Journal of Tropical Ecology (2008) **24**:49–56. Copyright © 2008 Cambridge University Press
doi:10.1017/S0266467407004671 Printed in the United Kingdom

$\delta^{13}C$ in *Pentaclethra macroloba* trees growing at forest edges in north-eastern Costa Rica

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(*Accepted 26 October 2007*)

Abstract Fragmented forest landscapes with large proportions of edge area are common in the tropics, though little is known about functional responses of trees to edge effects. Foliar $\delta^{13}C$ can increase our understanding of tree function, as these values reflect changes in c_i/c_a as trees respond to environmental gradients. We expected that foliar $\delta^{13}C$ would be enriched, indicating a decline in c_i/c_a, in *Pentaclethra macroloba* trees growing at forest edges in north-eastern Costa Rica. We also anticipated this isotopic shift in $\delta^{13}C$ values of soil carbon and soil respired CO_2. Three transects perpendicular to forest edges were established at three study sites, and six plots per transect were located 0–300 m from edges. Within plots, foliage, soil and soil respired CO_2 were collected for isotopic analyses. Foliar $\delta^{13}C$, thus c_i/c_a, and soil carbon $\delta^{13}C$ did not vary along the edge to interior gradient. $\delta^{13}C$ for canopy and understorey foliage averaged $-29.7‰$ and $-32.5‰$, respectively, while soil carbon $\delta^{13}C$ averaged $-28.0‰$. Soil respired CO_2 $\delta^{13}C$ ranged from $-29.2‰$ to $-28.6‰$ and was significantly depleted within 50 m of edges. The predominant lack of functional responses at forest edges indicates that *P. macroloba* trees are robust and these forests are minimally influenced by edge effects.

Key Words: edge effects, soil respired CO_2, stable carbon isotopes, tropical rain forest

INTRODUCTION

Land-use change has dramatically altered tropical forest landscapes, increasing both forest fragmentation and the amount of land area in edge zones. With this change comes a need to better understand how edges affect forest ecosystems. It is well known that abiotic and biotic conditions at forest edges typically differ from those in forest interiors, and many studies of edge effects have focused on changes in variables such as forest structure, species composition and dynamics. However, few studies have examined forest functional responses to edges as a means of exploring why these characteristics may change at forest edges.

Foliar stable carbon isotope values ($\delta^{13}C_{leaf}$) can be used as an indicator of a tree's functional response to environmental gradients (Ehleringer *et al.* 1986), such as those present at forest edges. Modified growing conditions at edges, including high light, temperature, and vapour pressure deficit (VPD), are common across ecosystems (Chen *et al.* 1995, Kapos 1989, Newmark 2001, Williams-Linera *et al.* 1998) and may influence tree growth and survival (Laurance *et al.* 2002). Specifically, these conditions can lead to increased stomatal closure and decreased ratios of intercellular to ambient CO_2 concentrations (c_i/c_a) within the leaf, thereby limiting carbon uptake. For a given rate of net photosynthesis, declines in c_i/c_a can lead to an increase in $\delta^{13}C_{leaf}$ as more $^{13}CO_2$ is assimilated by the leaf.

Changes in $\delta^{13}C_{leaf}$ can have downstream effects on plant $\delta^{13}C$ values. Specifically, as the products of photosynthesis are translocated within a plant, some become substrates for root and rhizosphere respiration. $\delta^{13}C$ values of soil respired CO_2 ($\delta^{13}C_{R\text{-}soil}$) represent a mixed signal, derived not only from sources that may rely on current photosynthate, but also from heterotrophic respiration in which soil organic carbon serves as the primary respiratory substrate. Despite the variation in inputs to soil respired CO_2, climatic and environmental changes influencing the $\delta^{13}C$ of carbon fixed in the canopy is detectable in $\delta^{13}C_{R\text{-}soil}$ 1–6 d following initial carbon fixation (Ekblad & Högberg 2001, Ekblad *et al.* 2005). These data indicate that both short-term environmental changes, such as seasonal increases in VPD, and

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long-term changes, such as those resulting from forest edge creation, will produce $\delta^{13}C$ signals evident both above-ground and below-ground.

We chose to examine $\delta^{13}C$ values in the ecosystem components described above: foliage, soil organic carbon and soil respired CO_2 in the Sarapiquí region of Costa Rica. A shade-tolerant, leguminous tree species, *Pentaclethra macroloba* (Willd.) Kuntze, was selected as a focal species for this study. Reduced stomatal conductance has been observed in *P. macroloba* following extended exposure to full sun conditions in both field and laboratory settings (Oberbauer 1983, Oberbauer *et al.* 1987). Because high light conditions can increase both temperature and VPD, it is probable that the stomatal sensitivity of *P. macroloba* under high light is linked to one or both of these factors. Therefore, it is likely that signs of environmentally induced stress on trees growing near forest edges will be apparent in *P. macroloba*.

With the goal of determining forest functional responses to the presence of an edge, we examined two primary hypotheses. First, $\delta^{13}C_{leaf}$ will be more enriched, indicating decreased c_i/c_a, in canopy and understorey *P. macroloba* trees growing close to forest edges, relative to those in the forest interior. Second, patterns in $\delta^{13}C_{leaf}$ detected in canopy trees will also be apparent in the mineral soil organic carbon $\delta^{13}C$ value ($\delta^{13}C_{SOC}$) and $\delta^{13}C_{R\text{-}soil}$. Additionally, $\delta^{13}C_{leaf}$ was examined relative to leaf mass per unit area (LMA, the reciprocal of SLA) and nitrogen concentration, with the expectation that differences in canopy and understorey $\delta^{13}C_{leaf}$ will reflect higher photosynthetic capacity in canopy foliage.

METHODS

Site description

This study was conducted in the Sarapiquí region of north-eastern Costa Rica. Rainfall averages approximately 4000 mm annually with a mean annual temperature of 26 °C (Sanford *et al.* 1994). There is little annual variation in either rainfall or temperature. The landscape is characterized by fragmented forests within an agricultural matrix comprised of active pastures and crop land (Butterfield 1994). Forests in Sarapiquí are primarily classified as tropical wet forest by the Holdridge life zone system (Tosi 1969). Between 32% and 35% of the basal area in these high-diversity forests is occupied by *P. macroloba* (Clark & Clark 2000).

Three sites with 20–30-y-old forest-pasture borders were selected for this study, and all sites were located between 50 and 120 m asl on gently rolling terrain. Two sites, Rojomaca and Selva Verde, were located on highly weathered, acidic Ultisols derived from volcanic parent material while the third site, Tosi, was located on an Inceptisol derived from Quaternary alluvial deposits (ITCR 2004). Rojomaca was 375 ha in size, with western and south-western edge aspects, Selva Verde was 196 ha in size, with a western edge aspect, and Tosi was 120 ha in size, with a north-eastern edge aspect. The indicated site sizes represent only the size of an individual landowner's forested property; each site was embedded in a larger surrounding area of forest.

Rojomaca and Tosi were selectively harvested within 15 y of the present study, but no harvesting is known to have occurred at Selva Verde. Forest structural characteristics were similar among sites, regardless of logging history (see Schedlbauer *et al.* 2007, Table 1, where Site 1 is Rojomaca, Site 2 is Tosi and Site 3 is Selva Verde).

Foliar sampling

At each site, we established three transects perpendicular to the forest edge. Transects were randomly located and spaced 22–100 m apart, depending on the length of the forest-pasture edge at each site. Samples were collected in 0.095-ha plots located at distances of 0, 25, 50, 100, 200 and 300 m from the forest edge. However, plots were not installed at two locations (25 m and 50 m in separate transects) at Rojomaca due to inundation. Each plot extended 20 m into the forest, parallel to the established transects. Two canopy and two understorey *P. macroloba* trees were sampled in each plot. If suitable trees could not be located within the plot, trees immediately adjacent to the plot were substituted.

A tree-climber cut one small branch from the upper third of each *P. macroloba* tree (dbh at 1.37 m range: 19.1–82.0 cm) sampled in the forest canopy. All leaves on these branches were in full sun and came from heights of 25–35 m above the forest floor. From each branch, we collected 10 fully expanded leaves and avoided sampling leaves with significant epiphytic growth. A similar procedure was used to collect foliage from shaded understorey *P. macroloba* trees (dbh range: 1.5–34.5 cm) at heights of 3–5 m above the forest floor.

A subset of the sampled trees, consisting of one canopy and one understorey tree per plot, was selected for measurements of LMA. These measurements were not made for all trees because of the difficulty associated with processing foliage from *P. macroloba*, a species with bipinnately compound leaves and very small pinnules. LMA was determined separately for three leaves per tree by selecting five pinnae per leaf for analysis. Pinnules were separated from the rachilla, scanned, dried at 70 °C for 48 h, and weighed to the nearest mg. The leaf area of each scanned pinnule was determined using Image-J image analysis software (v. 1.34s, National Institutes of Health, Bethesda, Maryland, USA).

https://doi.org/10.1017/S0266467407004671 Published online by Cambridge University Press

To determine $\delta^{13}C_{leaf}$ and nitrogen concentration (N_{mass}), 10 pinnae per sample tree were randomly selected for analysis. Pinnules were removed from the rachilla, dried at 70 °C for 48 h, and ground into a fine powder. $\delta^{13}C_{leaf}$ and N_{mass} were determined at the Idaho Stable Isotopes Laboratory (ISIL). Stable carbon isotope data are expressed in standard delta notation as

$$\delta^{13}C = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where R_{sample} is the ratio of ^{13}C to ^{12}C in the sample of interest and $R_{standard}$ is the ratio of ^{13}C to ^{12}C in a standard (PDB). Twenty-two randomly selected foliar samples (10% of all samples) were analysed in duplicate, and analyses yielded a mean difference for $\delta^{13}C_{leaf}$ of 0.25‰. The nitrogen concentration of each leaf, expressed as a fraction, was multiplied by LMA to calculate the quantity of nitrogen per unit leaf area (N_{area}).

Within-leaf $\delta^{13}C$ variability was examined in a small subset of leaves (n = 8). For this analysis, pinnae were selected from the upper, middle and lower third of leaves from five canopy and three understorey trees. Pinnae from the middle third of these leaves were also divided into apical and basal pinnules to examine within-pinna variation in $\delta^{13}C$. Foliage was processed and analysed as described above.

Mineral soil organic carbon and soil respired CO_2 sampling

Within each plot, three mineral soil samples from 0–10 cm depth were homogenized to form a single sample. All leaf litter was removed from the soil surface prior to sampling. Soils were air-dried to constant mass, sieved through a 2-mm mesh, and a subsample was homogenized for $\delta^{13}C$ analysis. The mean difference in $\delta^{13}C_{SOC}$ for six duplicate soil samples (11% of all samples) was 0.31‰.

Soil CO_2, representing both heterotrophic and autotrophic respiration, was also collected in each plot from plastic chambers fitted with rubber septa on the soil surface. Each chamber was inverted over an excavated circle in the soil, and all living plant material was removed from the area beneath the chamber, though litter was left in place. Soil from outside the study area was used to seal the edges of the chamber to minimize diffusion of atmospheric air into the chamber. Preliminary testing indicated that air beneath the chamber equilibrated with soil air within 4 d of installation (i.e. $\delta^{13}C$ and CO_2 concentration did not change with increased time) (data not shown). Therefore, chambers were left in place for at least 4 d before an air sample was collected. Soil CO_2 samples were drawn from the chamber with a syringe and injected into evacuated 12-ml septum-capped Exetainer vials (Labco Ltd., High Wycombe, UK).

Air samples were shipped to the ISIL and analysed for $\delta^{13}C$ 8–13 d after collection. $\delta^{13}C$ values of soil CO_2 samples were corrected by −4.4‰ to account for the difference in diffusion rates between $^{12}CO_2$ and $^{13}CO_2$ in the soil pore space (Cerling *et al.* 1991). This corrected value is hereafter referred to as the $\delta^{13}C$ value of soil respired CO_2, $\delta^{13}C_{R\text{-}soil}$.

An independent test was conducted to examine the effect of air transportation on the $\delta^{13}C$ values of CO_2 stored in Exetainer vials. At the ISIL, 30 vials were evacuated and filled with CO_2 gas of varying concentration (10 vials were filled with each of the following CO_2 concentrations: 382.5 ppm, 3000 ppm and 10 000 ppm). The vial evacuation and filling procedure was carried out twice for each vial to reduce the possibility of contamination. Five vials per gas concentration were left in the laboratory, and the remaining five vials were sent to and returned from Costa Rica via air transportation. The $\delta^{13}C$ values of the gas in the 30 vials were analysed 9 d after the vials were initially filled. Student's t-tests run for each CO_2 concentration indicated that the $\delta^{13}C$ of the 382.5 and the 10 000 ppm gas was not significantly changed following air transport (df = 8, 8, t = −0.763, −0.791, P = 0.47, 0.45, respectively). However, a slight isotopic shift was detected for the 3000-ppm gas. Vials shipped by air had a $\delta^{13}C$ value enriched by a mean of 0.15‰ relative to samples that had remained in the laboratory (df = 8, t = 2.33, P = 0.048). Considered together, we interpret these results as an indication that soil CO_2 samples collected in the field and sent to the ISIL for analysis were not significantly altered by air shipment.

Statistical analyses

The statistical language R (version 2.0.1, R Development Core Team) was used for all data processing and analyses. Linear mixed-effects models (Pinheiro & Bates 2000) were used to examine differences in $\delta^{13}C$ in pinnae and pinnules from varying leaf positions. 'Leaf position' was used as each model's fixed effect while each 'leaf' was used as the random effect. Analysis of variance (ANOVA) was used to test for differences in $\delta^{13}C$ among the different leaf positions. LMA, N_{mass}, N_{area}, $\delta^{13}C_{leaf}$, $\delta^{13}C_{SOC}$ and $\delta^{13}C_{R\text{-}soil}$ data were also analysed with linear mixed-effects models. 'Distance to forest edge' was analysed as a continuous variable as each model's fixed effect. Random effects for these models were 'transect' nested within 'fragment'. Prior to analysis, the LMA data for understorey foliage were reciprocal-transformed to normalize data, as suggested by Box–Cox tests (Box & Cox 1964). Individual ANOVAs were performed for each variable to test for differences along the forest edge to interior gradient.

https://doi.org/10.1017/S0266467407004671 Published online by Cambridge University Press

Table 1. Within-leaf variation in $\delta^{13}C$ measured for eight individual *Pentaclethra macroloba* trees. Trees are identified by their crown position as either canopy (C) or understorey (U). Among-pinna variation in $\delta^{13}C$ was measured in pinnae from the bottom, middle and top third of the leaf. Within-pinna variation in $\delta^{13}C$ was measured in basal and apical pinnules of pinnae from the middle third of each leaf.

	Among-pinna $\delta^{13}C$ (‰)			Within-pinna $\delta^{13}C$ (‰)	
Tree	Bottom	Middle	Top	Basal	Apical
C–1	−29.5	−29.5	−29.7	−29.6	−29.4
C–2	−28.4	−28.2	−28.3	−28.6	−28.6
C–3	−28.8	−28.6	−28.4	−28.7	−28.6
C–4	−29.3	−29.1	−29.0	−29.3	−28.9
C–5	−31.2	−31.1	−31.1	−31.0	−30.9
U–1	−31.2	−31.3	−31.3	−31.3	−31.3
U–2	−31.6	−31.5	−31.7	−31.6	−31.6
U–3	−31.4	−31.6	−31.7	−32.1	−31.6

Table 2. Mean leaf mass per unit area (LMA), nitrogen concentration (N_{mass}), and nitrogen per unit leaf area (N_{area}) ± 1 SE for canopy and understorey *Pentaclethra macroloba* foliage in relation to the distance of each plot to the forest edge.

Distance to forest edge (m)	LMA (g m^{-2})	N_{mass} (mg g^{-1})	N_{area} (g m^{-2})
Canopy foliage			
0	68.28 ± 0.82	26.20 ± 0.62	1.79 ± 0.06
25	62.26 ± 2.29	27.35 ± 0.59	1.72 ± 0.09
50	66.29 ± 1.48	26.83 ± 0.64	1.77 ± 0.05
100	65.43 ± 1.92	26.90 ± 0.60	1.76 ± 0.07
200	69.15 ± 1.65	26.68 ± 0.70	1.84 ± 0.05
300	68.08 ± 1.46	26.12 ± 0.51	1.78 ± 0.04
Understorey foliage			
0	36.67 ± 1.19	31.34 ± 0.66	1.15 ± 0.04
25	33.71 ± 0.89	31.91 ± 0.40	1.08 ± 0.03
50	35.27 ± 0.70	31.01 ± 0.71	1.10 ± 0.04
100	37.04 ± 2.07	31.95 ± 0.64	1.17 ± 0.05
200	34.72 ± 0.82	30.65 ± 0.59	1.06 ± 0.02
300	33.91 ± 0.82	31.80 ± 0.51	1.08 ± 0.03

Results

Within-leaf $\delta^{13}C$ variation

No significant within-leaf variability was detected in the $\delta^{13}C$ value of pinnae from the bottom, middle and top of *P. macroloba* leaves (ANOVA, df = 14, F = 0.456, P = 0.64) (Table 1). However, a small but significant difference in pinnule $\delta^{13}C$ values was detected (ANOVA, df = 7, F = 5.63, P = 0.0494), with basal pinnules more depleted than apical pinnules (mean difference: −0.16‰) (Table 1). It should be noted that this difference was below the level of precision in analysed duplicate foliar samples (0.25‰). The small variation in $\delta^{13}C$ detected in these analyses indicate that a random sampling of pinnae sufficiently captures the overall bulk $\delta^{13}C_{leaf}$.

Forest edge to interior gradients

$\delta^{13}C_{leaf}$ was invariant along the forest edge to interior gradient for both canopy (ANOVA, df = 95, F = 0.06, P = 0.80) and understorey foliage (ANOVA, df = 96, F = 1.20, P = 0.28) (Figure 1a). The mean ± 1 SE $\delta^{13}C_{leaf}$ for canopy foliage was −29.6 ± 0.07‰ and was −32.5 ± 0.08‰ for understorey foliage.

No differences in LMA were detected for either canopy (ANOVA, df = 43, F = 1.33, P = 0.25) or understorey (ANOVA, df = 43, F = 0.64, P = 0.43) foliage along the edge to interior gradient (Table 2). Similarly, no significant differences in N_{mass} or N_{area} were observed along this gradient for either canopy (ANOVA, df = 43, 43, F = 0.62, 0.28, P = 0.44, 0.60, respectively) or understorey foliage (ANOVA, df = 43, 43, F = 0.14, 1.35, P = 0.71, 0.25, respectively) (Table 2). LMA in canopy foliage averaged 66.6 ± 0.97 g m^{-2} and was nearly double the mean LMA of understorey foliage, 35.2 ± 0.69 g m^{-2} (Table 2). N_{area} was consistently higher in canopy foliage, relative to understorey foliage, while the opposite was true of N_{mass}.

$\delta^{13}C_{SOC}$ for samples collected from the top 10 cm of mineral soil did not vary along the forest edge to interior gradient (ANOVA, df = 42, F = 2.07, P = 0.16) (Figure 1b), and the mean value across all distance classes was −28.0 ± 0.05‰. However, $\delta^{13}C_{R\text{-}soil}$ did vary significantly across the edge to interior gradient (ANOVA, df = 42, F = 4.76, P = 0.0347) (Figure 1b). Values were more depleted at the forest edge than in the interior and ranged from −29.2 ± 0.39‰ at 0 m to −28.6 ± 0.28‰ at 300 m.

DISCUSSION

Above-ground responses to forest edge effects

In contrast to our expectations, we did not observe enriched $\delta^{13}C_{leaf}$ in canopy or understorey *P. macroloba* trees growing near forest edges. These data indicate that *P. macroloba* did not exhibit a decline in c_i/c_a related to environmental stress at forest edges. Studies that have investigated changes in $\delta^{13}C_{leaf}$ over environmental gradients have found irradiance and VPD to be significant factors in determining $\delta^{13}C_{leaf}$ (Ehleringer *et al.* 1986, Hanba *et al.* 1997). However, neither of these factors appears to be influential along forest-edge-to-interior gradients in these tropical forests.

Conclusions regarding $\delta^{13}C_{leaf}$ and c_i/c_a, must be considered in light of canopy boundary layer dynamics. Total conductance from the leaf to the atmosphere can be reduced by the presence of a large boundary layer in a forest canopy. Therefore, $\delta^{13}C_{leaf}$ and c_i/c_a can

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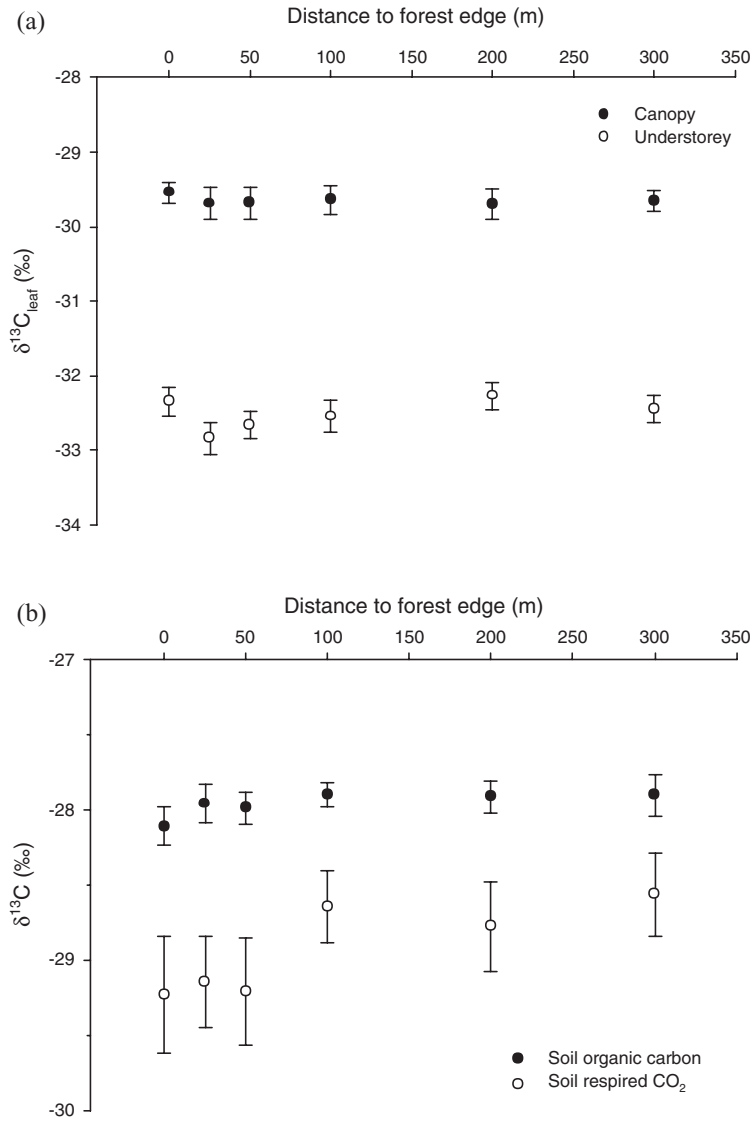

Figure 1. Above- and below-ground $\delta^{13}C$ values (mean ± SE) expressed in relation to the distance of each plot to the forest edge. Foliar $\delta^{13}C$ values of *Pentaclethra macroloba* canopy and understorey foliage ($\delta^{13}C_{leaf}$) (a). Soil organic carbon $\delta^{13}C$ values in the top 10 cm of mineral soil ($\delta^{13}C_{SOC}$) and soil respired CO_2 $\delta^{13}C$ values ($\delta^{13}C_{R\text{-}soil}$) (b).

be influenced by poor coupling of the canopy and the atmosphere. Forest canopies in Sarapiquí are typically uneven, ranging from 22–38 m in height, with canopy emergents reaching 50 m or greater (Clark & Clark 2001, Lieberman *et al.* 1996). Further unevenness is contributed by frequent gap formation in these forests (Denslow & Hartshorn 1994). Because aerodynamically rough canopies are typically well coupled to the atmosphere, it is unlikely that poor coupling and canopy boundary layer build-up played significant roles in determining total conductance, $\delta^{13}C_{leaf}$, and c_i/c_a in these canopies.

A study of edge effects conducted 5 y after forest edge creation in the Brazilian Amazon also failed to find variation in the $\delta^{13}C_{leaf}$ of two canopy tree species along forest-edge-to-interior transects (Kapos *et al.* 1993). However, enrichment in $\delta^{13}C_{leaf}$ was observed in an understorey species at these forest edges and this was linked to both increased canopy openness at these young forest edges and enriched $\delta^{13}C$ values in understorey air (Kapos *et al.* 1993). Fragmented forests in Sarapiquí develop edges that seal with dense vegetation in the 20–30 y following edge creation, and no difference in canopy openness has been found between edge and interior environments (Schedlbauer *et al.* 2007). The presence of a sealed edge decreases the likelihood that well-mixed air from outside the forest will penetrate edges and influence understorey $\delta^{13}C_{leaf}$. Our understorey $\delta^{13}C_{leaf}$ data are consistent with this idea.

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Although we did not detect evidence of edge effects in our measures of $\delta^{13}C_{leaf}$, there exists the possibility that *P. macroloba* trees growing directly adjacent to forest edges do exhibit enriched $\delta^{13}C_{leaf}$. Our sampling design was such that edge plots included trees growing anywhere between 0 and 20 m from the forest edge. Trees at the immediate edge of the forest are likely subject to a more extreme environment than trees growing close to an edge. The influence of hotter, drier conditions in adjacent pastures, as well as increased crown irradiance at edges could affect $\delta^{13}C_{leaf}$ on a scale smaller than that measured in the present study. While this possibility exists, it is unlikely that it would have any significant effect on the forest as a whole. The development of dense vegetation at forest edges in Sarapiquí appears to stabilize edges (Schedlbauer *et al.* 2007), and the potential for greater environmental stress on trees growing at immediate forest edges is unlikely to threaten this stability.

The overall lack of variation in $\delta^{13}C_{leaf}$ within both the canopy and understorey suggests that the $\delta^{13}C$ value of carbon available when leaves were developing was relatively constant. This is not surprising, given the relative aseasonality of temperature and precipitation in the Sarapiquí region (Sanford *et al.* 1994). Studies in tropical regions with distinct seasonality also report little to no change in $\delta^{13}C_{leaf}$ in canopy species between wet and dry seasons (Buchmann *et al.* 1997, Terwilliger 1997).

Comparison of canopy and understorey foliage

Photosynthetic capacity may influence $\delta^{13}C_{leaf}$ (Duursma & Marshall 2006, Hanba *et al.* 1997), and was assessed here indirectly via measures of LMA, N_{mass} and N_{area} (Field & Mooney 1986, Reich *et al.* 1992, 1997). None of these parameters exhibited significant changes with increased distance to forest edges for either canopy or understorey foliage, indicating that differences in photosynthetic capacity were not influential in determining $\delta^{13}C_{leaf}$. However, the variation in these parameters between canopy and understorey *P. macroloba* trees was important in explaining the differences in $\delta^{13}C_{leaf}$ from the canopy to the understorey.

A mean difference in $\delta^{13}C_{leaf}$ of 2.86‰ was observed between canopy and understorey *P. macroloba* foliage, with canopy foliage exhibiting a more enriched $\delta^{13}C_{leaf}$ than understorey foliage. This difference is slightly lower than is typically reported for tropical forests (Buchmann *et al.* 1997, Medina & Minchin 1980, Sternberg *et al.* 1989), perhaps because other studies introduce interspecific variation to measures of $\delta^{13}C_{leaf}$ and we report values for one species only. Buchmann *et al.* (2002), in a global analysis, determined that approximately 70% of the variation in $\delta^{13}C_{leaf}$ within forest canopies is related to changes in isotopic discrimination, while the remaining 30% is attributed to variation in the $\delta^{13}C$ of source air available for photosynthesis. Of the variation related to discrimination, there is a lack of consensus regarding the strongest drivers of the gradient in $\delta^{13}C_{leaf}$ within forest canopies. However, variation in light availability as well as differences in photosynthetic capacity between canopy and understorey foliage is often influential in determining $\delta^{13}C_{leaf}$ (Duursma & Marshall 2006, Hanba *et al.* 1997).

LMA nearly doubled in canopy foliage, relative to understorey foliage. This pattern is characteristic of sun and shade foliage (Lambers *et al.* 1998) and reflects the difference in light availability between the canopy and understorey. The high N_{area} observed in canopy foliage is also a manifestation of high light availability in the forest canopy, as most leaf nitrogen is associated with the photosynthetic apparatus of a leaf (Evans & Seemann 1989, Hanba *et al.* 1999). Although we detected higher N_{mass} in understorey foliage than canopy foliage, this pattern is likely related to the thinness of shade leaves. An enrichment in canopy tree $\delta^{13}C_{leaf}$ relative to understorey tree $\delta^{13}C_{leaf}$ is partially the result of high light availability and photosynthetic capacity, both of which lead to draw-downs in c_i. However, differences in water conducting path length, boundary layer conductance, leaf temperature, and leaf-to-air vapor pressure difference between foliage in canopy and understorey trees can also influence c_i by inducing earlier or more frequent stomatal closure in canopy foliage.

Below-ground responses to forest edge effects

Consistent with our hypothesis that canopy-level patterns in $\delta^{13}C_{leaf}$ would be reflected below-ground, $\delta^{13}C_{SOC}$ was found not to vary with distance to forest edges. However, a significant depletion in $\delta^{13}C_{R\text{-}soil}$ was detected in plots close to the forest edge. The magnitude of this depletion was approximately 0.5‰ and, though small, was consistent for all plots within 0–50 m of the forest edge. Evidence presented above decreases the likelihood that alterations in canopy processes such as photosynthesis and transpiration are responsible for these changes at the forest edge. Further, relatively constant $\delta^{13}C_{SOC}$ along the edge to interior gradient makes it unlikely that differences in the $\delta^{13}C$ of soil organic matter explain these changes.

As described previously, the edges of forest fragments in Sarapiquí seal with vegetation in the 20–30 y following edge creation (Schedlbauer *et al.* 2007), a process that is related to an increase in pioneer species at edges (Forero & Finegan 2002). Pioneer species and shade-tolerant or late-successional species in tropical forests do not vary consistently in $\delta^{13}C_{leaf}$ (Bonal *et al.* 2000a, Huc *et al.* 1994), although the direction of isotopic shifts among tree functional types often varies with the ecophysiological

https://doi.org/10.1017/S0266467407004671 Published online by Cambridge University Press

traits of individual species (Bonal *et al.* 2000b, Huc *et al.* 1994). In the forests of Sarapiquí, it is possible that the carbon available for root and rhizosphere respiration in pioneer species is more depleted than that of late-successional species. This depleted signal would then be evident in $\delta^{13}C_{R\text{-}soil}$ in areas of the forest dominated by early successional species, such as the forest edge. These possible shifts in $\delta^{13}C_{leaf}$ may not have been detected in $\delta^{13}C_{SOC}$ because these forest edges are not occupied solely by early successional species. Further research is needed to assess whether variation in $\delta^{13}C_{leaf}$ among tree functional groups is a potential driver of the pattern in $\delta^{13}C_{R\text{-}soil}$ observed at forest edges in Sarapiquí.

In the present study we were unable to accurately determine the CO_2 concentration of soil air samples. As such, it was not possible to correct $\delta^{13}C_{R\text{-}soil}$ for the effect of atmospheric CO_2 intrusion into the soil profile (Cerling 1991), though this can be done (Cernusak *et al.* 2004). Because the $\delta^{13}C_{R\text{-}soil}$ data presented here are uncorrected, there remains the possibility that variation in soil CO_2 concentration may have contributed to the reported variation in $\delta^{13}C_{R\text{-}soil}$.

Conclusions

Functional changes in *P. macroloba* trees growing close to forest edges were not detectable in the fragmented forests of Sarapiquí. Along edge to interior gradients, we did not observe significant changes in $\delta^{13}C_{leaf}$, thus c_i/c_a, or any measured leaf characteristics in canopy or understorey foliage. Therefore, we conclude that *P. macroloba* trees growing close to forest edges are robust and functionally similar to trees in the forest interior. Variation in $\delta^{13}C_{leaf}$ and other leaf characteristics between canopy and understorey foliage was typical of patterns commonly observed in forest canopies. Consistent with our above-ground data, no differences in $\delta^{13}C_{SOC}$ were found along edge to interior gradients. However, we did detect significantly greater depletion of $\delta^{13}C_{R\text{-}soil}$ at forest edges relative to interiors, but this pattern may be tied to shifts in species composition at edges rather than physiological responses to edge effects. Overall, these results are consistent with other research on edge effects in Sarapiquí reporting the development of a dense wall of vegetation at these forest edges (Forero & Finegan 2002, Schedlbauer *et al.* 2007). This dense vegetation appears to be effective in sealing the forest edge, as we detected few forest functional changes in edge zones.

ACKNOWLEDGEMENTS

Thanks to Leonel Coto for assistance in climbing trees and also to Bob Brander, Stacy Sesnie, Benjamin Miller, Erika Barrientos and Bertha Gonzalez for laboratory assistance. Germán Obando and Andrés Sanchún from FUNDECOR provided logistical support for fieldwork. Thanks to the landowners in Sarapiquí who graciously permitted access to their land. Helpful comments on earlier versions of this manuscript were provided by Bryan Finegan, John Marshall, Paul McDaniel and two anonymous reviewers. This research was supported by NSF-IGERT grant no. 0114304.

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