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ECOLOGICAL INTERPRETATION OF LEAF CARBON ISOTOPE RATIOS: INFLUENCE OF RESPIRED CARBON DIOXIDE[1]

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Abstract. In a Neotropical moist forest at Barro Colorado Island, Panama, $\delta^{13}C$ values of CO_2 in air and $\delta^{13}C$ values of leaf tissue exhibit parallel patterns of variation between the forest floor and the canopy. During the daytime, $\delta^{13}C$ values of CO_2 from air sampled at 1 m and 0.5 m were significantly less than that at 25 m. Based on mass balance equations, up to 18% of the CO_2 in air at 0.5 m above the forest floor is from respiration. Respired CO_2 is responsible for 31 and 37% of the variation in isotope composition in leaves of two species of herbaceous bamboo grown in a well-ventilated sun treatment and in the forest understory. Respired CO_2 accounts for 45–70% of the difference in $\delta^{13}C$ values between understory and canopy leaves for three tree species growing in large-scale irrigation and control treatments. Understory leaves of these species show $\delta^{13}C$ values consistent with higher ratios of intercellular to ambient CO_2 in irrigated relative to control treatments. Estimates of water-use efficiency from leaf carbon isotope content should be corrected for the contribution of the carbon isotope composition of respired CO_2 in closed-canopy forests.

Key words: respired carbon dioxide; stable carbon isotopes; tropical forest; water-use efficiency.

INTRODUCTION

Carbon isotope ratios of plant tissue in the forest understory are frequently lower than that of tissue in the canopy (e.g., Ehleringer et al. 1986). In part, this can result from the presence of high concentrations of respired CO_2 in the understory (Keeling 1961, Vogel 1978, Medina and Minchin 1980, Schleser and Jayasekera 1985, Medina et al. 1986). Because soil bacteria acting on plant detritus produce CO_2 with carbon isotope ratios similar to that of the substrate (Jacobson et al. 1970), such respiration could result in the input of respired CO_2 with $\delta^{13}C$ values between -25 and $-28‰$ (Peterson and Fry 1987). Forest air would otherwise have a $\delta^{13}C$ value close to that of the atmosphere at $-7.8‰$ (Farquhar et al. 1982, Evans et al. 1986). Stratification of understory CO_2 varies as a function of diurnal gas exchange between the boundary layer above the forest and the free troposphere, and is more pronounced near the ground where air mixing is slow (Wofsy et al. 1988).

Lower carbon isotope ratios in understory leaves are also caused by plant environmental responses which increase the ratio of intercellular to ambient CO_2 concentrations. Higher ratios of intercellular to ambient CO_2 result in greater discrimination against ^{13}C during photosynthesis in C_3 plants (Farquhar et al. 1982). This ratio is mediated by variation in stomatal and mesophyll conductance with respect to the rate of assimilation. Thus for leaves growing in air with similar humidity and carbon dioxide composition, variation in leaf $\delta^{13}C$ values should represent integrated long-term water-use efficiency (Farquhar and Richards 1984). Leaf carbon isotope ratios have been shown to be an index of water-use efficiency for plants grown in experimental manipulations of soil water (Farquhar and Richards 1984), humidity (Winter et al. 1982), and irradiance (Evans et al. 1986, Mulkey 1986).

Data relating carbon isotope ratios of CO_2 from forest understory air to height are crucial to determining the extent to which leaf ^{13}C content might reflect plant environmental responses over this gradient. Although several studies have shown elevated CO_2 concentrations in the forest understory (e.g., Medina et al. 1986), we know of only one study where both the CO_2 concentration and isotopic composition of forest air have been systematically sampled. Francey et al. (1985)

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[2] Order of first two authors determined by coin toss.

sampled CO_2 at various levels in a huon pine forest in Tasmania. Their data indicate a relationship between $\delta^{13}C$ values and concentration of CO_2 typical of the mixing of isotopically depleted CO_2 and atmospheric CO_2 (Keeling 1961). However, the CO_2 concentrations observed in their study sites were only slightly elevated relative to atmospheric concentration, and the authors concluded that the effect of respired CO_2 on the carbon isotope ratios of understory plants is only slight. Their results cannot be applied to tropical forest because litter decay in temperate coniferous forest is slower than in a typical humid tropical forest (Olson 1963, Anderson and Swift 1983). Moreover, forests can differ in their CO_2 flux from soil and root respiration (Goreau and Mello 1985), and it is likely that the relative contribution of respired CO_2 to the $\delta^{13}C$ values of forest understory air would also differ among forests. Here we report concentrations and $\delta^{13}C$ values of CO_2 from air sampled at different heights, and $\delta^{13}C$ values for leaf tissue in a mature closed-canopy moist Neotropical forest. Our results indicate that respired CO_2 can have an important effect on the carbon isotope ratios of leaf material in the understory.

METHODS

Study site

Air and leaf material were sampled from four 2.25-ha plots in the understory of mature (500-yr-old) lowland Neotropical forest on Barro Colorado Island (BCI), Panama (9°10′ N, 79°51′ W). This forest typically receives 2600 mm rainfall per year, and experiences a pronounced dry season from December through April. Detailed descriptions of the BCI forest can be found in Leigh et al. (1982). During the dry seasons of 1985–1986 and 1986–1987, plots 1 and 2 were irrigated in the understory for 1.5 h each day for 5 d each week, whereas plots 3 and 4 experienced normal dry season moisture regimes. Irrigation was accomplished with sprinklers arranged in a hexagonal array, each mounted 1.8 m above the ground. During a typical week in the dry season, each manipulated plot received at least 675 t of water. During both dry seasons, irrigation in plots 1 and 2 maintained soil water potentials above −0.04 MPa while in plots 3 and 4 soil water potentials fell to −1.60 MPa (Wright and Cornejo, *in press*).

Gas collection and analysis

Air was sampled in each of the four plots at heights of 1 and 25 m above the litter between 0800 and 1300 during March and July 1987. Samples during July also included gas from 0.5 m. Gas samples were collected in glass ampules ≈150 mL in volume. These were made of 22 mm OD (outside diameter) glass tubes ≈0.5 m long with 9 mm OD endings that were partially collapsed at 2 cm from their ends. In the field these ampules were attached to high-density polyethylene tubes 25 m long. A battery-operated pump drew air at high velocity for at least 5 min through the tube before a sample was collected by flame sealing the ampules with a portable torch. This period of time was empirically determined to exceed that necessary to replace air in the tube with ambient air. CO_2 concentration was measured in the air stream after it passed through the ampules with a portable infrared gas analyzer (Analytical Development Company, Hertfordshire, England) connected in parallel with the pump via a T joint. Samples were taken after CO_2 concentration remained stable (±2 μL/L) for at least 2 min.

Ampules were taken to the laboratory where the carbon dioxide was cryogenically purified and subjected to mass spectrometry analysis. Samples were introduced into a fully expanded bellow in the mass spectrometer and then fully compressed so as to increase the sample pressure as much as possible. Typical standard deviations for measurements within a sample were ±0.05 ‰. Carbon isotope ratios were corrected for possible N_2O in the lower strata of the forest assuming a concentration of 0.32 μL/L (Goreau and Mello 1985). Carbon isotope ratios are reported here as $\delta^{13}C$ which is the difference in carbon isotope ratios between a sample and the PDB standard (Craig 1957), in thousandths (‰) of the isotope ratio in the standard:

$$\delta^{13}C = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000, \quad (1)$$

where R_{sample} and R_{standard} are the $^{13}C/^{12}C$ ratio of sample and standard, respectively.

To test whether flame sealing would alter the ^{13}C content of a sample, we sampled tank air as described above and by directly introducing tank air into a pre-evacuated vessel which was closed with stopcocks instead of flame (Craig 1957, Keeling et al. 1979). Both aliquots were purified by the same method as described above for four replicate samples. Tank air sampled in the flame-sealed ampule had a mean (±SD) $\delta^{13}C$ value of −10.6 ± 0.3 ‰, and that of the pre-evacuated vessel was −10.6 ± 0.1 ‰.

Collection and analysis of leaf tissue

In March, leaves were collected between 1100 and 1400 from three saplings and three adults in each plot for *Hirtella triandra, Trichilia cipo,* and *Tetragastris panamensis* (nomenclature follows Croat 1978). Leaves were collected from 1 m tall saplings for all three species, from canopy adults located in full sun for *Trichilia* and *Tetragastris,* and from subcanopy adults for *Hirtella.* Leaves from canopy and subcanopy trees were brought down by shotgun. By noting leaf color, size, toughness, and the presence of weathering and epiphylls, we attempted to collect leaves that had developed during the dry season of 1986–1987. We selected leaves that were newly developed but mature. In July 1987, leaves of *Pharus latifolius* and *Streptochaeta sodiroana* were collected in all plots at 0.3 m above the litter. After

drying, leaves from tree and herb species were deveined, ground in a Wiley mill, and combusted with cupric oxide according to procedures reported elsewhere (Stump and Frazer 1973). The resulting CO_2 was cryogenically purified.

Experimental light treatment

P. latifolius and *S. sodiroana* were grown in sun and shade treatments in a screened growing house at BCI in 1984 (described in Mulkey 1986). A water-misting apparatus and two industrial fans located at opposite ends of the growing house were used to maintain humidity and temperature in the light treatments similar to that measured at 30 cm above the ground in the BCI forest. Periodic measurements indicated that temperature and humidity did not significantly differ between treatments. Because fans were operated on a daily basis (except during rain) and the growing house was directly exposed to the prevailing winds from Gatun Lake, we assume CO_2 concentrations were the same in the two light treatments, and similar to concentrations above the forest canopy. Periodic determinations of ambient CO_2 concentrations at midmorning near the growing house produced values between 330 and 350 μL/L. Shade-treatment ambient photon fluence was typical of wet-season diffuse radiation in the understory of BCI forest (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday), whereas sun-treatment fluence (430 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was above that necessary for maximum light-saturated photosynthesis in gap-grown plants of these species. Stable isotope analysis of leaves from these treatments has been previously described by Mulkey (1986).

Statistical analysis

Statistical analyses of leaf and gas data were completed using PC SAS (version 6.02; licensed to the University of Missouri). Gas data were analyzed by regression and analysis of variance, and tissue data for field grown plants were analyzed by analysis of variance. In either case, the analysis of variance employed a mixed model with two fixed effects (irrigation treatment and height) and nested random effect (plot within irrigation treatment). $\delta^{13}C$ values were transformed by square root and arcsine; CO_2 concentrations were transformed by square root. Season did not significantly contribute to variance in the gas data, and thus data from both collection periods were combined except where otherwise noted. Where terms with the nested factor were not significant ($P > .25$), expected mean square values for these terms were pooled with that of the error term in order to employ increased degrees of freedom (Sokal and Rohlf 1981: 285). Variances in tissue isotope composition between the understory and canopy were heteroscedastic and could not be stabilized by transformation. Accordingly, differences between canopy and understory tissue values were assessed through analysis of variance conducted on the mixed model using ranks of the data (Conover 1980: 337). Irrigation treatment effects were then assessed through separate analysis of variance of the transformed data within each height class.

Results and Discussion

Analysis of CO_2 in forest air

Measurements of $\delta^{13}C$ values of CO_2 at different heights showed that there were significant differences in $\delta^{13}C$ values at 25, 1, and 0.5 m. Average (± 2 SE) $\delta^{13}C$ values were -8.9 ± 0.3, -10.6 ± 0.3, and -11.4 ± 0.4 ‰, respectively (Fig. 1; $F = 53.8$, df = 2,39, $P < .0001$; Tukey multiple comparisons $P < .05$ for each height). A $\delta^{13}C$ value of -8.9 ‰ at 25 m is consistent with the estimate provided by Medina et al. (1986) for canopy air in Amazonian forest (-8.8‰).

Carbon dioxide concentration parallels the pattern shown by $\delta^{13}C$ values with values of 348.7 ± 3.5, 374.6 ± 6.3 and 388.9 ± 9.4 μL/L at 25, 1, and 0.5 m, respectively ($F = 23.08$, df = 2,2, $P < .04$). The carbon isotope composition of CO_2 in forest air ($\delta^{13}C_f$) can be expressed as $p \cdot \delta^{13}C_r + (1 - p) \cdot \delta^{13}C_a$, where $\delta^{13}C_a$ and $\delta^{13}C_r$ represent the carbon isotope composition of atmospheric air and respiration, respectively, and p is the proportion of CO_2 from respiration. Using the observed isotope ratios and the $\delta^{13}C$ values of atmospheric and respired CO_2 (-7.8 and -28 ‰), we calculate that respired CO_2 composes 5.4, 13.9, and 17.8% of forest air at 25, 1, and 0.5 m, respectively.

Carbon-13 composition and concentrations of CO_2 in this forest can be characterized by the mixing of CO_2 from two sources according to the mass balance equation:

$$(\delta^{13}C_f - \delta^{13}C_r)\,[CO_2]_f = (\delta^{13}C_a - \delta^{13}C_r)\,[CO_2]_a, \quad (2)$$

where $[CO_2]_f$ and $[CO_2]_a$ represent the CO_2 concentrations in forest air and the atmosphere, respectively. $\delta^{13}C_f$, $\delta^{13}C_r$, and $\delta^{13}C_a$ represent the $\delta^{13}C$ values of forest, respired, and atmospheric carbon dioxide, respectively. Eq. 2 can be simplified to

$$\delta^{13}C_f = ([\delta^{13}C_a - \delta^{13}C_r]\,[CO_2]_a)/[CO_2]_f + \delta^{13}C_r \quad (3)$$

Thus, in cases where carbon dioxide in the forest air is not consumed by photosynthesis, the $\delta^{13}C$ of CO_2 from forest air is related to the inverse of $[CO_2]_f$ by a linear relation having a slope of $[CO_2]_a \cdot (\delta^{13}C_a - \delta^{13}C_r)$, and an intercept at the $\delta^{13}C$ value of respired carbon dioxide ($\delta^{13}C_r$).

Regression analysis for $\delta^{13}C$ values and inverse CO_2 concentration results in a highly significant relationship expressed by the equation

$$\delta^{13}C_f = 6703 \cdot (1/[CO_2]) - 28.3 \text{ ‰} \quad (4)$$

(Fig. 2), indicating that respired CO_2 in this forest has an average $\delta^{13}C$ value of -28.3 ‰, a value very similar to that reported for tropical rainforest litter (Medina et al. 1986). This equation also shows that the atmospheric CO_2 concentration of 330 μL/L results in a $\delta^{13}C$

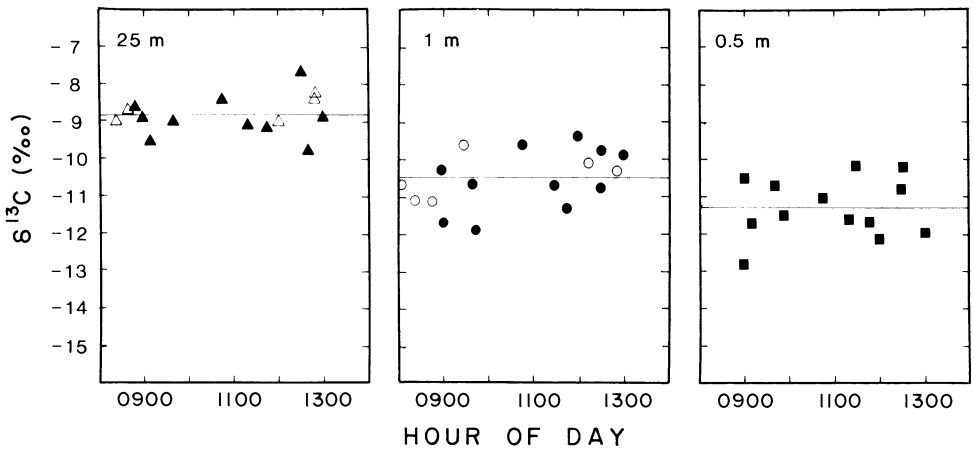

FIG. 1. $\delta^{13}C$ values of CO_2 from 0800 to 1300 in forest air at three different heights for all plots. Symbols are samples taken at 25 m (△, ▲), 1 m (○, ●), and at 0.5 m (■). Open symbols represent samples taken during the dry season, and closed symbols represent samples taken during the wet season. N = 15, 18, and 13 for 25, 1, and 0.5 m, respectively.

value of −8.0 ‰, which compares favorably with the accepted value of −7.8 ‰.

Several measurements have been made of CO_2 concentrations in the lower strata of forests (e.g., Medina et al. 1986). The results reported here and by Francey et al. (1985) show that in cases where the CO_2 concentration in forest air is higher than the atmospheric concentration, forest air is depleted in ^{13}C by the amount predicted in Eq. 3. Thus, using Eq. 3 and the assumption that forest litter and atmospheric CO_2 have $\delta^{13}C$ values of −28.0 and −8.0 ‰ (Medina et al. 1986), respectively, we calculate that the approximate $\delta^{13}C$ value of CO_2 from forest air at the concentration of 500 μL/L measured by Medina et al. (1986) has a value of ≈−15 ‰. The $\delta^{13}C$ value of CO_2 from forest air near the ground at the concentrations reported by Aoki et al. (1975) is −13 ‰. At El Verde at the near-ground concentrations reported by Odum et al. (1970), it ranges from −11 to −17 ‰.

Sources of isotopic variation in air

The coefficient of determination for Eq. 4 is 0.69, and thus ≈30% of the variation in ^{13}C cannot be explained by the increase in CO_2 due to input of respired carbon dioxide (Fig. 2). Further, irrigation has a significant effect on the adjusted least-squares means of ^{13}C values of ambient CO_2 (Table 1). At least three sources of variation may exist: (1) diurnal variation in photosynthetic CO_2 flux, (2) heterogeneous sources of respired CO_2 in the understory, and (3) measurement error with respect to CO_2 concentration.

Variation in $\delta^{13}C$ values of CO_2 from forest air may be partly independent of CO_2 concentration because CO_2 flux out of the forest air can occur either via pho-

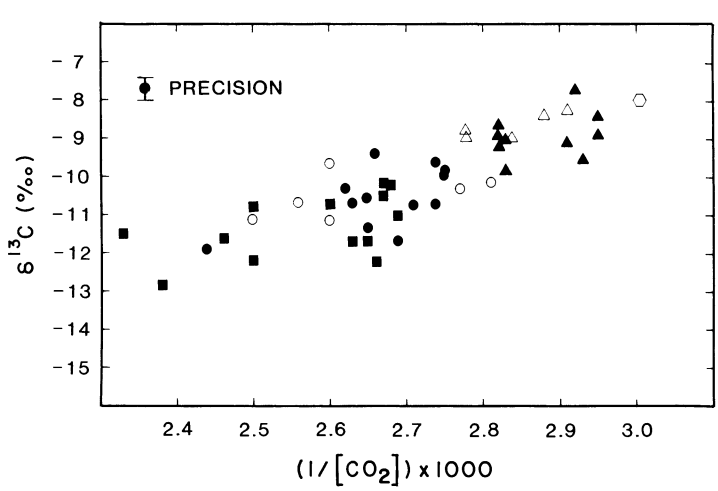

FIG. 2. Relationship between $\delta^{13}C$ values of forest air as a function of the inverse of CO_2 concentration (μL/L). Symbols are described in Fig. 1. Statistics are given in Table 1. Precision (SD) is ±0.3 ‰. The $\delta^{13}C$ value and concentration of atmospheric CO_2 is shown by the open hexagon in the upper right of the figure.

TABLE 1. Regression analysis of $\delta^{13}C$ values and CO_2 concentration of forest air for wet season data from irrigated and control plots separately, and for all plots together (wet and dry season data). N = 20 and 14 for control and irrigated treatments, respectively; N = 45 for the overall regression.

I. Analysis of covariance*

Source of variation	df	ss	F	$P<$
Homogeneity of slopes:				
Treatment	1	5498.07	5.40	.03
$[CO_2]$ (μL/L)	1	83 507.64	82.03	.0001
Treatment × $[CO_2]$	1	945.23	0.93	.34
Error	30	30 540.43		
Analysis of covariance:				
Among adjusted treatment means	1	5498.07	5.41	.03
Regression of covariance	1	86 280.54	84.95	.0001
Error	31	31 485.66		

II. Treatment regressions

Treatment	Equation	$\delta^{13}C$ at 330 μL/L CO_2 (‰)	Adj. LS mean†
Control (wet season data)	$8370(1/[CO_2]) - 33.2$ ($r^2 = 0.67$, $P < .0001$)	−7.8	−10.7
Irrigated (wet season data)	$6776(1/[CO_2]) - 28.3$ ($r^2 = 0.78$, $P < .0001$)	−7.8	−10.1
All (wet season and dry season data)	$6703(1/[CO_2]) - 28.3$ ($r^2 = 0.69$, $P < .01$)	−8.0	

* Analysis of covariance of wet season data is based on transformed $\delta^{13}C$ values.

† Adjusted least squares means for treatments are back transformed and significantly different at $P < .03$, indicating that there are treatment differences in regression elevations after adjustment for treatment differences in CO_2 concentration.

tosynthesis with a fractionation factor of ≈20 ‰, or by turbulent mixing without any isotopic discrimination. Thus, variation in photosynthetic flux relative to turbulent mixing at steady-state CO_2 concentrations could account for the isotopic variability reported here.

Another possible explanation is that forest air at any CO_2 concentration can have CO_2 with different carbon isotope ratios because of different CO_2 sources. Samples of forest air used for our regression equation were from two different irrigation treatments, each having a characteristic soil moisture content (Wright and Cornejo, *in press*) which should produce seasonally-dependent treatment differences in litter composition and decay rates. Isotope ratios of respired carbon dioxide will vary depending on the type of litter being decomposed and the stage of decomposition. For example, decomposition of stems and trunks could yield carbon dioxide with a different proportion of ^{13}C than that of leaf litter, because these different plant parts have different isotope ratios (Francey et al. 1985). In addition, isotope fractionations associated with the diffusion of CO_2 from the subsurface of the soil to the forest air could differ between treatments as a result of changes in soil structure associated with seasonal variation in water content.

Treatment differences in either photosynthetic flux or sources of respired CO_2 would produce separate regression lines for the irrigated and control plots. As shown in Table 1, the irrigation treatment has a significantly different adjusted least-squares mean with respect to the control, and the two lines pass through $\delta^{13}C$ values matching the accepted atmospheric $\delta^{13}C$ value at a CO_2 concentration of 330 ppm. Thus, during the wet season sampling period, control plots had ambient CO_2 depleted in ^{13}C relative to irrigated plots and this contributes to the unexplained variation of the overall regression (Eq. 4). Further measurements, particularly of respired CO_2, will be necessary before we can determine whether the variation in $\delta^{13}C$ value per CO_2 concentration is caused by treatment differences in photosynthetic flux vs. turbulent mixing, or by heterogeneous sources of respired CO_2.

A third source of variation is measurement error with respect to CO_2 concentration in the ampules at the time they were sealed. Although concentrations were stable (±2 ppm) for at least 2 min prior to sampling, it is possible that the CO_2 concentration in the ampule may have been slightly different from the parcel of air measured by the infrared gas analyzer at the time of sealing.

Isotopic analysis of plant material

Because discrimination against ^{13}C can vary as a function of environmental parameters such as light and humidity (Farquhar and Richards 1984, Evans et al. 1986, Mulkey 1986), one way to test for the effect of respired carbon dioxide on the isotope ratios of plants is to compare $\delta^{13}C$ values of plants grown in the understory with those of plants grown under the same environmental conditions as the understory, but exposed

TABLE 2. Isotope ratios of leaf tissues for two herbaceous bamboo species, *Pharus latifolius* and *Streptochaeta sodiroana*, and differences in $\delta^{13}C$ values due to growing conditions.* Leaves from the forest were collected from the four plots during July (wet season), 1987, and do not show an irrigation treatment effect or significant among-plot variation. Differences within species for sun and shade treatments are significant at $P < .05$ (Mulkey 1986).

Treatment	*P. latifolius*		*S. sodiroana*	
	$\bar{X} \pm 2$ SE	(*N*)	$\bar{X} \pm 2$ SE	(*N*)
A) Isotope ratios ($\delta^{13}C$, ‰)				
1) Sun in growing house	−28.3 ± 0.3	(8)	−27.7 ± 0.4	(8)
2) Shade in growing house	−32.5 ± 0.5	(8)	−32.6 ± 0.3	(6)
3) Shade in forest understory	−35.0 ± 0.5	(15)	−34.8 ± 0.3	(9)
B) Changes in $\delta^{13}C$†				
Effect of respired CO_2 (2 − 3)	2.5‰		2.2‰	
Effect of respired CO_2 and light environment (1 − 3)	6.7‰		7.1‰	
Percent change in $\delta^{13}C$ due to respired CO_2	37.3%		31.0%	

* Plants were grown in a well-ventilated screen growing house under two light treatments (Mulkey 1986), and leaves were collected from the same species growing in the forest understory under light conditions similar to those of the growing-house shade treatments.

† Changes in $\delta^{13}C$ values of plants due to respired CO_2 are hypothetical changes in $\delta^{13}C$ values of plant material caused by both respired CO_2 and increased discrimination due to lower light levels, and proportions of this change which would be due to lower carbon isotope ratios of CO_2 from forest air.

to carbon dioxide similar in composition and concentration to that above the canopy. We measured leaf isotope ratios of individuals of two species of herbaceous bamboo growing in the forest understory during the wet season, and compared these values to those of individuals of the same species grown under typical forest diffuse light and wet-season humidity conditions in a well-ventilated growing house (Mulkey 1986). If we assume that sunflecks in the understory contribute little to variation in isotopic discrimination, the differences in carbon isotope ratios between the forest and the shade treatment of the growing house should be primarily a function of differences in the $\delta^{13}C$ values of available CO_2.

The species of herbaceous bamboo grown in the forest understory had $\delta^{13}C$ values 2.5 and 2.2 ‰ less than shade-treatment individuals grown under local atmospheric carbon dioxide in the growing house (Table 2). The value 2.5 ‰ is consistent with the difference between $\delta^{13}C$ values of CO_2 from the forest air at 0.5 and 25 m levels (11.4 ‰ − 8.9 ‰ = 2.5 ‰). Thus our measurements of $\delta^{13}C$ values for CO_2 of forest air appear to be representative of the $\delta^{13}C$ value of the CO_2 available for plant photosynthesis during the dry-season and wet-season sampling periods. The difference in $\delta^{13}C$ values between plants grown in the sun treatment of the growing house and in forest shade is 6.7 and 7.1 ‰, and we estimate that respired CO_2 accounts for on average ≈34% of the depletion in ^{13}C in plants growing in the forest understory. If we are wrong in our assumption that sunflecks are unimportant to isotopic discrimination, then the contribution of respired CO_2 to the carbon isotope ratios of these understory plants is even greater.

Similar to results from other forests (Ehleringer et al. 1986), individuals of three tree species growing in the canopy and understory at BCI exhibit a marked difference in ^{13}C content (Fig. 3) between the canopy and the understory. Leaf samples from *Hirtella, Tetragastris,* and *Trichilia* for the understory were on average 3.5, 4.2, and 2.8 ‰, respectively, lower than those from the canopy. Again, this difference may be partially a function of light and humidity, and partially a function of the isotopic composition of the source CO_2. The difference between $\delta^{13}C$ values of canopy (25 m) and understory (1 m) carbon dioxide is ≈1.7 ‰. Expressing this as a proportion of the average difference in carbon isotope composition of leaf tissue at these heights, the contribution of respired air in the control plots is calculated to be 55, 37, and 70% for each of the tree species respectively. The corresponding calculation for the irrigated plots results in percentages of 45, 45, and 53 for the three species.

The ^{13}C content of understory leaves of *Hirtella* and *Trichilia* shows a small but significant irrigation treatment effect (Fig. 3, $F = 89.1$ and 22.3, respectively, df = 1,2, $P < .05$). Leaves of control plants of these two species may have fixed relatively more ^{13}C because of stomatal closure associated with treatment differences in water availability and humidity, but treatment differences in dry-season rates of decomposition would produce a similar pattern because air of the irrigated sites should be relatively depleted in ^{13}C due to higher rates of decomposition. Control plots had higher amounts of undecayed litter after the dry season, indicating a slower rate of decomposition and soil respiration (49.8 ± 6.8 and 38.0 ± 6.9 g dry mass per 0.25 m^2; mean ± 2 SE; $N = 20$).

CONCLUSIONS

Isotopic measurements of CO_2 in the forest understory at BCI show that respired CO_2 can make up 13–18% of the total CO_2 at 1 m and below. Our measurements of carbon isotope content of leaves from the understory, canopy, and BCI growing house indicate that respired CO_2 near the forest floor can account for

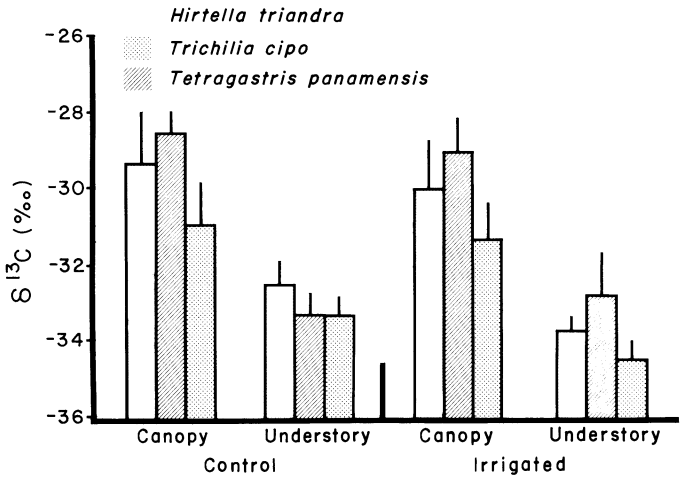

FIG. 3. Variation in $\delta^{13}C$ values between irrigation treatments and understory and canopy individuals of *Hirtella, Tetragastris,* and *Trichilia.* For each species, understory and canopy individuals are significantly different as determined by a mixed model analysis of variance on ranks of the data ($F = 63.1$, 84.7, and 65.0; df = 1,21, 1,24, and 1,20, respectively; $P < .0001$ for each species). Error bars are 2 SE. Statistics for treatment effects are given in Results and Discussion: Isotopic Analysis of Plant Material.

a large fraction of the lower carbon isotope ratios of forest understory plants. The implications of these findings are important for the use of $\delta^{13}C$ values of plant organic matter to estimate water-use efficiency. Although there is little doubt that fractionation is partially determined by environmental factors that influence stomatal control of water loss, the effect of respired CO_2 on isotope ratios of understory plants must be considered when analyzing forest understory leaf tissue in an ecological context. Because our results and those of Francey et al. (1985) indicate a direct relationship between $\delta^{13}C$ and CO_2 concentrations, carbon isotope ratios of CO_2 from forest air can be estimated from concentration measurements and $\delta^{13}C$ values of decomposing litter. Thus, it may be possible to avoid isotope analysis of CO_2 from the air near study plants in order to use $\delta^{13}C$ values as an indicator of water-use efficiency.

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