

## Vertical stratification of $\delta^{13}\text{C}$ values in closed natural and plantation forests in the Luquillo mountains, Puerto Rico

Ernesto Medina<sup>1, 3, \*</sup>, Leonel Sternberg<sup>2</sup>, and Elvira Cuevas<sup>1, 3</sup>

<sup>1</sup> Centro de Ecología, IVIC, Aptdo. 21827, Caracas 1020-A, Venezuela

<sup>2</sup> University of Miami, Department of Biology, Coral Gables, FL 33124, USA

<sup>3</sup> Institute of Tropical Forestry, USDA Forest Service, Southern Experiment Station Rio Piedras, Call Box 25000, Puerto Rico 00928-2500

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**Summary.** The variability of  $\delta^{13}\text{C}$  values was measured in leaf, stem and root tissues of several tree species growing in closed natural and plantation forests in the Luquillo mountains of Puerto Rico. Results confirm a significant decrease of  $\delta^{13}\text{C}$  values from the tree canopy to the forest floor. The values measured in understory plants growing in gaps were not significantly different from the average for plants growing under the forest shade. Seedling leaf values tended to be more positive than those of saplings, probably reflecting the contribution of organic matter from the mother tree. Photosynthetic independence on the forest floor results in a reduction in  $\delta^{13}\text{C}$  value. Stem and root tissue values of seedlings and saplings were less negative than those of the leaves of the same plants. It is suggested that this difference results from the slower change in isotopic composition experienced by the woody tissue, as the seedlings become photosynthetically independent in the forest floor.

**Key words:**  $\delta^{13}\text{C}$  value – Tropical forests –  $\text{CO}_2$  recycling

In closed natural or planted forests it has been shown that  $\delta^{13}\text{C}$  values of whole leaf tissue decrease with depth in the canopy (Vogel 1978; Medina and Minchin 1980; Ehleringer et al. 1986). This effect has been attributed to a reduction in light intensity resulting in higher ratios of intercellular to ambient  $\text{CO}_2$  concentrations ( $c_i/c_a$  ratio) (Farquhar et al. 1982) and/or to the assimilation by understory plants of  $\text{CO}_2$ -impoverished in  $^{13}\text{C}$  originating from soil respiration (Medina et al. 1986; van der Merwe and Medina 1989). The higher  $\text{CO}_2$  concentration near the forest floor might be important in counteracting the effect of lower light intensity. The problem has been approached through the systematic measurement of  $\text{CO}_2$  concentration and  $\delta^{13}\text{C}$  value of forest

air and the  $\delta^{13}\text{C}$  values of the vegetation. Results have been somewhat contradictory. In a temperate forest in Tasmania little influence of changes in forest air  $\delta^{13}\text{C}$  values was detected (Francey et al. 1985), while in tropical forests of Venezuela and Panamá substantial contributions of  $\text{CO}_2$  originating from soil respiration have been reported (van der Merwe and Medina 1989; Sternberg et al. 1989).

The actual fraction of  $\delta^{13}\text{C}$  value decrease due to soil respiration is still a matter of debate, because the effect of lower light intensity appears to be quite strong. However, there are no sufficiently precise measurements of this effect under field conditions. On the other hand, the influence of undercanopy air has been detected in agricultural plantations near the forest border, that is, in plants grown under full sun exposure, in a tropical forest of the Rio Negro region (van der Merwe and Medina 1989).

To provide additional field evidence to clarify these influences we collected leaf, stem and root samples from woody species occupying different positions in the canopy in two forests located in the Luquillo mountains of Puerto Rico. The goals were to verify the stratification of  $\delta^{13}\text{C}$  values in closed montane forests with relatively low frequency of gaps (Pérez-Viera 1986), and to test the presumed influence of undercanopy air in plants growing at relatively higher light intensity in forest gaps.

### Materials and methods

The forests sampled were: the experimental area of El Verde forest located at 450 m altitude (Odum and Pigeon 1970), and a nearly 50-year-old mahogany plantation (*Swietenia macrophylla*) nearby (Marrero 1948). The natural forest corresponds to the Tabonuco forest type (*Dacryodes excelsa*) (Briscoe and Wadsworth 1970). Leaf, stem, and root material was sampled from seedlings, saplings, and mature trees, from forest understory and a large gap, whenever possible, and dried in a ventilated oven at 60° C. The gap sampled was about 30 m long (downhill) by 8 m wide, allowing full sun exposure of the ground around noon. Before 10 a.m. and after 3 p.m. only sky light reached the ground. Seedlings and saplings were visually distinguished. "Seedlings" were always less than 2-

\* Present address and address for offprint requests: Centro de Ecología IVIC, Apartado 21827, Caracas 1020-A, Venezuela

**Table 1.** List of species sampled and corresponding  $\delta^{13}\text{C}$  values. Ranking by basal area of species in the natural forests corresponds to the evaluation of Briscoe and Wadsworth (1970)

Species	Ranking by basal area		$\delta^{13}\text{C}$ values (‰)		
			Leaf	Stem	Root
El Verde					
<i>Buchenavia capitata</i> (Vahl) Eichl	8	seedling	-33.25	-32.21	-32.48
		seedling	-34.58	-33.02	-33.42
		sapling	-34.41	-34.17	-33.59
		canopy	-30.09		
<i>Cordia borinquensis</i> Urban	26	sapling	-34.76		
<i>Dacryodes excelsa</i> Vahl.	1	seedling	-31.66	-29.6	-30.66
		seedling, gap	-31.63	-32.54	-30.97
		sapling, 4 m	-33.27		
		canopy	-28.64		
<i>Drypetes glauca</i> Vahl.	28	seedling	-33.63	-29.64	-32.31
		seedling, gap	-34.72	-34.14	-34.06
		canopy	-30.25		
<i>Guarea quidonia</i> (L.) Sleumer	21	seedling	-36.28	-34.42	-32.53
<i>Inga fagifolia</i> (L.) Willd.	17	seedling, gap	-34.55	-34.13	-33.03
		sapling, gap	-35.35	-35.03	-33.59
		canopy	-27.59		
<i>Manilkara bidentata</i> (A. DC.) Cher.	10	seedling	-31.29	-30.91	-31.53
		sapling	-33.67	-34.10	-33.37
		sapling, 3 m	-31.86		
		canopy, old leaves	-28.65		
		canopy, young leaves	-26.69		
<i>Ormosia krugii</i> Urgan	4	seedling	-31.97	-30.39	-29.49
		sapling	-33.28	-32.31	-31.41
		canopy	-28.51		
<i>Palicourea riparia</i> Benth.		shrub	-34.76		
		shrub, gap	-32.01		
<i>Prestoea montana</i> (R. Grah.) Nichols	2	seedling	-30.24	-28.33	
		tree	-32.54		
		tree, gap	-31.28		
<i>Rourea glabra</i> H.B.K.		seedling, vine	-34.58	-34.14	-33.43
<i>Sloanea berteriana</i> Choisy	9	seedling	-32.57	-31.24	-31.72
<i>Tabebuia heterophylla</i> (DC) Britton	14	sapling	-33.25		
Mahogany plantation. Harvey					
<i>Swietenia macrophylla</i>		seedling	-32.29	-30.47	-30.54
		sapling	-32.51	-33.60	-32.08
		understory tree	-32.65		
		canopy tree	-29.67		

year-old plants, frequently bearing remainings of primary leaves and/or cotyledons, while "saplings" already had a well developed woody stem. The tree species sampled are among the most frequent and characteristic of the Tabonuco forest (Table 1).

Measurements of  $\delta^{13}\text{C}$  values of the dried plant samples were done in the National Science Foundation Isotope Laboratory at the University of Utah, Salt Lake City. Values are expressed per mille (‰).

## Results

Canopy leaves in the natural forest showed significantly higher (less negative)  $\delta^{13}\text{C}$  values than leaves from the understory saplings or seedlings (Fig. 1), the average dif-

ference between canopy and understory leaves being 5.1‰. The values obtained from the leaves growing in well illuminated forest gaps were within the same range as the understory leaves. The same tendency was observed with the mahogany samples from the Harvey plantation;  $\delta^{13}\text{C}$  values of mahogany were well within the variation range of the categories differentiated in the natural forest (Fig. 1). The average  $\delta^{13}\text{C}$  value of seedlings was not significantly different from that of understory leaves. However, in several of the species sampled there was a clear tendency for the seedling leaves to show higher  $\delta^{13}\text{C}$  values than the understory leaves of saplings in the same forest (Table 2). On average  $\delta^{13}\text{C}$  values of sapling leaves were  $1.3 \pm 0.8\%$  more negative

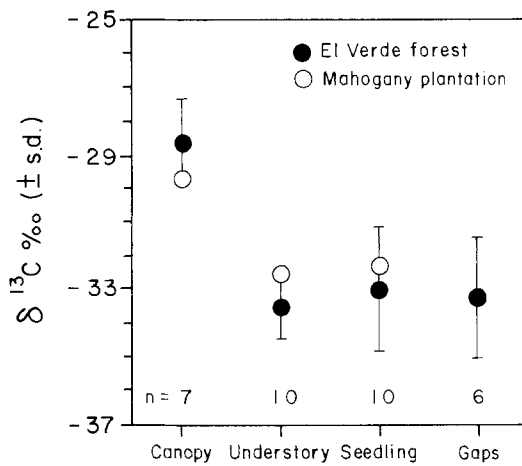


Fig. 1. Average  $\delta^{13}\text{C}$  values of leaf tissues collected at different levels within closed forests in the Luquillo mountains, Puerto Rico. Bars represent SD

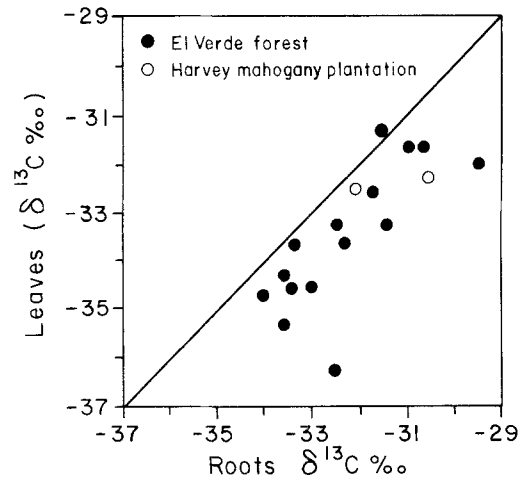


Fig. 2. Differences between  $\delta^{13}\text{C}$  values of leaf and root tissues in seedlings and saplings of closed forest in Puerto Rico

Table 2.  $\delta^{13}\text{C}$  values of seedlings saplings and canopy leaves of selected species

Leaf material	<i>S. macrophylla</i>	<i>B. capitata</i>	<i>D. excelsa</i>	<i>M. bidentata</i>	<i>O. krugii</i>
Seedling	-32.3	-33.3	-31.7	-31.3	-32.0
Sapling	-32.5	-34.3	-33.3	-33.7	-33.3
Canopy	-29.7	-30.1	-28.6	-28.7	-28.5

Average difference Seedling-Sapling = 1.3; P (two-tailed) = 0.02, Seedling-Canopy = 3.0; P (two-tailed) = 0.02

than the seedling leaves, in the species *S. macrophylla*, *Buchenavia capitata*, *Dacryodes excelsa*, *Manilkara bidentata* and *Ormosia krugii*, for which a complete set of data was available. For this set the difference between saplings and canopy leaves was also significant and averaged  $3.0 \pm 0.4\%$ .

Root samples had  $\delta^{13}\text{C}$  values consistently less negative than leaves (Fig. 2). The average difference was  $-1.23\% \pm 0.93$ , and it was highly significant ( $t_{\text{paired}} = 5.29$ ,  $P = 0.0001$ ). The same tendency was observed in the difference between stem and leaf values, although its statistical significance was lower (average difference =  $1.01\%$ ,  $t_{\text{paired}} = 3.69$ ,  $P = 0.002$ ).

## Discussion

### The stratification of leaf $\delta^{13}\text{C}$ values and light conditions

The  $\delta^{13}\text{C}$  values reported here confirm previous observations on the vertical decrease, from the canopy to the forest floor, of the relative  $^{13}\text{C}$  content of leaf tissues. From measurements of the  $\delta^{13}\text{C}$  values of air and leaves, and also the  $\text{CO}_2$  concentration of the air surrounding the leaves, Sternberg et al. (1989) concluded that respired  $\text{CO}_2$  is responsible for a large percentage (45–70%) of the difference in  $\delta^{13}\text{C}$  values between the canopy and understory leaves of tree species grown under different irrigation regimes in a tropical deciduous forest, thus

confirming earlier hypotheses (Vogel 1978; Medina and Minchin 1980) and in agreement with results obtained in tropical rain forests in southern Venezuela (van der Merwe and Medina 1989). Data reported here provides additional information on the possible dominant influence of the  $\delta^{13}\text{C}$  value of the forest air in determining leaf  $\delta^{13}\text{C}$  values, also in forest gaps. The gap sampled allowed direct sunlight penetration to the floor between 10 a.m. and 3 p.m.; during the rest of the day only diffuse sky light was available. Photosynthetic active radiation flux density in the gap was, therefore, much larger than in the undisturbed forest understory. The gap was probably not large enough to allow rapid turbulent mixing of the air, particularly during the early morning. We did not find consistent differences between leaves in the understory and those growing in the gap. It appears that air impoverished in  $^{13}\text{C}$  is contributing significantly to the net carbon gain of small woody plants growing under high-light conditions but surrounded by closed forests. The direct measurement of  $c_i/c_a$  ratios of plants in forest understory and gaps, together with the measurement of the  $\delta^{13}\text{C}$  value of the forest air, would be necessary to calculate the contribution of light and  $\text{CO}_2$  isotopic composition to the actual  $\delta^{13}\text{C}$  value of the leaves. This is of great practical importance if the model of Farquhar et al. (1982) of the relationship between water use efficiency and  $^{13}\text{C}$  discrimination is to be applied to species growing under different light intensities in closed forests.

In some of the species, leaf samples from canopy, saplings and seedling were obtained (Table 2). It appears that seedling leaves have significantly higher  $\delta^{13}\text{C}$  values than the saplings, a tendency that would be expected if seedlings were still reflecting in their  $\delta^{13}\text{C}$  values the isotopic composition of the food reserves provided by the mother tree. This effect should be reduced with seedling age, as the newly formed leaves make the plant photosynthetically independent. Systematic measurements of  $\delta^{13}\text{C}$  values of seedlings growing under natural conditions should provide a precise estimation of the acquisition of photosynthetic independence of the seedlings.

#### *Increase of $\delta^{13}\text{C}$ values from leaves to roots*

The roots of the natural forest species (seedlings and saplings) were consistently less negative than the leaves of the same species (Table 1). Similar results have been reported earlier for Amazonian trees (Medina et al. 1986) and several coniferous species in temperate regions (Francey et al. 1985; Leavitt and Long 1986). However, the average difference between leaf and twig  $\delta^{13}\text{C}$  values observed in 15 species of non-twig-photosynthetic shrubs in arid areas was not significant (Ehleringer et al. 1987). A possible explanation for the root-leaf difference reported here has been advanced before (Medina et al. 1986), based on the probable difference in chemical composition, particularly the lipid content, of stems and roots compared with the leaves (Preston and Phillips 1911; Kramer and Kozlowski 1979; Ziegler 1979). However, the same pattern of higher  $\delta^{13}\text{C}$  values in wood compared with leaves of the same species has been found when measuring pure cellulose, although the absolute values for cellulose were higher (Leavitt and Long 1986). Similar findings were reported by Schlessner (1990) from a detailed analysis of the variations of  $\delta^{13}\text{C}$  values in *Fagus sylvatica* leaves. Alternative explanations are respiratory effects (Leavitt and Long 1986), and the possibility that leaves synthesize their own cellulose during periods of high leaf conductance, while carbohydrate export from the leaf takes place during periods of low conductance (Francey et al. 1985). Another possible explanation is that the roots of seedlings and saplings still reflect the isotopic composition of the food reserves provided in the seed by the mother tree. As the seedlings become photosynthetically independent the leaves are the first organs where the influence of the lower  $\delta^{13}\text{C}$  values of the forest air will be detected. Woody organs, with a significant fraction of their mass being immobilized in structural components, might show a slower change in their isotopic composition. This alternative is supported by the observation that differences in  $\delta^{13}\text{C}$  values between leaves and woody tissues are smaller in adult trees than in seedlings and saplings (Medina et al.

1986). Further measurements in this direction are required for a definitive answer.

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