

Changes in leaf properties across an elevation gradient in the Luquillo Mountains, Puerto Rico

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Few data exist on the leaf physiological characteristics of tropical tree species. Quantifying variation in leaf CO₂ exchange within a forest canopy improves our understanding of forest carbon cycling, especially if this quantification is done in such a way that explores leaf response to environmental variables. The main goal of this study was to investigate how leaf structural and functional properties change in response to the changing environmental conditions that occur both within a tree canopy and along a gradient of elevation in a subtropical forest ecosystem in Puerto Rico. We employed novel field techniques that allowed measurement of leaf CO₂ exchange in different canopy positions as well as over a large geographic extent. Our results indicate that leaf properties such as leaf metabolic rates [light-saturated net carbon assimilation (A_{max}) and dark respiration (R_d) rates], leaf chemistry (chlorophyll and nitrogen concentration), and leaf structure [leaf mass per area (LMA)] differed significantly among species of varying successional status, between leaf positions within a tree, and spatially across the elevation gradient. Data presented here contribute to the worldwide 'leaf economics' database and will be useful to those interested in changes in canopy carbon sequestration across gradients.

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For the most part, our ability to estimate large-scale productivity patterns through simulation modeling depends on parameters that originate from small-scale, leaf-level observations, but rarely do such parameters account for variability within species in their physiological responses to different environmental factors. Species-specific physiological data for tropical tree species are rare (Pearcy 1987, Chazdon et al. 1996, Rijkers et al. 2000, Pons and Welschen 2003), and the majority of leaf-level observations in tree canopies have been limited either to fully sunlit, fully expanded leaves (Hogan et al. 1995, Santiago et al. 2004) or to small sample sizes in terms of the number of individual trees measured (Oberbauer and Strain 1986). As a result, the photosynthetic rate of an entire forest canopy is represented frequently by a single generic equation which, in fact, may be based on few empirical data (Running and Gower 1991, Wang et al. 2003).

One reason for these spatial constraints to data collection is that non-destructive, in situ measurements are ex-

tremely difficult to obtain. Some studies have investigated rates of photosynthesis in tree seedlings and saplings under controlled or natural conditions (Lugo 1970, Huc et al. 1994, Wen et al. 2008), but extrapolating these values to mature forests is problematic because substantial differences in photosynthesis and leaf structure exist between seedlings and mature trees (Koike 1988, Thomas and Winner 2002). Several new methods have been developed to place researchers physically into tree canopies using devices such as cranes, rafts and scaffolding towers, but these methods are expensive, geographically limited and may have their own logistical problems in certain contexts.

In this study, we overcame many of these difficulties by using inexpensive but effective tree climbing techniques that allowed us to measure rates of photosynthesis and respiration in situ for upper and lower canopy leaves of five tree species located along a 1000 m gradient of elevation in a subtropical forest in Puerto Rico. By measuring leaves in different canopy positions on a relatively large sample

of trees located throughout each species' range, we could relate leaf structural and functional responses to environmental inputs that vary with elevation while quantifying the variability in exchange processes among leaves, trees and species. We examined across- and within-species differences in leaf metabolic rates (photosynthesis and dark respiration), leaf chemistry (nitrogen and chlorophyll concentration), and leaf structure [leaf mass per area (LMA)]. We measured full light response curves for each leaf, rather than single values for maximum photosynthetic capacity (A_{max}), to determine differences in the shapes of light response curves.

Methods

Study site

Research was conducted in and around the Luquillo Experimental Forest (LEF, 18°18'N, 65°50'W), a secondary subtropical forest located in northeastern Puerto Rico. The forest spans more than 11 000 ha and elevations range from about 15 to 1075 m above mean sea level (Brown et al. 1983, Scatena and Lugo 1995). The climate is considered subtropical (*sensu* Holdridge 1967), with mean annual temperatures ranging from 23° to 19°C along the elevational gradient (Weaver and Murphy 1990, García Martínó et al. 1996) and annual rainfall increasing with elevation from approximately 2300 mm at 35 m elevation to almost 4500 mm at the mountain peaks (Brown et al. 1983, García Martínó et al. 1996). Above the cloud condensation level at approximately 600 m elevation, the forest is enveloped frequently in clouds that reduces mean annual solar radiation to about 63% of nearby coastal areas (Briscoe 1966). However, instantaneous insolation values, UV radiation loads and air saturation deficits can be high at upper elevations during the relatively dry months of February and March.

Plot and species selection

Thirteen study sites were established over two transects spanning an elevation gradient ranging from near sea level to over 1000 m. Each site was geo-referenced using a handheld GPS and its elevation was determined using an altimeter and verified on a 30 × 30 m resolution digital elevation model (DEM) digitized from a 1:24 000 USGS Forest Service map. Sites were located at 100–200 m elevation intervals (Fig. 1).

Species were chosen based in part on their life history characteristics and their importance values, calculated as the sum of relative basal area and relative stem density (Frangi and Lugo 1998). *Cecropia schreberiana* is a gap-colonizing pioneer species present in disturbed and/or open

sites throughout the forest; *Dacryodes excelsa* and *Manilkara bidentata* are late-successional canopy emergents that inhabit elevations below 600 m; *Micropholis garciniifolia* is a late successional canopy emergent that prevails at high elevations above 600 m; and *Prestoea montana* dominates the understory at low to mid-elevations but will grow into an emergent if exposed to sufficient light. *Prestoea* also dominates areas with steep slopes and saturated soils above 600 m. We measured individual trees that spanned each species' elevational range so that for example, leaves of *Dacryodes* were sampled at plots located only between 300 and 600 m whereas leaves of the widespread species *Prestoea* were sampled at virtually all of the sites. Total sample sizes of sites, trees and leaves measured for each species are given in Table 1.

Gas exchange measurements

Gas exchange measurements were conducted using a portable infrared gas analyzer (LI-6400). Since the ability of leaves to photosynthesize after excision has been shown to be compromised in some species (Santiago and Mulkey 2003), leaves were sampled in situ from those accessible from existing towers at sites 3, 8 and 9 (Fig. 1). At other locations where the canopy was inaccessible, tall trees were rigged to climb by using a modified arborists' technique. Briefly, a giant slingshot was used to shoot a 3 oz. fishing weight attached to 12 lb. test fishing line over a branch high in the canopy. Once the fishing line was rigged over the branch, it was replaced first with nylon parachute cord and then with a 12 mm diameter rock climbing rope. One end of the rope was tied to a nearby tree, and the climber ascended into the canopy on the other end of the rope using a harness and mechanical ascenders. At each climbing site, a system of ropes and pulleys was erected to lift a sturdy canvas case that contained all equipment required to work in the canopy, including the photosynthesis measurement system.

Leaves were sampled at different heights in each tree, but each was categorized as an 'upper canopy' or 'lower canopy' leaf ($n \geq 2$ for each leaf type per species × site combination, $n = 130$ leaves). Leaves were considered 'upper canopy' if they were exposed to unobstructed sunlight for some part of the day and were located in the upper third of the canopy. Leaves were considered 'lower canopy' if they were exposed to shady conditions for most of the day and were located at the base of the canopy. Because the natural light inside the canopy was unreliably constant (or variable) minute-to-minute, we used the measurement system's LED light source (red+blue) to control light levels at fixed values and generated a light response curve for each leaf sampled. One cycle, from light saturation to complete darkness [photosynthetically active radiation photon flux density (PAR PFD) = 2000 to 0 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, respectively], took approximately 20 min to

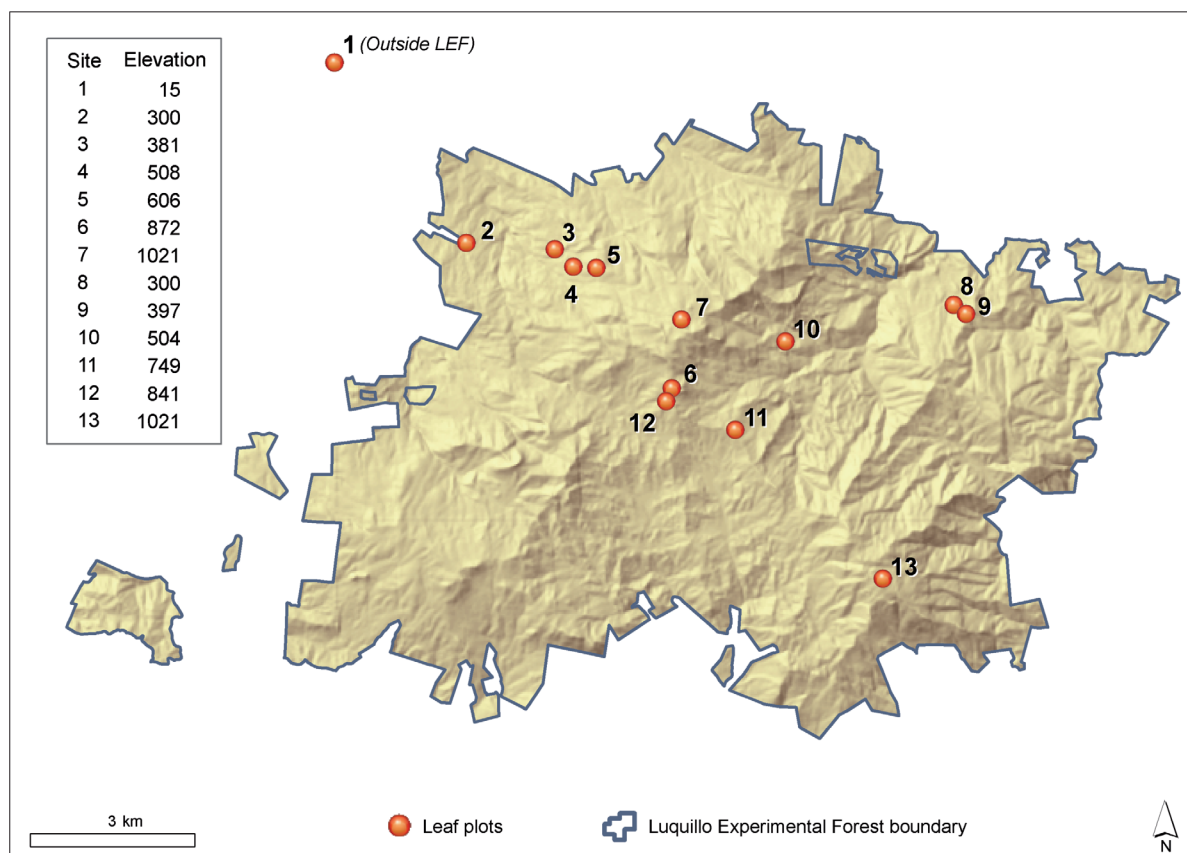


Figure 1. Map of thirteen sites established for leaf physiological measurements in the Luquillo Experimental Forest, Puerto Rico. Site 1 is an isolated stand of *Manilkara bidentata* trees near sea level (15 m).

complete. The rate of photosynthesis (or dark respiration) at each light level was logged when the combined coefficient of variation (ΔCO_2 , $\Delta\text{H}_2\text{O}$, and $\Delta\text{flow rate}$) fell below 0.5%. Leaf temperature was held constant at $30 \pm 1^\circ\text{C}$ by a leaf temperature thermocouple located inside the leaf chamber and relative humidity remained at ambient levels.

After sampling was complete, leaves were harvested and their areas were determined using a leaf area meter (LI-

3100). Chlorophyll was extracted from 0.1 g leaf material within 6 h of sampling using the method of Hiscox and Israelstam (1979). Dry mass of the remaining leaf portion (without petioles) was determined after oven drying at 50°C for 2 d and leaf mass per unit area (LMA) was calculated. Organic nitrogen content of leaves was determined in an elemental analyzer (LECO CNS 2000) at the International Inst. of Tropical Forestry in Río Piedras, Puerto Rico.

Table 1. Site, tree and leaf sample sizes for the five tree species measured along an elevation gradient in the Luquillo Experimental Forest, Puerto Rico. Total leaves measured were separated into lower and upper canopy leaves, respectively (shown in parentheses).

Common name	Scientific name	Sites	Trees	Leaves (low/up)
Yagrumo	<i>Cecropia schreberiana</i>	6	17	34 (14/20)
Tabonuco	<i>Dacryodes excelsa</i>	6	9	28 (14/14)
Ausubo	<i>Manilkara bidentata</i>	4	8	18 (10/8)
Caimitillo	<i>Micropholis garciniifolia</i>	4	6	16 (9/7)
Sierra palm	<i>Prestoea montana</i>	10	26	34 (20/14)
Total		13	66	130

Statistical analysis

We conducted a nested multivariate analysis of variance with species and leaf position as main plot factors and trees nested within species as the sub-plot factor to evaluate whether leaf structural and functional properties differed among light environments and/or species. Ordinary least squares regressions were also conducted to evaluate how leaf characteristics such as nitrogen content and leaf mass per area (LMA) contributed to intraspecific and interspecific variation in photosynthetic and respiratory rates. We used data from upper canopy leaves to analyze changes in leaf structural properties across the elevation gradient. Because these properties also varied in response to vertical canopy structure, data from lower canopy leaves were not included in this analysis.

To generate forest-wide estimates of photosynthetic parameters for each species, light response curves for upper and lower canopy leaves of each species were analyzed using a nonlinear mixed model procedure in SAS. Curves were fit to photosynthetic data from each species \times position combination according to the function (Peek et al. 2002):

$$A = A_{\max} [1 - e^{-A_{\text{qe}} (\text{PPF} - \text{LCP})}] \quad (1)$$

where A_{\max} represents the light-saturated rate of photosynthesis at high light levels, A_{qe} corresponds to the initial slope of the curve at low light levels (i.e. the apparent quantum yield), LCP denotes the x-intercept when net photosynthesis is equal to zero (i.e. the photosynthetic light compensation point), PPF is the incident photosynthetic photon flux and A is net photosynthesis, the response variable. Since three of the parameters (A_{\max} , A_{qe} , LCP) vary by individual leaf, we assumed that the mean of these three terms varied by treatment and that these coefficients followed a multivariate normal distribution (Peek et al. 2002).

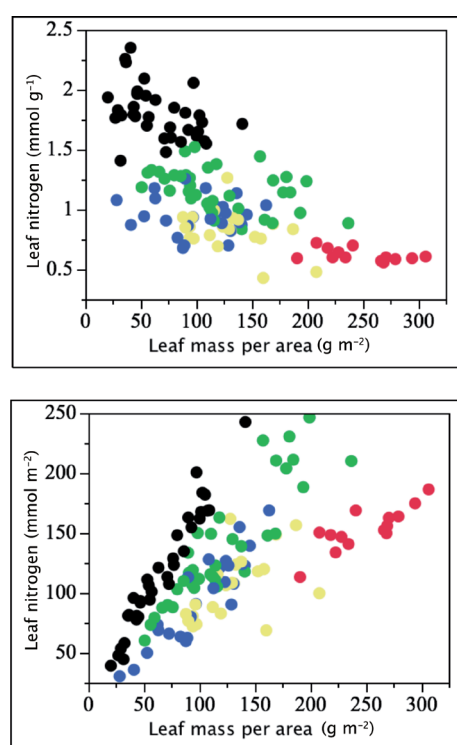
Results

Leaf structure and chemistry

Differences among species and leaf positions ('upper' vs 'lower' canopy leaves) were significant across all structural and chemical parameters measured [Wilk's λ criterion for species = 0.0061 ($p < 0.0001$) and for leaf position = 0.3344 ($p = 0.0002$)]. Table 2 summarizes these differences with respect to each parameter. Leaf properties varied most among species, and differences were generally larger among leaves within a tree than among trees within a species, since the error ratio (leaves:trees) ranged from 1.0 to 4.2 in a variance component analysis. Across all sites sampled, thick leaves of *Micropholis* had the high-

est LMA, which resulted in the lowest mean chlorophyll and nitrogen concentrations on a mass basis but the highest concentrations on an area basis (Table 2). In contrast, thin *Cecropia* leaves contained approximately three times more nitrogen per unit mass (N_{mass}) but less N per unit area (N_{area}) than *Micropholis* leaves. All other species had intermediate values for the variables measured (Table 2). *Prestoea* leaves were most variable with respect to the leaf characteristics measured.

Across all species, leaves in the lower canopy had generally higher chlorophyll concentrations per unit mass and higher chlorophyll to nitrogen (Chl/N) ratios than leaves in the upper canopy. N_{mass} was not significantly different between upper and lower canopy positions, but shade-adapted leaves in the lower canopy had lower N_{area} and chlorophyll a to b (Chl a/b) ratios (Table 2). In contrast, leaves growing in high light environments at the top of the canopy were thicker (i.e. high LMA) and had high



● *Cecropia schreberiana* *[^]; ● *Manilkara bidentata* *;
● *Prestoea montana* *[^]; ● *Dacryodes excelsa* *;
● *Micropholis garciniiifolia* *[^]

Figure 2. Nitrogen concentration (mass and area basis) vs leaf mass per area for five tree species in the Luquillo Experimental Forest. Species are indicated with different symbols. The regression is: N_{mass} = 1.723 - 0.0046 \times LMA, R² = 0.44, $p < 0.0001$; N_{area} = 66.73 + 0.4562 \times LMA, R² = 0.41, $p < 0.0001$. Significant regressions within a species ($p < 0.05$) are indicated by an * for area-basis and ^ for mass-basis in the legend.

Table 2. Structural characteristics of upper and lower canopy leaves of five tree species located in the Luquillo Experimental Forest. Species-specific values are means and standard errors (in parenthesis) across all replicate trees. Different letters within a row indicate a significant difference ($p < 0.1$) among species within a leaf position and an asterisk within a column indicates a significant difference ($p < 0.1$) between canopy position within a single species.

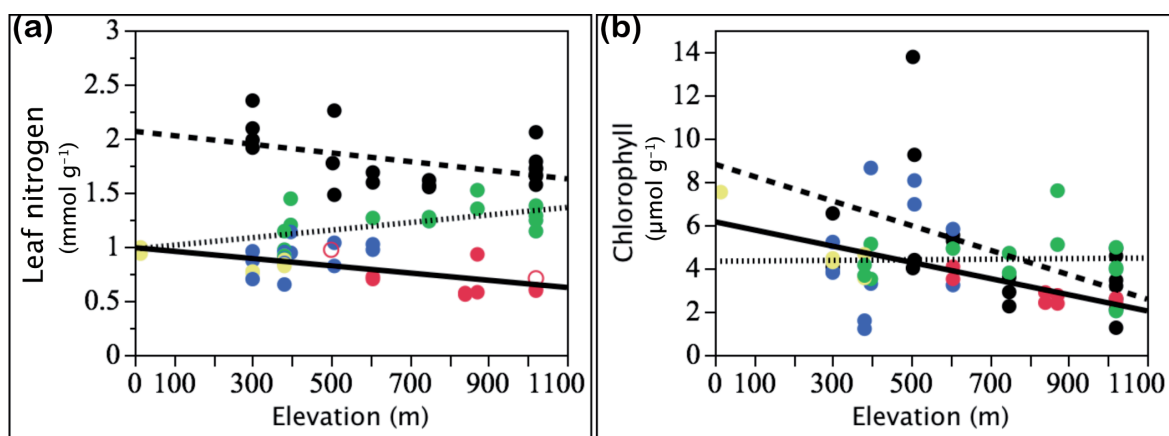
	Leaf position	<i>Cecropia schreberiana</i>	<i>Dacryodes excelsa</i>	<i>Manilkara bidentata</i>	<i>Micropholis garciniiifolia</i>	<i>Prestoea montana</i>	All
Sample size	Upper	20	14	8	7	14	63
	Lower	14	14	10	9	20	67
LMA (g m ⁻² leaf)	Upper	71.5 (7.5) a*	114.4 (9.4) b*	140.7 (15.0) b	252.8 (8.8) c	119.6 (16.8) b	139.8 (5.5)
	Lower	46.7 (8.6) a	84.5 (9.4) b	133.9 (10.7) c	249.4 (10.7) d	116.7 (11.8) c	126.2 (4.6)
Chlorophyll (μmol g ⁻¹ leaf)	Upper	5.23 (0.41) a*	5.21 (0.52) a*	5.58 (0.82) a	2.95 (0.49) b	2.82 (0.93) b*	4.36 (0.30)
	Lower	7.29 (0.47) a	7.74 (0.52) a	6.48 (0.59) a	3.72 (0.59) b	7.37 (0.65) a	6.52 (0.25)
Chlorophyll (μmol m ⁻² leaf)	Upper	280 (45.0) a	512 (56.1) bd	797 (89.9) c	740 (52.9) c*	359 (101) ad*	537 (32.4)
	Lower	325 (51.6) a	669 (56.1) bd	832 (63.9) c	893 (63.9) c	764 (70.9) cd	697 (27.6)
Chl a/Chl b	Upper	3.79 (0.16) a*	2.13 (0.20) b	1.80 (0.32) b	3.36 (0.19) cd	3.28 (0.36) ad*	2.86 (0.12)
	Lower	3.29 (0.21) a	2.05 (0.29) b	1.74 (0.27) b	2.64 (0.26) ab	2.14 (0.29) b	2.37 (0.12)
Nitrogen (mmol g ⁻¹ leaf)	Upper	1.80 (0.05) a	0.98 (0.06) b	0.79 (0.09) c	0.67 (0.05) c	1.11 (0.10) b	1.07 (0.03)
	Lower	1.75 (0.05) a	0.92 (0.06) b	0.72 (0.07) c	0.57 (0.07) c	1.23 (0.07) d	1.04 (0.03)
Nitrogen (mmol m ⁻² leaf)	Upper	125 (7.6) a*	112 (9.5) a*	106 (15.2) a	163 (9.0) b	133 (17.0) a	128 (5.5)
	Lower	82 (8.7) a	78 (9.5) a	93 (10.8) a	146 (10.8) b	140 (12.0) b	108 (4.7)
Chl/N (mmol mol ⁻¹)	Upper	2.85 (0.38) a*	5.32 (0.48) b*	8.00 (0.77) c	4.45 (0.45) bd*	2.83 (0.86) ad*	4.69 (0.28)
	Lower	4.12 (0.44) a	8.35 (0.48) b	9.55 (0.54) b	6.32 (0.54) c	5.99 (0.60) c	6.87 (0.23)

N concentrations per unit leaf area and low chl/N ratios (Table 2).

When leaves of all species sampled over the elevation gradient were plotted individually, significant correlations between LMA and leaf nitrogen content became apparent (Fig. 2); Narea was positively correlated with LMA, while Nmass was negatively correlated with LMA. Across the elevation gradient, increases in LMA with elevation were more pronounced for *Dacryodes*, *Manilkara* and *Micropholis* than for *Cecropia* or *Prestoea* (Fig. 3c). Mass-based nitrogen and chlorophyll concentrations decreased significantly with elevation (Fig. 3a–b), but these decreases were offset by increases in LMA (Fig. 3c) such that area-based values either did not change significantly (as for chlorophyll, results not shown) or even increased with elevation, as was the case for nitrogen (Fig. 3d). Leaf properties of *Prestoea* did not change significantly with elevation.

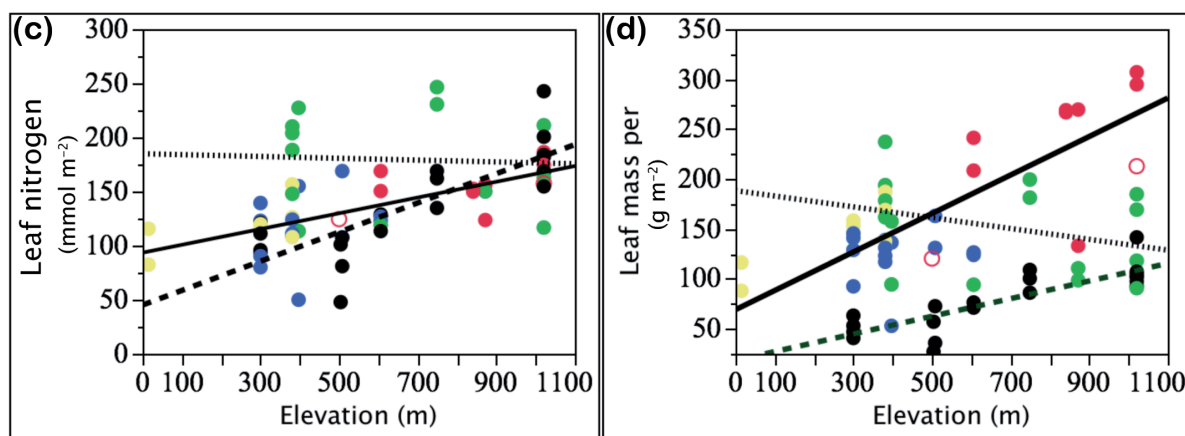
Functional responses

Modeled light response curves (Eq. 1) plotted against field data of leaf CO₂ exchange for each species were used to obtain parameter estimates from Eq. 1, and are listed in Table 3. Among different species, Amax values were highest for *Cecropia* and lowest for *Prestoea*, while rates for the three late-successional species *Dacryodes*, *Manilkara* and *Micropholis* were relatively similar (Table 3). *Dacryodes* and *Manilkara* inhabit similar locations in the LEF, but leaves of *Manilkara* are more shade-tolerant than *Dacryodes* as reflected by lower rates of dark respiration in upper canopy leaves, lower chl a/b ratios and higher chl/N ratios (Table 2). Rates of dark respiration (Rd) across all species varied from 1.52 μmol m⁻² s⁻¹ for upper canopy leaves of *Cecropia* to 0.2 μmol m⁻² s⁻¹ for lower canopy leaves of *Manilkara*, *Dacryodes* and *Prestoea*. These rates translated into leaf res-



(1) $n = 20$; $R^2 = 0.22$; $p = 0.04$
 (2) $n = 15$; $R^2 = 0.27$; $p = 0.04$
 (3) $n = 31$; $R^2 = 0.31$; $p = <0.001$

(1) $n = 18$; $R^2 = 0.26$; $p = 0.03$
 (2) $n = 13$; $R^2 = -0.09$; $p = 0.93$
 (3) $n = 26$; $R^2 = 0.26$; $p = 0.008$



(1) $n = 20$; $R^2 = 0.67$; $p = <0.001$
 (2) $n = 15$; $R^2 = -0.07$; $p = 0.85$
 (3) $n = 29$; $R^2 = 0.38$; $p = <0.001$

(1) $n = 20$; $R^2 = 0.70$; $p = <0.001$
 (2) $n = 15$; $R^2 = -0.10$; $p = 0.24$
 (3) $n = 30$; $R^2 = 0.60$; $p = <0.001$

(1) - - ● *Cecropia schreberiana*; ● *Manilkara bidentata*;
 ● *Dacryodes excelsa*;
 (2) ||||| ● *Prestoea montana*
 (3) — ● *Micropholis garciniifolia*;
 ○ Medina et al. 1981

Figure 3. Elevational trends in leaf structural properties of upper canopy leaves of the species studied along an elevation gradient in the Luquillo Experimental Forest. Also shown are data from Medina et al. (1981) collected in the same forest.

Table 3. Summary of parameter estimates from the nonlinear mixed model analysis. The light saturation point was estimated from the model as the lowest light value at which the rate of photosynthesis fell within 1% of the estimated light-saturated photosynthetic rate (A_{\max}). Estimated leaf respiration was calculated at zero PFD and converted to a percentage of A_{\max} .

	Leaf position	<i>Cecropia schreberiana</i>	<i>Dacryodes excelsa</i>	<i>Manilkara bidentata</i>	<i>Micropholis garciniifolia</i>	<i>Prestoea montana</i>
A_{\max}						
($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Upper	12.78 (0.2)	7.29 (0.2)	7.25 (0.3)	5.62 (0.3)	4.09 (0.2)
	Lower	7.81 (0.2)	3.94 (0.1)	4.32 (0.1)	2.42 (0.1)	2.32 (0.1)
Light compensation point						
(PFD)	Upper	28 (4.1)	24 (6.1)	12 (9.5)	20 (7.7)	17 (6.7)
	Lower	14 (6.0)	7 (7.2)	4 (4.8)	16 (5.4)	6 (4.5)
Light saturation point						
(PFD)	Upper	1176	849	784	668	615
	Lower	739	557	442	331	295
Dark respiration						
($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Upper	1.52	1.06	0.52	0.88	0.57
	Lower	0.73	0.24	0.2	0.63	0.21
Respiratory cost						
(% of A_{\max})	Upper	12	15	7	16	14
	Lower	9	6	5	26	9

piratory costs ranging from 5 to 26% of maximum photosynthetic capacity (Table 3).

Rates of light-saturated photosynthesis (A_{\max}) were significantly higher ($p < 0.0001$) in upper canopy leaves relative to lower canopy leaves, and photosynthesis saturated at higher light levels (Table 3) in upper vs lower levels. Table 4 shows significant correlations between N_{area} and A_{\max} as well as N_{area} and R_d for 4 of the 5 species measured.

Mean photosynthetic nitrogen-use efficiency (PNUE), or the amount of CO_2 fixed per unit N accumulated in leaves at a given time, is a useful metric that relates leaf structure to leaf function. PNUE was highest in *Cecropia* ($115.5 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$), intermediate in *Dacryodes* and *Manilkara* (72.0 and $57.1 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$, respectively) and lowest in *Micropholis* and *Prestoea* (26.3 and $27.8 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$, respectively). Differences in PNUE were significant among species but not between upper and lower canopy leaves. Across all species, PNUE was negatively correlated to both LMA (Fig. 4a) and to N_{area} (Fig. 4b).

Discussion

Leaf structure and function

During a plant's growth, its carbon economy is connected closely with its nitrogen economy. This association is generally attributed to the large amount of leaf organic nitrogen present in the photosynthetic machinery within the chloroplasts of leaves. Alternatively, Kull (2002) argues that this relationship appears because a certain amount of energy must be captured through photosynthesis to maintain the nitrogen within a leaf. Either way, the correlation between a leaf's light-saturated rate of photosynthesis and its nitrogen content has been shown by many authors to exist, first on a biochemical basis (Medina 1970, 1971) and later based on gas exchange measurements (Field and Mooney 1986, Reich et al. 1994, Anten et al. 1995, Bassow and Bazzaz 1997). Recently we have gained a more mechanistic understanding of this 'leaf economics spectrum' (Reich et al. 1998, Wright et al. 2004, 2005, Shipley

Table 4. Regressions between light-saturated photosynthesis (Amax) and dark respiration (Rd) both per unit area ($\mu\text{mol m}^{-2} \text{s}^{-1}$), and leaf nitrogen concentration per unit area (mmol m^{-2}) for five tree species in the Luquillo Experimental Forest. Significant regressions indicated by asterisks (* $p < 0.065$, ** $p < 0.01$)

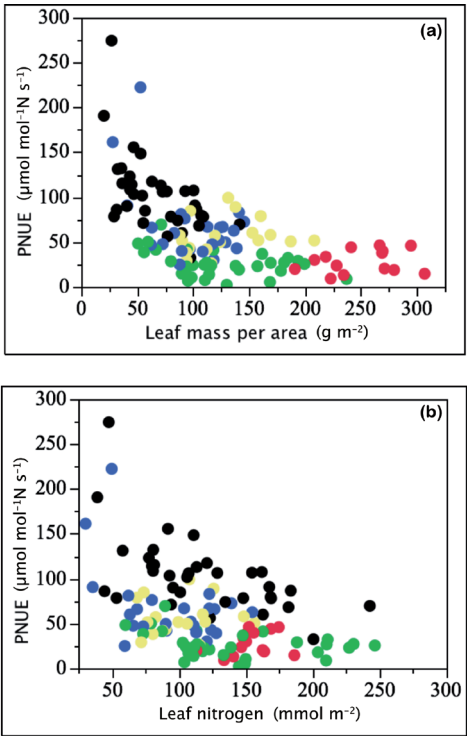
Species	Intercept	Amax			Rd	
		Slope	R ²		Slope	R ²
<i>Cecropia schreberiana</i>	6.12	0.042	0.32**		0.58	0.17*
<i>Dacryodes excelsa</i>	2.41	0.036	0.22**		0.20	0.15
<i>Manilkara bidentata</i>	-0.43	0.061	0.34**		0.008	0.59**
<i>Micropholis garciniifolia</i>	-3.80	0.054	0.19		0.013	0.41**
<i>Prestoea montana</i>	1.00	0.017	0.20*		0.006	0.59**

et al. 2006) of correlated leaf traits that affect global patterns of primary productivity. The worldwide database is extensive, but only a few of the data points are from tropical regions or from secondary forests. The data presented here therefore provide a valuable addition to the database.

Across all species combined, Amax and Rd rates were related to leaf nitrogen concentration more strongly on a

mass basis than on an area basis, as has been shown previously by Field and Mooney (1986) and Wright et al. (2004). However, these mass-based correlations were influenced strongly by *Cecropia*, while variation in Nmass among the other species measured was relatively low. Within an individual species, Amax-N correlations were stronger on an area basis, as indicated by significant correlations for 4 of the 5 species measured for both Amax-Narea and Rd-Narea relationships (Table 4). One possible explanation for this finding is that the species measured in this study may be more plastic with respect to light availability as reflected by LMA than to Nmass.

Upper and lower canopy leaves of each species had similar Nmass values (Table 2), and the observed variation in Narea among both species and leaf positions was due mainly to variation in LMA (Fig. 2) rather than the relatively small change in Nmass within the canopy or across the elevation gradient (Table 2, Fig. 3a). Across all species, lower canopy leaves had high chlorophyll concentrations per unit weight and chlorophyll to nitrogen (Chl/N) ratios, which reflect relatively higher nitrogen investment in chlorophyll production for light harvesting in shady environments. Nmass was not significantly different between upper and lower canopy positions (Table 2), but shade-adapted leaves had lower Narea and Chl a/b ratios (Table 2), which may allow them to capture the deficient red light in the forest shade more efficiently (Björkman 1981). In contrast, leaves growing in high light environments at the top of the canopy were thicker, likely as a result of excess incident light energy and increased mesophyll cell density (Witkowski and Lamont 1991). These leaves also had high Narea and low Chl/N ratios (Table 2; Seeman et al. 1987, Terashima and Evans 1988, Sims et al. 1998, Poorter et al. 2000). Since light is rarely limiting for these sun-adapted leaves, more nitrogen is invested in carboxylation enzymes such as Rubisco or other Calvin cycle enzymes and electron carriers (Terashima and Evans 1988, Evans 1989, Hikosaka and Terashima 1995) rather than in light harvesting components. This partitioning allows sun-adapted leaves to achieve high rates of light-saturated photosynthesis per unit leaf area for a given Narea.



● *Cecropia schreberiana*; ● *Manilkara bidentata*;
● *Prestoea montana*; ● *Dacryodes excelsa*;
● *Micropholis garciniifolia*

Figure 4. Photosynthetic nitrogen-use efficiency (PNUE) as a function of (a) leaf mass per area (LMA) and (b) area-based leaf nitrogen concentration for leaves of five tree species in the Luquillo Experimental Forest.

The significant trends in leaf structure that were apparent along the elevation gradient in our study (Fig. 3) are corroborated by results of Tanner et al. (1998), who summarized a general trend of decreasing Nmass with increasing elevation using data collected from several wet tropical mountain sites. Results also confirm site-specific results of Medina et al. (1981), who showed that leaf Nmass decreases and Narea increases with increasing elevation in the Luquillo Mountains. Since their dataset consisted of only two elevation points (500 and 1000 m) while ours spanned a broader elevational range (15 to 1021 m), our results increase the statistical power of this trend (Fig. 3).

Evolutionary tradeoffs in the Luquillo Mountains

There is strong evidence that nitrogen is the most limiting resource in montane tropical rain forests (Tanner et al. 1998) such as at high elevations in the LEF. Even under non-limiting conditions, nitrogen acquisition involves considerable energy costs (Field 1988). Therefore, how a plant uses its nitrogen resources is believed to affect its overall fitness (Field and Mooney 1986). High PNUE is associated with a high relative growth rate (Poorter et al. 1990) and a faster turnover of plant parts that allows for flexible response to the spatial patchiness of light and soil resources (Grime 1994), so species with high PNUE leaves (such as *Cecropia*) should outcompete species with low PNUE leaves (such as *Micropholis* or *Prestoea*) (Fig. 4). Why, then, should species with low-PNUE leaves ever be selected for?

There is an inherent trade-off in nitrogen allocation between leaf photosynthesis and leaf longevity. A leaf cannot maximize both PNUE and leaf life span simultaneously; low-PNUE leaves generally have longer life spans (Wright et al. 2004). To achieve high PNUE, more nitrogen is invested in photosynthetic structure and leaves have thin cell walls to maximize CO₂ diffusion (Hikosaka 2004). In contrast, species with longer leaf life spans produce tougher, thicker leaves (Reich et al. 1991, Wright and Cannon 2001, Wright and Westoby 2002) by allocating more nitrogen to the production of leaf proteins but at the expense of lower PNUE (Fig. 4, Reich et al. 1991, Onoda et al. 2004, Takashima et al. 2004). These thicker, longer-lived leaves may also contain more secondary defense compounds (such as alkaloids) and have extended mean residence time for nutrients (Eckstein et al. 1999, Aerts and Chapin 2000), which could permit a progressively greater share of nitrogen pools in these species (Aerts and van der Peijl 1993). This is especially important in nitrogen-limiting environments, as the slow decomposition of high-LMA leaf litter may restrict opportunities for potentially fast-growing competitors (Cornelissen et al. 1999).

What does this apparent tradeoff between leaf photosynthesis vs leaf longevity indicate in the context of species

distribution in the Luquillo landscape? Optimal leaf traits differ along the elevational gradient in response to resource availability, herbivory and frequency of disturbance. The mountain peaks of the LEF are characterized by saturated soils with low nutrient availability from poor soil development and slow decomposition. Frequent cloud cover results in low light availability and an atmosphere with a low saturation deficit (Medina et al. 1981, Weaver et al. 1986, Weaver and Murphy 1990, Silver et al. 1999). Due to limitations by these environmental factors, tree species that inhabit these environments have slow growth rates (Weaver et al. 1986), low leaf area (Weaver and Murphy 1990) and low rates of new leaf production. Therefore, any new leaves that are produced must persist for a relatively long time. Therefore, species present at high elevations in the LEF, such as *Micropholis* and *Prestoea*, invest more mass into cell walls and accumulate excess carbohydrate (i.e. they have high construction costs), leading to a high LMA (Fig. 3d) and a low PNUE (Fig. 4). Even within a single species with a wide range distribution (*Cecropia*), leaves at high elevations had twice the leaf mass per area than leaves measured at lower elevations (102 vs 51 g m⁻² at 1021 and 300 m elevation, respectively), suggesting that different leaf properties may be selected for in locations with different environmental conditions. Plants can achieve these properties either by being selected for in a genetic sense or through a plastic response to environment without any genetic changes (Fetcher et al. 2000). On the other hand, pioneer species that inhabit disturbed environments, such as *Cecropia*, must achieve high growth rates (mean growth rate = 9.87 cm cm⁻¹ yr⁻¹ basal diameter, Myster 2002) to outcompete other seedlings competing for the same high light environment. Therefore, leaves of this species have higher PNUE (Fig. 4) but the large leaves are highly susceptible to attack by herbivores (up to 11% loss of leaf area per month, Myster 2002). Species with intermediate PNUE, such as *Dacryodes* and *Manilkara* (Fig. 4), dominate low to mid-elevations where net primary production is higher (Wang et al. 2003, Harris et al. 2013). This correlation provides leaf-level support for H. T. Odum's maximum power hypothesis (Odum and Pinkerton 1955), which states that the production of useful energy is highest when systems (made up of leaves, in this case) operate at intermediate efficiencies.

Conclusion

In summary, we have shown quantitatively that both leaf structure and function differ in response to environmental conditions that vary vertically through the canopy as well as across an elevation gradient. We have demonstrated the utility of tree climbing for physiological studies and have compiled extensive gas exchange data for future comparisons. We have also attempted to explain physiological differences in the context of well-known adaptive physiologi-

cal strategies. Since leaf structural and chemical parameters are often easier to measure than CO₂ exchange for large numbers of leaves and over large spatial regions (Cavaleri et al. 2010), the correlations between structure (leaf mass per area, leaf nitrogen concentration) and function (A_{max}, R_d) presented in this study may be used to parameterize ecosystem carbon models and/or to validate remotely sensed imagery.

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