1,59} = 2.878$; $P > 0.09$) whereas bulk $\delta^{13}\text{C}$ was significantly related to height [$\delta^{13}\text{C} = -17.48 + 0.006 \times \text{height (cm)}$; $r^2 = 0.114$; $F_{1,95} = 13.986$; $P < 0.0003$].

In the multivariate ANCOVA analysis, our reduced model included all five categorical variables and seven interactions (Table 4). The other variables (height, RH, temperature, and VPD) and interactions were dropped because of collinearity or no effect. Overall, the model had an r^2 of 0.607 and an adjusted r^2 of 0.566 (Table 4). The estimates for the indicator variables told us how different they were from the baseline variables (baseline estimates were 0 since they were included in the intercept). For example, conditional on the other factors, the bulk tissue

Table 3 Seasonal shift in $\delta^{13}\text{C}$ for bulk tissue and sugars of four *Tillandsia* species in the Yucatan peninsula, Mexico

Material	$\delta^{13}\text{C}$ (‰)	
	Dry season	Wet season
Bulk tissue	-16.75 ± 0.17	-16.94 ± 0.15
Sugars	-15.73 ± 0.14	-16.42 ± 0.16

Means \pm 1SE

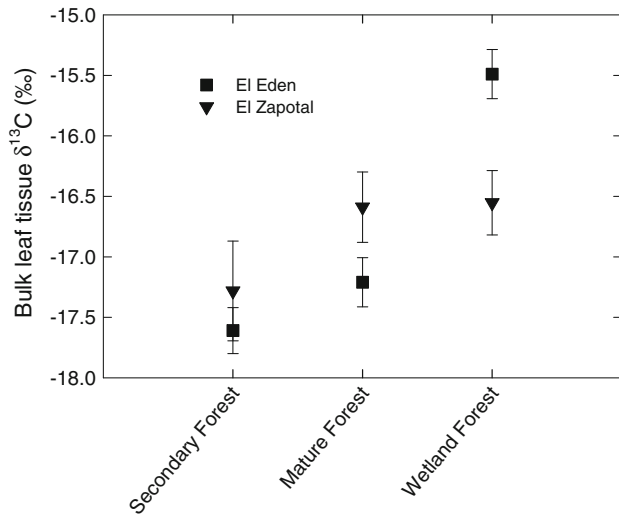


Fig. 2 Wet season bulk tissue C isotopic composition for *Tillandsia* species sampled from three forest types in two seasonally dry tropical forest of Mexico, El Edén and El Zapotal. Each point is the mean \pm 1SE

estimate for *T. dasyliriifolia* was very close to 0, while the estimates for *T. brachycaulos* and *T. fasciculata* were 0.054 and 0.046, respectively. At an $\alpha = 0.05$ significance level, *T. brachycaulos* and *T. fasciculata* were statistically equivalent and significantly different from both *T. dasyliriifolia* and *T. balbisiana* (the baseline variable). Similarly, the model demonstrated that prop_{C_3} differed between species and between forest types, and the species effect on prop_{C_3} was modified for the sugar material (Table 4; $P < 0.008$). Forest type by season and reserve by forest type interactions were also important. The estimates for the main effects were consistent with expected trends of more enriched $\delta^{13}\text{C}$ values under drier or greater VPD conditions. For example, El Zapotal, the drier reserve, had a negative estimate (-0.037 ; $P = 0.066$), suggesting there was less proportional contribution of C_3 compared with El Edén, the wetter reserve. The wetland area also had a negative estimate (-0.041 ; $P = 0.004$), suggesting less C_3 contribution than the other forest types, consistent with greater VPD in this forest type. The wet season showed a positive estimate (0.046 ; $P = 0.002$), indicating that during the wet season, the proportional contribution of C_3 photosynthesis increased.

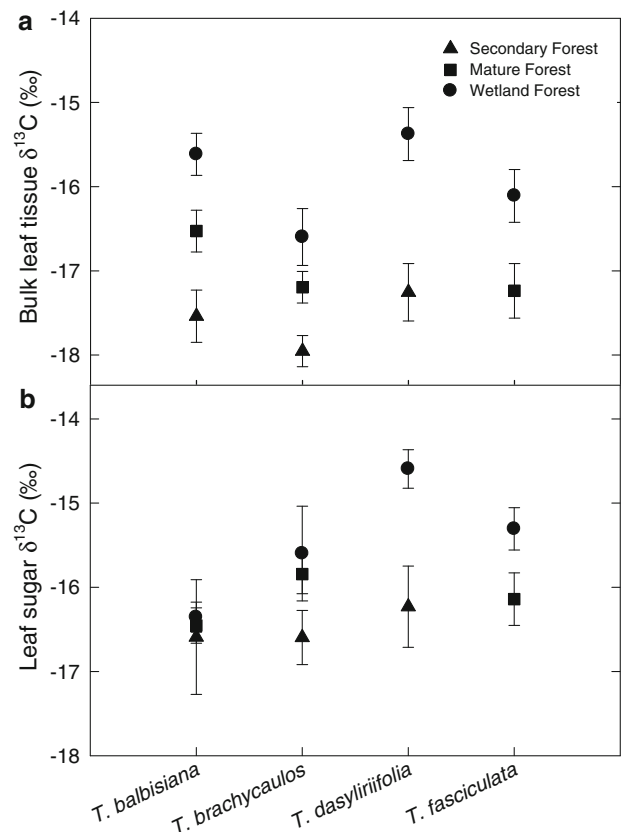


Fig. 3 Bulk (a) and sugar (b) C isotopic composition (mean \pm 1SE) for four species of *Tillandsia* in three forest types (secondary, mature, wetland) of seasonally dry tropical forests in the Yucatan peninsula, Mexico

The ANCOVA model estimates for the proportional contribution of C_3 photosynthesis varied for the 24 possible combinations of species, forest type, and reserve (Fig. 4). For all species occurring in the mature forest, the estimated contribution of C_3 was predicted to increase by about 4% during the wet season. Plants in the secondary forest were also predicted to utilize $\sim 3.5\%$ more C_3 in the wet season compared to the dry season. In contrast, the proportional contribution of C_3 photosynthesis in wetland plants changed little with season. For *T. balbisiana* and *T. brachycaulos*, the two species found in mature forest of both reserves, individuals in El Zapotal were predicted to utilize 4% less C_3 than those in El Edén. Similarly, *T. brachycaulos* in secondary forests of both reserves was predicted to utilize $\sim 4.5\%$ less C_3 in El Zapotal (Fig. 4).

Discussion

This study utilized bulk tissue and sugar isotope ratios to test plasticity of photosynthetic pathways. Contrary to our expectations, the magnitude of isotope shifts observed

Table 4 Reduced fitted model to explain measured $\delta^{13}\text{C}$ values in four species of *Tillandsia* growing throughout mature, secondary, and wetland areas of the Yucatan peninsula

Coefficients	Estimate	SE	<i>t</i> -value	<i>P</i> ($\geq t$)	Significance
Intercept	0.213	0.0120	17.745	0.0000	***
Material = sugar	0.011	0.0138	0.800	0.4249	
Season = wet	0.046	0.0143	3.184	0.0018	**
Reserve = Zapotal	-0.037	0.0197	-1.854	0.0658	†
Forest = secondary	0.051	0.0148	3.478	0.0007	***
Forest = wetland	-0.041	0.0141	-2.928	0.0040	**
Species = TIBR	0.054	0.0123	4.388	0.0000	***
Species = TIDA	-0.005	0.0176	-0.280	0.7796	
Species = TIFA	0.046	0.0165	2.763	0.0065	**
Sugar \times TIBR	-0.095	0.0198	-4.797	0.0000	***
Sugar \times TIDA	-0.068	0.0252	-2.693	0.0079	**
Sugar \times TIFA	-0.070	0.0247	-2.847	0.0051	**
Wet \times secondary	-0.012	0.0215	-0.544	0.5870	
Wet \times wetland	-0.057	0.0212	-2.667	0.0085	**
Zapotal \times secondary	-0.008	0.0339	-0.250	0.8033	
Zapotal \times wetland	0.099	0.0272	3.630	0.0004	***

TIBA, *T. balbisiana*; TIBR, *T. brachycaulos*; TIDA, *T. dasyliriifolia*; TIFA, *T. fasciculata*;

** $P = 0.01$, *** $P = 0.001$, † $P = 0.1$

Residual SE, 0.0492 on 144 *df*; multiple r^2 , 0.6071; adjusted r^2 , 0.5662; *F*-statistic, 14.83 on 15 and 144 *df*; *P* value, $< 2.2 \times 10^{-16}$

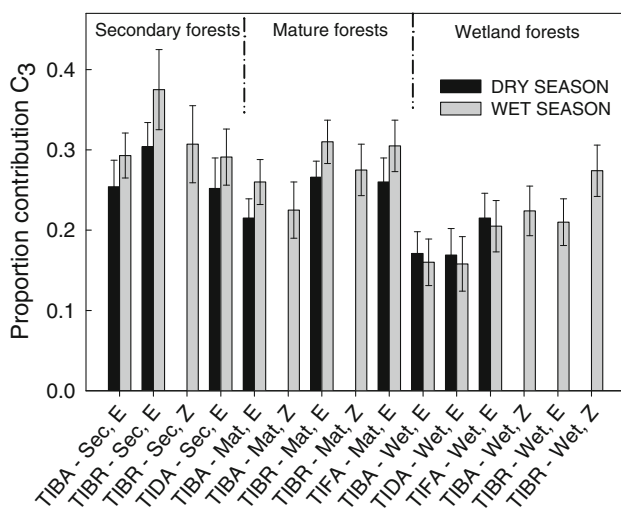


Fig. 4 Model-based estimated contribution of C_3 photosynthesis \pm SE for different scenarios (combinations of variables) based on observed data from bulk tissue of *Tillandsia* species growing in seasonally dry tropical forests of the northern Yucatan peninsula, Mexico. TIBA *T. balbisiana*, TIBR *T. brachycaulos*, TIDA *T. dasyliriifolia*, TIFA *T. fasciculata*, Sec secondary forest, Mat mature forest, Wet wetland area, E El Edén, Z El Zapotal

across habitat types and seasons was small, indicating that any seasonal and habitat-related shifts in the use of C_3 and CAM pathways are moderate among *Tillandsia* species in our study sites. We found the $\delta^{13}\text{C}$ values of dry season sugars to be significantly more enriched in ^{13}C than wet

season sugars, consistent with assimilation of relatively more CO_2 through the C_3 pathway during the wet season, and potentially indicating differences in the use of photosynthetic pathways. However, the shifts in $\delta^{13}\text{C}$ also fall within the range expected by physiological factors that affect CO_2 concentration at the site of carboxylation, such as variation in stomatal conductance caused by changes in light environment, water availability, or VPD (Farquhar et al. 1982; Zimmerman and Ehleringer 1990). Nonetheless, the use of isotopic data derived from sugars holds promise as a method for assessing C isotope discrimination during photosynthesis on shorter timescales than those offered by bulk leaf sampling.

In CAM plants, differences in $\delta^{13}\text{C}$ can be due to a number of factors, in addition to shifts in the degree of CAM activity. For example, there are established ontogenic and size-related effects on $\delta^{13}\text{C}$ (Troughton et al. 1977; Kalisz and Teeri 1986; Zotz and Ziegler 1999; Holtum and Winter 2005; Winter et al. 2008). The plants tested in our sampling scheme were all of similar size and developmental stage, determined by the number and length of the leaves, thus eliminating size differences as a factor in our observed $\delta^{13}\text{C}$ changes. Light environment can also influence $\delta^{13}\text{C}$. For example, Skillman et al. (2005) showed that increased light resulted in more negative $\delta^{13}\text{C}$ values for CAM plants and less negative $\delta^{13}\text{C}$ values for C_3 plants. The *Tillandsia* species we studied exhibit intermediate $\delta^{13}\text{C}$ values around -16‰ for bulk tissue, indicating they

are CAM plants with some atmospheric CO₂ assimilation through the C₃ pathway. In our study, bulk tissue δ¹³C values were less negative in higher light (wetland forest), consistent with the direction of change in δ¹³C among C₃ plants in the Skillman et al. (2005) study. Therefore, the isotopic differences between plants growing in the wetland forest type and those in the mature and secondary forest types may be reflective of the C₃ mode operating at a lower internal CO₂:ambient CO₂ (*c_i/c_a*) ratio in the higher light environment (Zimmerman and Ehleringer 1990).

Leakage of CO₂ during decarboxylation could also lead to more negative δ¹³C values (Farquhar 1983; Pierce et al. 2002). If this were a factor in our study, we would expect leakage to occur similarly during both the wet and the dry seasons. However, leakiness cannot be ruled out from influencing the C isotope ratios across seasons due to the possibility for variation in CO₂ leakage to occur with changes in stomatal conductance or the amount of Rubisco (von Caemmerer et al. 1997), both of which may vary with environmental conditions and nutrient availability (Tissue et al. 1993), and the concentration of CO₂ in the bundle sheath cells.

The isotopic composition of source air can contribute to differences in δ¹³C of plant tissue because respired air from the soil surface is depleted in δ¹³C (Medina and Minchin 1980; Medina et al. 1991; Berry et al. 1997). Variation in leaf δ¹³C can therefore be caused by: (1) physiological processes, and/or (2) source δ¹³C of the atmosphere. In a comparison across canopy types, Buchmann et al. (2002) reported that 70% of the variation in δ¹³C was due to differential discrimination of the heavier isotope and 30% was due to source air. They show that for forests with a LAI less than 2.5 m² m⁻² and for canopy height over 1 m, differences in δ¹³C would be negligible. The seasonally dry tropical forest types in our study are relatively open, with LAI ≤ 2.5 m² m⁻², and most of our samples were collected between 1- and 2.5-m height. Thus, our sampling scheme suggests that the effects of respired CO₂ influencing the δ¹³C of source air is likely negligible, and that the observed δ¹³C values reflect physiological processes, similar to Mooney et al. (1989) who conducted their study in a comparable seasonally dry forest in Mexico.

Finally, we noted that the reduction in isotope values during the wet season was quite small, and could possibly be explained by greater stomatal conductance during the C₃ mode of photosynthesis, rather than a shift in the ratio of initial fixation by PEPC versus Rubisco. Because stomatal conductance can vary depending on irradiance and VPD (Farquhar et al. 1982), we cannot rule this out as a factor affecting δ¹³C. Direct measurements of gas exchange are necessary to distinguish between variation in C isotope ratios caused by changes in *c_i/c_a* versus photosynthetic pathway.

Changes in the C isotopic composition of *Tillandsia* species over a range of environmental conditions were observed. To our knowledge, this is the first study to use sugar and bulk C isotope ratios in contrasting habitats within tropical forests to investigate photosynthetic plasticity and epiphyte physiology. We emphasize that gas exchange measurements will improve the ability to pinpoint mechanisms of seasonal or habitat-mediated shifts in C isotopic composition of sugars. Although in our system, any seasonal or habitat-mediated variation in photosynthetic pathway appears to be quite moderate, C isotopic analysis of sugars in conjunction with bulk leaf isotopic measurements offers a valuable future approach for incorporating short- and long-term measurements of C isotope discrimination during photosynthesis.

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