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Trait-based scaling of temperature-dependent foliar respiration in a species-rich tropical forest canopy

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Summary

1. The scarcity of empirical data on leaf respiration (R) and its temperature sensitivity (e.g. Q_{10}, defined as the proportional increase in R per 10 °C warming) causes uncertainty in current estimates of net primary productivity of tropical forests.

2. We measured temperature response curves of R on 123 upper-canopy leaves of 28 species of trees and lianas from a tropical forest in Panama and analysed variations in R and Q_{10} in relation to other leaf functional traits.

3. Respiration rates per leaf area at 25 °C (R_A) varied widely among species and were significantly higher in trees than in lianas. R_A was best predicted by a multiple regression model containing leaf phosphorus concentration, photosynthetic capacity and leaf mass per area ($r^2 = 0{\cdot}64$). The mean Q_{10} value (2·4) was significantly higher than the commonly assumed value of 2·0. Q_{10} was best predicted by the combination of leaf carbohydrate concentration and growth form (trees vs lianas) ($r^2 = 0{\cdot}26$).

4. The night-time leaf respiratory carbon flux from this tropical forest was calculated from these multiple regression models to be 4·5 Mg C ha^{-1} $year^{-1}$, with an estimated additional 2·9 Mg C ha^{-1} $year^{-1}$ being released by respiration during the day.

5. Trait-based modelling has potential for estimating R, thus facilitating carbon flux estimation in species-rich tropical forests. However, in contrast to global analyses, leaf phosphorus content was the most important correlate of R and not leaf nitrogen, so calibration of trait models to the tropics will be important. Leaf traits are poor predictors of Q_{10} values, and more empirical data on the temperature sensitivity of respiration are critically needed to further improve our ability to scale temperature-dependent respiration in species-rich tropical forests.

Key-words: carbon flux, climate change, gas exchange, leaf functional traits, NPP, Panama, Q_{10}, temperature response of respiration

Introduction

Tropical forests account for more than one-third of global terrestrial gross primary productivity (GPP) (Beer *et al.* 2010), but 30% of the photosynthetically fixed carbon (C) is released back into the atmosphere by leaf respiration (R) (Chambers *et al.* 2004; Malhi 2012). Global rise of temperature, especially during night-time (Easterling *et al.* 1997) may have major impacts on net primary productivity (NPP = GPP – autotrophic respiration), as autotrophic respiration increases with temperature. Tropical forests are likely to experience unprecedented warming within the next two decades (Diffenbaugh & Scherer 2011), but lack of empirical data on R and its temperature sensitivity hinders efforts to reliably model current and future carbon fluxes in tropical forests (Malhi *et al.* 2009).

R of canopy trees is challenging to measure, especially in tall and diverse tropical forests. Eddy covariance techniques capture nocturnal respiration fluxes poorly (Goulden *et al.* 1996; Lavigne *et al.* 1997) and do not allow for straightforward partitioning of ecosystem respiration to its component sources. Thus, more direct measurements of respiration are necessary to estimate forest canopy respiration. In tropical forests, R accounts for *c.* 50% of total autotrophic respiration (Chambers *et al.* 2004; Malhi 2012), and R is highest at the top of the canopy where leaves are exposed to full sun (Meir, Grace & Miranda 2001; Cavaleri, Oberbauer & Ryan 2008). Therefore, to

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quantify the leaf respiratory carbon flux from tropical forests, fully exposed upper-canopy leaves should be measured. Such data at the species level are very scarce considering the diversity and importance of tropical forests, and the uncertainty associated with R flux estimates is consequently large (Malhi *et al.* 2009). Furthermore, to calculate the respiratory carbon flux from forest canopies, both R at a set temperature [e.g. R at 25 °C (R_{25})] and the short-term temperature sensitivity of R need to be known. R is generally assumed to double with 10 °C temperature increase; that is, it has a Q_{10} (the proportional increase in R with 10 °C warming) of 2·0. Warming for extended periods can result in acclimation of R, including in tropical species (Slot *et al.* 2014), so the Q_{10} cannot be used to predict future R fluxes, but is essential for calculation of current fluxes. Available data from tropical forests suggest that R and Q_{10} differ widely among species (Meir, Grace & Miranda 2001; Slot, Wright & Kitajima 2013) and growth forms (Cavaleri, Oberbauer & Ryan 2008), but not enough data are currently available to identify systematic patterns in Q_{10} among tropical forest trees and lianas.

The leaf economics spectrum (LES; Wright *et al.* 2004) describes general correlations among photosynthesis and R, and leaf structural and chemical properties, including leaf mass per unit leaf area (LMA), leaf longevity, and concentration of nitrogen (N) and phosphorus (P). Because these structural and chemical traits are easier to determine reliably than photosynthesis and respiration, these trait correlations are useful for estimating C flux in species-rich forest canopies. Further, in many global vegetation models, R is defined as a fixed fraction of maximum photosynthesis (e.g. HyLand, Levy, Cannell & Friend 2004; IBIS, Foley *et al.* 1996; LPJ, Sitch *et al.* 2003), or as a function of leaf N (e.g. HYBRID, Friend *et al.* 1997; NCAR LSM, Bonan *et al.* 2003). What is less certain is whether correlations with other leaf traits can be identified for the temperature sensitivity of R, for example for the Q_{10}, which is widely used in C flux models.

Our ability to estimate R fluxes from forest canopies would greatly improve if the Q_{10} could be captured by a simple trait-based model in the way that R can be modelled from N or photosynthesis. Observational evidence suggests that Q_{10} values vary among species and with environmental conditions (Griffin, Turnbull & Murthy 2002; Slot, Zaragoza-Castells & Atkin 2008). It remains unknown whether this variation is predictable. The physiological factor that limits the rate of R changes with temperature (Atkin & Tjoelker 2003). At low temperature, the respiratory capacity is most limiting (because enzymatic reaction rates are low), while at high temperature, enzymatic reaction rates are high and R is thus more likely to be limited by the availability of respiratory substrate (primarily carbohydrates), or by the demand for respiratory product [e.g. ATP: when the demand for ATP is low, the rate of respiratory electron transport becomes limited by the availability of ADP (Noguchi & Terashima 1997)]. Two species with the same respiratory capacity but different substrate availability or demand for respiratory products are thus likely to have different temperature response curves. Furthermore, the factor that controls R may differ among species in relation to their plant functional type (PFT). For example, R in fast-growing, light-demanding species is more likely to be limited by substrate availability (because fast growth guarantees high demand for respiratory products), while slow-growing, shade-tolerant species are more likely to be limited by the demand for respiratory products (Noguchi & Terashima 1997). If species differ in the factor that controls R based on their respiratory substrate content and their PFT, it is likely that variation in Q_{10} values can be explained by traits associated with respiratory control and life history.

Here, we set out to (i) quantify respiration at a set temperature of 25 °C and temperature sensitivity of respiration for upper-canopy leaves of 28 common tropical forest trees and lianas, (ii) statistically explain interspecific variation in respiration 25 °C and Q_{10} in relation to leaf chemical and structural traits and (iii) estimate the annual flux of respiration at the stand level in our study forest, using trait-based models identified under the second objective. We chose to use R_{25} because it is widely used for comparison of respiration rates of plants from different biomes, and 25 °C is very close to the ambient night-time temperature at our study site. We previously measured R *in situ*, using changing ambient temperatures in the morning to estimate Q_{10} values at the species level (Slot, Wright & Kitajima 2013). In the current study, we utilized the greater degree of temperature control and greater accuracy of respiration measurements achieved with laboratory measurements to obtain leaf-level Q_{10} values. In line with Slot, Wright and Kitajima (2013), we hypothesized that R_{25} and Q_{10} would be high compared to published values from other tropical forests, and thus that the stand-level flux would be high. We further hypothesized that traits associated with the global LES would also be important at the local scale, such that R_{25} per unit leaf area (R_A) would scale positively with photosynthetic capacity, LMA and concentrations of N and P, whereas species differences in Q_{10} – not considered in the LES – would correlate with variation in traits associated with respiratory control and life history, such as concentrations of N and soluble carbohydrates, and PFT. We focused on area-based leaf traits as they are thought to be biologically more relevant than mass-based traits (Lloyd *et al.* 2013; Osnas *et al.* 2013), and area-based traits can be scaled to the canopy using leaf area index (LAI; total leaf area per unit ground area) as a scalar.

Materials and Methods

STUDY SITE

The study was conducted in Parque Natural Metropolitano (PNM, 8°59′N, 79°33′W, 100 m a.s.l.), a semideciduous moist tropical forest near Panama City on the Pacific coast of the Republic of Panamá. Mean annual temperature at the site is 26·3 °C, and annual rainfall averages 1740 mm, most of which falls during the rainy season (May through December). The park

is a 256-hectare natural reserve consisting of 80- to 150-year-old secondary forest with tree heights up to 40 m. The soil is classified as an ultisol/ustult (Santiago, Schuur & Silvera 2005). A 42-m-tall construction crane with a 51-m jib enables access to canopy leaves. Twenty-eight species were selected from the upper forest canopy, representing a mix of PFTs based on growth form (trees vs lianas) and successional status; lianas (14 species), and trees classified as early-successional (five species), mid-successional (seven species) and late-successional (two species) (Table 1). Together, these 28 species cover >75% of the canopy area in reach of the crane (Avalos & Mulkey 1999).

RESPIRATION MEASUREMENTS

For each species, three to five sun-exposed terminal shoots were selected, where possible from multiple individuals (mean 1·6, range 1–4). Twigs were collected pre-dawn at *c*. 6 a.m., cut under water, or cut and immediately recut under water, and brought back to the laboratory in darkness and stored at *c*. 18 °C until measured. *R* was measured on 3–8 whole, mature leaves per species, in a Walz gas-exchange cuvette with Peltier temperature control (GWK 3M, Walz, Mess- und Regeltechnik, Eiffeltrich, Germany), connected with a LI-6252 infrared gas analyzer (Licor, Lincoln, NE, USA) operating in open-flow mode. Starting at 20 °C, the temperature was increased in steps of 2 or 3 °C to 32 °C, and *R* and leaf temperature (measured abaxially with a thermocouple wire) were recorded at each step. Mean annual night-time temperature at the site is 24·5 °C, so the temperature range used brackets environmentally relevant temperatures. Petioles were cut under water and sealed with Parafilm in a 5-ml glass vial with water to prevent the leaves from drying out during the measurement. These 5–7 *R* measurements per leaf took *c*. 60–90 min to complete. All leaves were measured within 10 h of collection. No trend in respiration rates with time since collection was detected within this period. Prior to collection of leaves, we measured *R in situ* with an LI-6400 (Licor) at 25 °C for all tree species and eight of the liana species. *In situ* and laboratory-obtained R_{25} values were not significantly different (paired *t*-test. $P > 0{\cdot}05$ for all 22 species. Data not shown). Measurements were made in the wet season, between late August and early October, when all selected species had mature but non-senescing leaves.

FUNCTIONAL TRAIT DATA

Leaf area was measured with a LI-3000 leaf area metre (Licor), and leaves were dried at 60 °C to a constant mass. Leaf N was determined with an elemental analyzer (Costech Analytical, Los Angeles, CA, USA). Concentrations of soluble carbohydrates (simple sugars) and starch were determined following Dubois *et al.* (1956) with modifications. In short, simple sugars were extracted in 80% (v/v) ethanol by shaking at 27 °C, followed by two incubations at 30 °C of 2 h each. For each sample, the supernatant from the three incubations was combined in a volumetric flask and brought to 10 ml. Glucose concentrations were determined colorimetrically at 487 nm via the phenol–sulphuric acid method. Starch was hydrolysed to glucose from the pellet in 1·1% hydrochloric acid at 100 °C. Starch concentrations were determined as glucose equivalents. Summing the concentrations of simple sugars and starch gives total non-structural carbohydrates (TNC).

We measured light-saturated photosynthetic capacity (A_{sat}) *in situ* on a separate set of 3–6 sun-exposed, upper-canopy leaves per species at a saturating irradiance of 1200 μmol m^{-2} s^{-1}, and 400 p.p.m. CO_2 at ambient temperature (range 26–29 °C) with an LI-6400. Relative humidity in the cuvette was controlled to 70–90% resulting in leaf-to-air vapour pressure difference of 1·1 ± 0·8 kPa. Photosynthesis was measured before midday stomatal closure was observed, and intercellular CO_2 concentration was never below 285 p.p.m. Species means of foliar P were collected previously at our study site, along with N, A_{sat} and LMA (S. Joseph Wright, unpublished data). Species means for N, A_{sat} and LMA in this independent data set correlated strongly with the species means determined in the current study ($r^2 > 0{\cdot}65$ for each trait), so we felt confident using the P data previously collected at this site.

QUANTIFICATION OF *R* AND Q_{10}

For each leaf, a linear regression line was fit to the $\log_{10}$-transformed leaf *R* vs. leaf temperature (T_{Leaf}) according to:

$$\log_{10}(R) = a + bT_{Leaf} \quad \text{eqn 1}$$

where *a* and *b*, respectively, the intercept and the slope of the response curve, are leaf-specific constants. The data fit to this equation were strong for all leaves (mean $r^2 = 0{\cdot}98$, $r^2 > 0{\cdot}88$ for all leaves. See also Fig. S1, Supporting information). Q_{10} values were calculated from the slope of these equations as:

$$Q_{10} = 10^{10b} \quad \text{eqn 2}$$

Subsequently, R_{25} was calculated for each of the 5–7 set cuvette temperatures of each leaf as:

$$R_{25} = \frac{R_{T_{Leaf}}}{Q_{10}^{(T_{leaf}-25)/10}} \quad \text{eqn 3}$$

where $R_{T_{leaf}}$ is *R* at the T_{Leaf}. We averaged these 5–7 R_{25} values to get one leaf-level R_{25} for our analyses.

MULTIPLE REGRESSION ANALYSES

Relationships of R_{25} and Q_{10} with other traits were examined with bivariate correlation and multiple regression models. We developed regression models for R_{25} and Q_{10} using leaf traits averaged at the species level, rather than using the leaf-level data to avoid pseudoreplication (as multiple leaves within a species are not independent data points). To identify the best combination of predictor variables, we used the subset selection method for multiple regression (Miller 2002) from the Leaps package, version 2.9 in the statistical analysis language R (R Development Core Team 2011). In this method, the best combination of predictors for a subset of *n* predictors is identified from all combinations of those predictors [using r^2, $r^2_{adjusted}$ and Mallows' C_p (Mallows 1973)], and this is repeated for all possible subset sizes. We developed models for R_A using functional traits expressed per unit area (subscript A), for R_{25} per unit mass (R_M) using traits expressed per unit leaf mass (subscript M), and for $Q_{10,}$ we used both area- and mass-based traits.

SCALING RESPIRATION FROM LEAF TO CANOPY

Our unpublished monitoring data at the study site show that leaf temperature (T_{Leaf}) is coupled closely to air temperature (T_{Air}) during the night across tree and liana species ($T_{Leaf} = 0{\cdot}99 * T_{Air}$, $r^2 = 0{\cdot}91$; T_{Leaf} measured abaxially with copper–constantan thermocouple wires, monitored 5–8 days for each of eight species). To estimate stand-level night-time *R* flux, we could therefore use a 17-year air temperature record from the site (collected at 25 m height on the crane at 60-minute (1995–2005) or 15-minute (2006–2011) intervals. http://biogeodb.stri.si.edu/physical_monitoring/research/metpark). We used four alternative approaches to scale *R* of canopy leaves to the stand level, which differed in how R_{25} and Q_{10} were estimated and averaged across species. In models 1A and

Table 1. Species codes, names, families, plant functional type (PFT. ES: early-successional, MS: mid-successional, LS: late-successional, L: lianas) and the number of leaves measured per species (n), dark respiration rate at 25 °C per unit area (R_A) and mass (R_M), leaf mass per unit area (LMA), photosynthetic capacity (A_{sat}), and concentrations of nitrogen (N), phosphorus (P) and total non-structural carbohydrates (TNC)

Code	Species	Family	PFT	n	R_A, μmol m^{-2} s^{-1}	R_M, nmol g^{-1} s^{-1}	Q_{10}	LMA, g m^{-2}	A_{sat}, μmol m^{-2} s^{-1}	N, %	P, %	TNC, mg g^{-1}
ALBG	*Albizia guachapele* (Kunth) Harms	Fabaceae	ES	4	0·76	8·2	2·50	94	12·4	3·8	0·10	159
ANNS	*Annona spraguei* Saff.	Annonaceae	ES	3	0·98	11·2	2·15	88	12·8	2·6	0·17	241
CECL	*Cecropia longipes* Pittier	Urticaceae	ES	3	1·82	19·5	2·20	94	20·6	2·5	0·20	155
CECP	*Cecropia peltata* L.	Urticaceae	ES	3	1·99	16·8	2·60	119	19·8	2·7	0·17	245
PITT	*Pittoniotis trichantha* Griseb.	Rubiaceae	ES	4	0·73	10·2	2·04	74	13·5	2·45	0·14	158
ASTG	*Astronium graveolens* Jacq.	Anacardiaceae	MS	3	1·27	12·9	2·15	98	12·6	2·4	0·20	160
CAS4	*Castilla elastica var. costaricana* (Liebm.) C.C. *Berg*	Moraceae	MS	6	1·23	11·2	2·19	110	19·4	2·6	0·17	207
FIIS	*Ficus insipida* Willd.	Moraceae	MS	9	1·44	10·8	2·30	133	23·4	2·8	0·17	161
LUE	*Luehea seemannii* Triana & Planch.	Tiliaceae	MS	6	0·92	8·5	2·40	108	19·4	2·1	0·15	191
PSES	*Pseudobombax septenatum* (Jacq.) Dugand	Malvaceae	MS	5	0·90	7·0	2·70	127	16·2	2·0	nd	188
SPOM	*Spondias mombin* L.	Anacardiaceae	MS	4	0·93	12·1	2·48	78	16·5	2·6	0·13	109
ZUEL	*Zuelania guidonia* (Sw.) Britt. & Millsp.	Salicaceae	MS	3	0·91	8·1	2·30	114	17·3	2·0	nd	164
ANAE	*Anacardium excelsum* Bertero & Balb. ex Kunth Skeels	Anacardiaceae	LS	8	0·97	8·1	2·24	121	13·8	1·8	0·14	166
CHRC	*Chrysophyllum cainito* L.	Sapotaceae	LS	4	0·63	5·1	2·28	124	17·1	1·9	0·10	177
AMPP	*Amphilophium paniculatum* (L.) kunth	Bignoniaceae	L	4	1·00	15·4	2·19	64	9·5	2·9	0·17	220
ARIC	*Aristolochia tonduzii* O.C. Schmidt	Aristolochiaceae	L	3	0·91	15·6	2·39	62	12·1	3·3	0·15	239
BONT	*Bonamia trichantha* Hallier f.	Convolvulaceae	L	7	0·73	10·0	2·93	74	10·4	2·3	0·18	266
CISE	*Cissus erosa* Rich.	Vitaceae	L	5	0·60	10·9	2·61	54	17·2	2·7	nd	210
COMF	*Combretum fruticosum* (Loefl.) Stuntz	Combretaceae	L	5	0·80	12·0	2·75	69	17·7	2·8	0·16	196
GOUL	*Gouania lupuloides* (L.) Urb.	Rhamnaceae	L	4	0·68	14·8	2·30	47	12·7	4·2	0·18	233
MIKL	*Mikania leiostachya* Benth.	Asteraceae	L	3	0·66	10·7	2·01	63	9·8	2·1	0·13	102
ODOM	*Odontadenia macrantha* (Willd. ex Roem. & Schult.) Markgr.	Apocynaceae	L	3	0·64	8·8	2·59	74	nd	2·1	0·09	128
PASV	*Passiflora vitifolia* Kunth	Passifloraceae	L	3	0·73	11·2	2·37	62	6·7	3·4	0·20	189
PHRC	*Phryganocydia corymbosa (*Vent.) Bureau ex K. *Schum.*	Bignoniaceae	L	3	0·76	6·9	2·53	110	12·2	3·2	0·15	94
PITC	*Pithecoctenium crucigerum* (L.) A.H. Gentry	Bignoniaceae	L	3	0·84	11·7	2·51	72	13·4	2·9	0·14	167

Table 1 (continued)

Code	Species	Family	PFT	*n*	R_A, μmol m^{-2} s^{-1}	R_M, nmol g^{-1} s^{-1}	Q_{10}	LMA, g m^{-2}	A_{sat}, μmol m^{-2} s^{-1}	N, %	P, %	TNC, mg g^{-1}
SERM	*Serjania mexicana* (L.) Willd.	Sapindaceae	L	4	0·64	9·0	2·56	71	12·9	2·9	0·15	177
STIH	*Stigmaphyllon hypargyreum* Triana & Planch.	Malpighiaceae	L	4	1·01	15·3	2·43	67	16·3	2·3	0·14	126
VITT	*Vitis tiliifolia* Humb. & Bonpl. ex Schult.	Vitaceae	L	7	0·83	14·2	2·22	59	13·0	2·4	0·13	158

nd: no data available.

1B, trait-based estimates of R_{25} and Q_{10} were used to assess the utility of the trait-based scaling approach when data are unavailable from direct measurements. In model 1A, R_{25} and Q_{10} were estimated at the species level from their multiple regression relationships with other leaf traits. Model 2A, in contrast, used species averages of *measured* R_{25} and Q_{10}. To assess the functionality of a PFT-level flux estimate, both models were also run after substituting species-level estimates with R_{25} and Q_{10} values modelled from leaf traits that were averaged by PFT (1B), or with measured R_{25} and Q_{10} values averaged by PFT (2B). In all models, CO_2 efflux from R was calculated by species for every 15- or 60-minute interval between 6 p.m. and 6 a.m. Where available, we used species-specific estimates of LAI (five species in Kitajima, Mulkey & Wright (2005) to calculate total CO_2 flux per unit ground area. For the remaining species, we used mean LAI per growth form from Clark *et al.* (2008).

R_A is lower in shade leaves than in sun leaves, and we used site-specific data to estimate the degree of reduction in R_A in relation to LAI of the trees. Transmittance of photosynthetically active radiation (PAR) decreases exponentially with LAI in the canopy of five of our focal tree species (Kitajima, Mulkey & Wright 2005) such that:

$$\%\mathrm{PAR} \approx 100e^{-0.41\times\mathrm{LAI}} \qquad \text{eqn 4}$$

($r^2 = 0{\cdot}50$) where LAI refers to the number of leaf layers that shade the focal leaf layer. R_A decreases linearly with a decrease in % daily PAR (Fig. S2, Supporting information) such that R_A of a shaded leaf is estimated as:

$$R_A[\%\ \text{of}\ R_{A\ \text{at full sun}}] = 22 + 0{\cdot}78 \times \%\mathrm{PAR} \qquad \text{eqn 5}$$

($r^2 = 0{\cdot}43$). Combining Eqns 4 and 5 gives:

$$R_A[\%\ \text{of}\ R_{A\ \text{at full sun}}] = 22 + 78e^{-0.41\times\mathrm{LAI}} \qquad \text{eqn 6}$$

Leaves in the second and third leaf layer of a tree canopy thus respire at 74% and 56% of leaves in the first (sun-exposed) layer, respectively.

Evergreen species were assumed to maintain LAI year-round, deciduous species had LAI = 0 in the 4-month dry season (January through April), and semideciduous species maintained LAI for 10 months. Because dry-season data were not available for the evergreen species in our data set, we kept R_A constant throughout the year. R_A was scaled to the stand level by determining the relative abundance of the canopy trees from their basal area in 2010 census data [collected by the Smithsonian Tropical Research Institute's Forest Dynamics Project (Condit 1998; Hubbell, Condit & Foster 2005; Condit *et al.* 2013)], and setting the sum of the abundance of the species we measured (83% of basal area) to 100%. Liana abundance was unknown at the species level, but lianas cover *c.* 30% of the crown area (Avalos & Mulkey 1999), so each liana species was assigned an abundance of 2·14% to add up to 30% total cover. For each calendar year, species-level R per hectare was summed up and multiplied by the molecular mass of C to calculate Mg C respired at night ha^{-1} $year^{-1}$.

Light inhibits R, so R_{Light} is lower than R_{Dark} at a given temperature (Sharp, Matthews & Boyer 1984). In the light, photosynthesis produces ATP and reducing agents, providing for some of the energy demands that in the dark are provided by R alone, and in the light, products of photorespiration directly inhibit the carbon flux into the Krebs cycle (Hurry *et al.* 2005). We estimated R during the day (R_{Day}) for saplings of four of our focal species. We determined R_{Light} using the Kok method (Sharp, *et al.* 1984) with an LI-6400 (Licor) and found an average reduction in respiration of 46% compared to R_{Dark} at a given temperature ($n = 17$ leaves). Light also reduces the $Q_{10,}$ but the extent of the reduction is variable and the mechanism is not clearly understood (Atkin *et al.* 2000; Pons & Welschen 2003). For calculation of $R_{Day,}$ we reduced the Q_{10} by 25% compared to the Q_{10} of R_{Dark}, taking a rough average of the degree of reduction reported in the literature. The C flux from R_{Day} was calculated using daytime air temperature data and a 46% reduction in R and 25% reduction in Q_{10} for all species. During the day, leaf temperature of sun leaves often exceeds air temperature, so leaf temperature, and thus R_{Day}, is likely to be slightly underestimated.

STATISTICAL ANALYSES

Comparisons among species, PFTs and growth forms were made using one-way ANOVAS and Tukey HSD *post hoc* tests, or unpaired, two-tailed Student's *t*-tests. Where necessary, data were transformed to improve normality and homoscedasticity. The variance in respiration and Q_{10} data was broken down to variance at the species, PFT and growth-form level using partial r^2 analysis. All statistical analyses were performed in R, version 2.14.1.

Results

RESPIRATION AT 25 °C

R_A differed significantly among species (Fig. 1; Table 1), exhibiting threefold variation from the early-successional tree *Cecropia peltata* (1·99 ± 0·06 μmol m^{-2} s^{-1}; mean ± SD) to the liana *Cissus erosa* (0·60 ± 0·15 μmol m^{-2} s^{-1}). R_A of lianas (0·77 ± 0·13 μmol m^{-2} s^{-1}) was lower than R_A of trees (1·11 ± 0·41 μmol m^{-2} s^{-1}: *t*-test, $P = 0{\cdot}007$). On average, lianas had lower R_A than

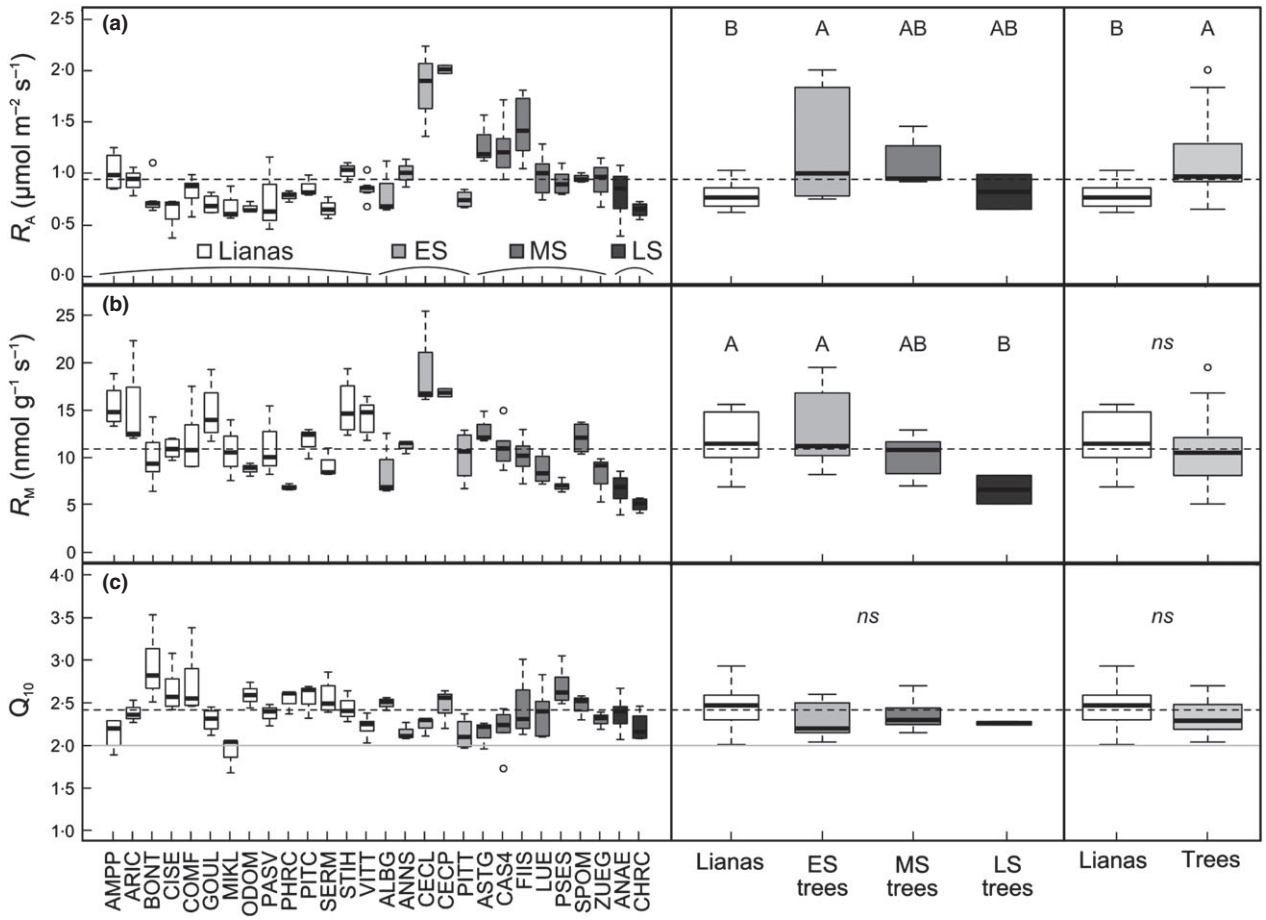

Fig. 1. Variation in respiration at 25 °C per unit leaf area (R_A) (a), and per unit leaf mass (R_M) (b), and Q_{10} (c) within and among species (species codes as in Table 1); within and among plant functional types (PFT), and within and between growth forms. The overall means are indicated by the dashed lines. In (c), the grey line indicates Q_{10} = 2·0, the value widely used in C flux models. The tree species are early-successional (ES, five species), mid-successional (MS, seven species) and late-successional (LS, two species). The box plots indicate the median, 25th and 75th percentile for each species, PFT and growth form. Whiskers extend to 1·5 times the interquartile range. Box plots for PFT and growth form are calculated from species means. Different letters indicate groups that are significantly different from one another ($P < 0·05$) (one-way analysis of variance with Tukey *post hoc* testing).

early-successional tree species, but the other PFTs were not significantly different from each other. R_{25} expressed on a mass basis (R_M) also differed widely among species and among PFTs. Lianas and early-successional tree species had significantly higher R_M than late-successional species, with mid-successional species showing intermediate values that were not significantly different from the other PFTs (Fig. 1).

Q_{10} VALUES BY SPECIES, PLANT FUNCTIONAL TYPE AND GROWTH FORM

Q_{10} values varied among species (mean 2·39, range 2·01–2·93), but did not differ systematically between trees (2·35 ± 0·18, mean ± SD) and lianas (2·45 ± 0·26) (Fig. 1). Different successional stages did not differ in mean Q_{10} values either, with early-successional (2·30 ± 0·19), mid-successional (2·36 ± 0·19) and late-successional species (2·26 ± 0·03) all falling within a narrow range. For all PFTs, Q_{10} values were significantly higher than commonly assumed value of 2·0 (*t*-test, $P < 0·05$ for all PFTs). For 19 of the 28 species, Q_{10} was also significantly greater than 2·0 at the species level.

VARIANCE OF RESPIRATION AND Q_{10}

Variation in R_A and R_M was considerable (Fig. 1), with leaf-level values ranging more than sixfold, and species means ranging threefold. Most of the variance existed among species (Fig. 2), but differences among leaves within species also explained 20–30% of the total variance. By comparison, Q_{10} values were less variable (leaf-level values ranged twofold and species means 1·5-fold). About 45% of the total Q_{10} variance could be attributed to variation in Q_{10} values among leaves within species plus measurement error, with a similar percentage of variance explained by Q_{10} differences among species within PFT (Fig. 2). LMA, in contrast, differed significantly between trees and lianas (trees 106 ± 19, lianas 68 ± 15;

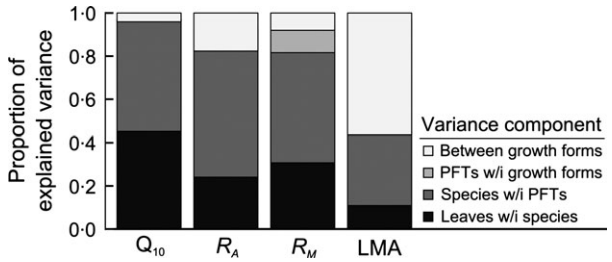

Fig. 2. Proportion of variance in Q_{10}, respiration at 25 °C per unit leaf area (R_A), per unit leaf mass (R_M) and leaf mass per unit leaf area (LMA) explained by variance within species, among species within plant functional type (PFT), among PFTs and between growth forms, as determined by partial r^2 analysis.

$P < 0{\cdot}001$. *t*-test), and growth form explained most of the variance in this trait.

TRAIT CORRELATIONS AND MULTIPLE REGRESSION MODELS FOR RESPIRATION AND Q_{10}

Significant positive pair-wise correlations were found between R_A and area-based $A_{sat\ A}$, N_A, P_A, total non-structural carbohydrates (TNC_A) and LMA (Table 2). These individual traits accounted for 21–55% of the explained variation in R_A. To avoid overfitting of the model, we chose to have maximally three predictors plus intercept in the model. The best three-parameter model for R_A based on Mallows' C_p contained the significant predictors P_A, A_{sat} and LMA:

$$R_A = 0{\cdot}14 + (7{\cdot}18 * P_A) + (0{\cdot}042 * A_{sat\,A}) - (0{\cdot}009 * LMA) \quad \text{eqn 7}$$

where R_A and A_{sat} are in μmol m^{-2} s^{-1}, P_A is in g m^{-2} and LMA in gram per square metre.

This model accounted for 64% of the variance in R_A in a subset of 24 species (12 tree, 12 liana species) for which data on all leaf traits were available (Table 3). LMA correlated negatively with the residual variance when P_A and $A_{sat\ A}$ were accounted for (Fig. 3), despite being a positive correlate of R_A in bivariate regression (Table 2). Phosphorus and A_{sat}, when expressed on a mass basis (R_M and $A_{sat\ M}$ in nmol g^{-1} s^{-1}, P in mg g^{-1}), constituted the best model for R_M:

$$R_M = -0{\cdot}14 + (6{\cdot}1 * P_M) + (0{\cdot}04 * A_{sat\,M}) \quad \text{eqn 8}$$

The best model for Q_{10} explained 26% of the variance and included TNC_A and growth form (Table 3):

$$\text{Lianas}: Q_{10} = 2{\cdot}21 + (0{\cdot}021 * TNC_A) \quad \text{eqn 9a}$$

$$\text{Trees}: Q_{10} = 2{\cdot}21 + (0{\cdot}021 * TNC_A) - 0{\cdot}281 \quad \text{eqn 9b}$$

where TNC_A is in gram per square metre. Models using mass-based traits and pair-wise correlations between Q_{10} and other leaf trait were not significant (Table 2), and models with more than two predictors included non-significant predictors.

Table 2. Pair-wise correlation of respiration at 25 °C per unit leaf area (R_A), per unit mass (R_M), and Q_{10}, with other traits: leaf phosphorus (P) and nitrogen (N) concentration, photosynthetic capacity (A_{sat}), leaf mass per unit area (LMA) and total non-structural carbohydrate concentration (TNC). R_A and Q_{10} are correlated with area-based P, N and TNC; R_M with mass-based P, N and TNC

	R_A				R_M				Q_{10}			
	Pearson's *r*	r^2	*P*	*n*	Pearson's *r*	r^2	*P*	*n*	Pearson's *r*	r^2	*P*	*n*
P	0·74	0·55	<0·001	24	0·53	0·28	0·02	24	0·11	0·01	ns	24
N	0·46	0·22	0·02	28	0·30	0·09	ns	28	0·06	0·00	ns	28
A_{sat}	0·57	0·32	<0·01	27	0·47	0·22	<0·01	27	0·08	0·01	ns	27
LMA	0·46	0·21	0·01	28	−0·42	0·17	0·03	28	0·02	0·00	ns	28
TNC	0·52	0·27	<0·01	28	0·24	0·06	ns	28	0·12	0·01	ns	28
Growth form	0·50	0·25	<0·01	28	0·09	0·03	ns	28	0·29	0·08	ns	28

Table 3. Parameter estimates of multiple regression analysis of R_A (μmol CO_2 m^{-2} s^{-1}), R_M (nmol CO_2 g^{-1} s^{-1}) and Q_{10}, against leaf phosphorus (P), photosynthetic capacity (A_{sat}), leaf mass per area (LMA: g m^{-2}), total non-structural carbohydrates (TNC) and growth form (lianas or trees), where: Response variable = Intercept + *a*P + *b*A_{sat} + *c*LMA + *d*TNC + *e*(Growth form). R_A is regressed on area-based leaf traits (P: g m^{-2}. A_{sat}: μmol m^{-2} s^{-1}); R_M on mass-based traits (P: mg g^{-1}. A_{sat}: nmol g^{-1} s^{-1}). In the Q_{10} model, TNC is expressed in gram per square metre. Number of species (*n*), model r^2 and *P* values of the models are also shown

		P	A_{sat}	LMA	TNC	Growth form			
Response variable	Intercept	*a*	*b*	*c*	*d*	*e*	*n*	r^2	*P*
R_A	0·14	0·72**	0·042*	−0·009*			24	0·64	<0·001
R_M	−5·14**	6·1**	0·04**				24	0·52	<0·001
Q_{10}	2·21***				0·021*	Lianas: 0 Trees: −0·28**	28	0·26	<0·05

*, ** and *** indicate significance of the variable with $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$.

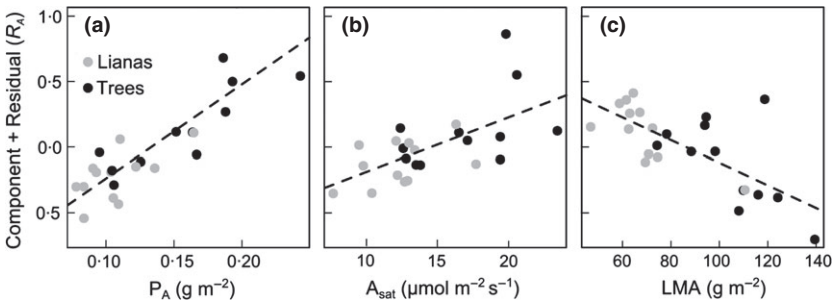

Fig. 3. Partial residual plots for the best three-trait regression model of R_A with (a) phosphorus content per unit area (P_A), (b) photosynthetic capacity (A_{sat}) and (c) leaf mass per unit area (LMA). These plots show the predictor variable on the *x*-axis, and on the *y*-axis, the residuals of the full model plus the partial regression coefficient of the predictor variable multiplied by the predictor variable. The dashed line indicates the best fit of the partial regression. Trees and lianas are combined in these analyses as growth form was not significant.

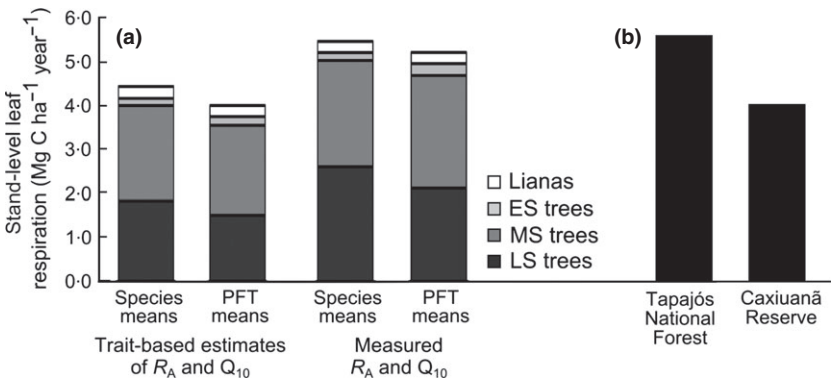

Fig. 4. Stand-level annual nocturnal leaf respiratory carbon flux in a tropical moist forest in Panama (a) and in two forests in Brazil (b). Fluxes are based on trait-based estimates (models 1A,B. See main text) or measured values of respiration (R_A) and Q_{10} (models 2A,B), using species means (models 1A, 2A) or PFT means (models 1B, 2B). In (a) contributions by lianas, early-successional (ES), mid-successional (MS) and late-successional (LS) tree species are indicated. Data for Tapajós and Caxiuanã (b) were taken from Malhi *et al.* (2009) and Metcalfe *et al.* (2010), respectively.

STAND-LEVEL LEAF RESPIRATORY CARBON FLUX

We scaled R to the stand level using R_A. Mean annual flux of nocturnal respiration between 1995 and 2011 was 4·5 ± 0·34 Mg C ha^{-1} (mean ± SD across years) when calculated using R_A and Q_{10} for each species estimated from trait-based multiple regression models (model 1A), and 4·1 ± 0·33 Mg C ha^{-1} when using leaf traits averaged by PFT (model 1B) (Fig. 4; Table 4). The annual flux estimate based on actual species-specific R_A and Q_{10} *measurements* (model 2A) was 5·5 ± 0·35 Mg C ha^{-1}, and 5·3 ± 0·34 Mg C ha^{-1} when PFT-level means were used. In all estimates, *c.* 95% of the respiratory carbon flux came from trees and 5% from lianas. Of the C flux from trees, 4–7% (range across the four models) came from early-successional species, 46–52% from mid-successional species and 42–50% from late-successional species.

R_{Day} was 2·9 ± 0·13 Mg C ha^{-1} year^{-1} when R was estimated from P_A, A_{sat} and LMA, and 2·7 ± 0·11 Mg C ha^{-1} year^{-1} when using PFT averages of these leaf traits (Table 4). When species-level measurements of R_A and Q_{10} were used, R_{Day} was 3·5 ± 0·09 Mg C ha^{-1} year^{-1}, while the R_{Day} flux estimated using PFT averages of measured R_A, and Q_{10} was 3·4 ± 0·11 Mg C ha^{-1} year^{-1} (Table 4). Estimates of the total carbon flux from foliar respiration ranged from 6·7 (model 1B) to 8·9 Mg C ha^{-1} year^{-1} (model 2A).

Discussion

SPECIES AND PFT DIFFERENCES IN RESPIRATION TRAITS

Based on *in situ* measurements by Slot, Wright and Kitajima (2013), we hypothesized respiration rates to be high. R_A of trees was indeed equally high as *in situ* (1·11 and 1·17 μmol m^{-2} s^{-1} in the current and previous study, respectively). R_A of trees was higher than R_A of lianas (Fig. 1), which is the opposite of what Domingues, Martinelli and Ehleringer (2007) and Cavaleri, Oberbauer and Ryan (2008) found in other Neotropical forests. The LMA of lianas in the current study was, however, significantly lower than the LMA of trees, which was not the case in the above-mentioned studies. This lower LMA probably indicates a lower quantity of metabolically active tissues per unit leaf area. R_M of lianas was equal to that of

Table 4. Contribution to stand-level foliar respiration flux during night and day by the four plant functional types (PFT): lianas, and early- (ES), mid- (MS) and late-successional (LS) tree species. Fluxes are based on trait-based estimates (models 1A,B. See main text) or measured values of respiration (R_A) and Q_{10} (models 2A,B), using species means (models 1A, 2A) or PFT means (models 1B, 2B)

Stand-level respiratory C flux (Mg C ha^{-1} $year^{-1}$)								
	Trait-based estimates of R_A and Q_{10}				Measured R_A and Q_{10}			
	Species means (Model 1A)		PFT means (Model 1B)		Species means (Model 2A)		PFT means (Model 2B)	
	Night	Day	Night	Day	Night	Day	Night	Day
Lianas	0·26	0·17	0·26	0·17	0·25	0·16	0·26	0·16
ES trees	0·17	0·11	0·20	0·13	0·18	0·11	0·28	0·18
MS trees	2·20	1·46	2·06	1·37	2·42	1·54	2·59	1·65
LS trees	1·83	1·20	1·53	1·01	2·61	1·64	2·16	1·36
Sum	4·46	2·94	4·05	2·68	5·46	3·45	5·29	3·35

early-successional species, indicating that lianas have comparatively high metabolic activity on a unit mass basis, indicative of fast-growing species.

HIGH TEMPERATURE SENSITIVITY OF RESPIRATION IN TROPICAL FOREST LEAVES

Q_{10} values were significantly higher than 2·0, the commonly assumed value that has been adopted in ecosystem process models (e.g. Thornton *et al.* 2002; Wang *et al.* 2009), supporting our hypothesis of relatively high Q_{10} values. Because Q_{10} has been observed to decline with rising temperature interval over which R is measured (Tjoelker, Oleksyn & Reich 2001; Atkin & Tjoelker 2003), a negative temperature-dependent Q_{10} has been incorporated in some global vegetation and ecosystem models (e.g. Ziehn *et al.* 2011; Chen & Zhuang 2013; Wythers, Reich & Bradford 2013). While it may be appropriate to expect the Q_{10} to decrease in relation to the measurement temperature within a given plant, or plants within a given climatic region (as originally suggested by Tjoelker, Oleksyn & Reich 2001), using this trend to estimate NPP of tropical vegetation in a global model, would result in considerable underestimation of Q_{10} compared to our data. Underestimation of Q_{10} leads to underestimation of R whenever leaf temperature exceeds the reference temperature in the model and in the tropics that is likely to occur very frequently. Q_{10} values >2·0 may not be uncommon in tropical forests (see Slot, Wright & Kitajima 2013; and references therein), so it will be important to assess the generality of this observation to improve estimates by global models of current and future C fluxes associated with foliar respiration.

LEAF PHOSPHORUS, PHOTOSYNTHESIS AND LMA, BUT NOT NITROGEN BEST PREDICT R_A

We found support for our hypothesis that the traits identified in the LES as correlates of variation in R_A across the globe also explain variation in R_A at the local scale. However, although N_A and A_{sat} both correlated positively with R_A in pair-wise correlations, the fit was relatively poor across these co-occurring tropical species. Interestingly, P_A was a much stronger correlate of R_A. Meir, Grace and Miranda (2001) found that phosphorus correlated more strongly with R in a P-limited forest in Jarú, Brazil, than in a less P-limited system in Cameroon, while Cavaleri, Oberbauer and Ryan (2008) found P to be an equally strong correlate of R as N in a P-limited rain forest in Costa Rica. Plant available P at PNM is on average 5·8 mg kg^{-1} (B.L. Turner, pers. comm.), which is relatively high for a tropical forest soil. Indeed, the P_A values in the current study were considerably higher than those in Meir, Grace and Miranda (2001) and Cavaleri, Oberbauer and Ryan (2008), and leaf N:P ratios averaged 18 (data not shown), suggesting no strong limitation of P. Thus, the greater strength of the correlation between R_A and P_A than between R_A and N_A is unlikely to reflect P limitation of this forest, but may instead be caused by interspecific variation in *non*-metabolic N (e.g. nitrogen-based defences) that obscured the correlation between R_A and N associated with metabolism.

The best three-trait regression model with P_A, A_{satA} and LMA explained 64% of the variance in R_A, improving significantly on the bivariate regression models. Meir, Grace and Miranda (2001) used stepwise regression to model R_A of leaves of tropical forest trees, and their best models also included P and LMA, but A_{sat} was not considered in their analysis. Similarly, Reich *et al.* (1998) did not consider P and A_{sat} when they found that N and LMA together explained 50–79% of the variance in R (area-based and mass-based, respectively) across sites and biomes, including tropical forest. Although the observation that respiration correlates with phosphorus is not new, the fact that N was not included in our best models suggests that – perhaps especially in tropical forests – P should be considered as a predictor of R, even if N is the better correlate across biomes (Wright *et al.* 2004).

Q_{10} POORLY PREDICTED FROM LEAF TRAITS

A maximum of 26% of the observed variance in Q_{10} was explained in a model that included TNC_A and growth

form, showing that higher Q_{10} values are found in species with higher TNC_A. Thus, R in species with high TNC increases more with temperature than in species with low TNC, suggesting that R in species with low TNC becomes substrate-limited at high temperature. This supports our hypothesis that Q_{10} could be predicted from traits related to substrate availability and life history. However, using the concentration of simple sugars (the more immediate substrate for R) instead of TNC in the model causes the trend to disappear (i.e. Q_{10} does not significantly increase with the concentration of simple sugars), suggesting that R was unlikely to be substrate-limited at higher temperatures. TNC concentrations are important for R and potentially for Q_{10}, but they are not commonly measured as leaf traits of forest trees. This calls into question the utility of a regression model that requires TNC and growth form to estimate Q_{10}, especially given that Q_{10} values of 85% of the species fall within the relatively narrow range between 2·15 and 2·70 and are not systematically different among PFTs.

TRAIT-BASED SCALING OF LEAF RESPIRATION; DISCREPANCIES AMONG APPROACHES

Estimates of annual nocturnal leaf respiratory carbon release were *c*. 20% lower for trait-based models that used multiple regression models to estimate R_A and Q_{10} than for models that used *measured* values of R_A and Q_{10}, despite the fact that the regression models were parameterized on the measured values. These differences could be explained by the difference in flux estimates for *Anacardium excelsum*, the species with the highest abundance at the site and the largest single contributor to stand-level C flux. *A. excelsum* had A_{sat} and P_A similar to other species, but one of the highest LMA values in our study (121 g m^{-2}; Table 1). As a result, R_A value for *A. excelsum* estimated from the multiple regression model – where LMA has a negative parameter estimate – was lower than the measured value for this species. The use of PFT means, either of measured or modelled respiration traits resulted in a small reduction in flux estimates compared to the use of species-level data. To reduce the discrepancy between species-level and PFT-level estimates, PFT averages can be weighted by the relative abundance of the different species in each PFT, if known (e.g. trait-based estimates of night and day flux are 4·51 and 2·97 Mg C ha^{-1} $year^{-1}$, respectively, when using PFT averages weighted by species (data not shown), almost identical to the 4·46 and 2·94 Mg C ha^{-1} $year^{-1}$ of models 1A and 1B). At the leaf level, differences among PFTs were small and species *within* PFT explained much more variation in trait values, so using models using species-level data may be more promising.

To overcome the discrepancy between models using measured and trait-based estimates of R_A and Q_{10}, the regression models should take into account the relative abundance of the trees and lianas at the study site. This seems impractical and would render such trait-based models highly site specific. In the following discussion, we will use the flux calculations based on species-level measurement of R_A and Q_{10} to represent the best estimate of the respiratory carbon flux in the study forest.

ANNUAL LEAF RESPIRATORY CARBON FLUX AT THE STAND-LEVEL

Per year, night-time R of canopy trees and lianas at PNM is estimated to release 5·5 Mg C ha^{-1}. This is almost identical to the night-time R efflux of 5·6 Mg C ha^{-1} $year^{-1}$ reported for the Tapajós National Forest in the Brazilian Amazon by Malhi *et al.* (2009), but higher than the mean of 4·0 Mg C ha^{-1} reported for the Caxiuanã reserve in the eastern Amazon (Metcalfe *et al.* 2010) (Fig. 4). Although the dry-season length at Tapajós is comparable to that at our site, the LAI estimates are higher (5·44 m^2 m^{-2}) than the values we used (mean for trees 3·66, with 30% cover of lianas with LAI of 0·73 m^2 m^{-2}). Furthermore, we conservatively assumed the deciduous species (11 of 14 tree species) to be leafless for the full 4 months of the dry season, whereas the Tapajós site shows increased metabolic activity during dry-season leaf flush (Huete *et al.* 2006). These considerations suggest that the night-time R flux we calculated may be conservative, even though it is comparable to fluxes in other tropical forests. Leaf-level respiration rates at PNM are at the high end of the spectrum of values reported for tropical forest trees (Slot, Wright & Kitajima 2013), which supports the notion that the nocturnal leaf respiratory C flux we report may be a slight underestimate of the true value for the study site because of our conservative (dry season) LAI estimates. R_A of early-successional species was not significantly higher than of late-successional species at PNM, but we recently measured R_A of upper-canopy leaves of 20 tree species from Parque San Lorenzo, an old-growth evergreen rain forest on the Atlantic side of Panama with a greater richness of late-successional tree species (slot *et al.*, 2014), and average R_A was significantly lower than R_A of trees at PNM (0·81 and 1·11 μmol m^{-2} s^{-1}, respectively). This suggests that per unit leaf area, total R may be lower in late-successional forest, and differences in total annual fluxes will depend on LAI (which tends to asymptote early in succession and should thus not differ much between early- and late-successional forests) and on deciduousness of the species.

R_{Day} added 3·5 Mg C ha^{-1} $year^{-1}$, bringing the total R flux to 8·9 C Mg ha^{-1} $year^{-1}$. This daytime flux is higher than reported in Malhi *et al.* (2009), who assumed R_{Light} to be 67% lower than R_{Dark} based on measurements on *Eucalyptus pauciflora* (Atkin *et al.* 2000). Our estimate of 46% reduction of R in the light from the measurements with sapling leaves of four tropical tree species is almost identical to the 47% reduction that Pons and Welschen (2003) report for seedlings of *Eperua grandiflora*, another tropical tree species. Not enough data on R_{Light} are

currently available to generalize the extent of reduction of R_{Light} relative to R_{Dark} in tropical vs. temperate species, nor do we know whether saplings and mature trees differ in the extent of light inhibition of R. Total C flux was comparable to that at Tapajós ($7{\cdot}4 \pm 4{\cdot}0$ Mg C ha^{-1} year^{-1}). For Caxiuanã, the site where Metcalfe *et al.* (2010) estimate night-time R to be 4·0 Mg C ha^{-1} year^{-1}, Malhi *et al.* (2009) report a total leaf respiratory carbon flux of $8{\cdot}9 \pm 4{\cdot}0$ Mg C ha^{-1} year^{-1}, identical to our total flux estimate. This suggests either a very high daytime R flux, or, more likely, it illustrates how differences in scaling methods result in grossly different flux estimates. In the central Amazon near Manaus, at a site with a shorter dry season than PNM, the total annual flux is $10{\cdot}1 \pm 4{\cdot}0$ Mg C ha^{-1} year^{-1} (Chambers *et al.* 2004; Malhi *et al.* 2009). Due to the large uncertainty in the estimates, it is currently not possible to conclusively summarize forest differences in total leaf respiration fluxes.

UNCERTAINTY IN CANOPY RESPIRATION FLUX ESTIMATES FOR BOTH BOTTOM-UP AND TOP-DOWN APPROACHES

Estimating canopy respiration fluxes necessarily depends on multiple assumptions, and consequently, the uncertainty in the flux estimates tends to be large both when scaling from leaf to canopy (bottom-up approach), and when extracting respiration components from ecosystem-level flux measurements (top-down). In our bottom-up approach, we were able to quantify uncertainty associated with R_A and Q_{10} at the species level, but unknown uncertainty associated with estimates of LAI and dry-season gas-exchange traits could affect the canopy flux estimates. The greatest metabolic activity occurs in the sun-exposed upper canopy, but when LAI is high, the multiple layers of shaded leaves can contribute considerably to the total respiratory carbon flux. Indirect estimates of LAI are prone to error (Olivas *et al.* 2013), whereas direct measurements are especially laborious in tall tropical forests (Clark *et al.* 2008). Improvements in remote sensing of LAI (Song 2013) should help reduce uncertainty in stand-level LAI estimates and associated C flux scaling over large geographic areas in the future. Dry-season gas-exchange traits may differ from traits observed in the wet season, but dry-season R contributed only *c.* 15% to the total estimated flux because of the dominance of dry-season deciduous species. Our estimates are likely a small underestimate of *total* C flux from foliar respiration as we focused on maintenance respiration only. By measuring fully mature leaves at the end of the night, we excluded respiration associated with growth (in young, developing tissue) and with phloem loading [which is higher early in the night when most photosynthates are exported from the leaves (Noguchi *et al.* 2001)]. While growth respiration can be considerable in young leaves, leaves tend to mature rapidly in the tropics, and the total contribution of growth respiration to the total respiratory carbon flux is probably small.

Nocturnal respiration fluxes estimated with eddy covariance techniques – a top-down approach – also have large uncertainty associated with them and are generally lower than fluxes measured concurrently with chambers (Goulden *et al.* 1996; Lavigne *et al.* 1997) because turbulence – essential for eddy flux measurements – is intermittent at night, and advective fluxes of CO_2, for example related to local topography, are usually not detected. Statistical approaches are available to reconcile eddy covariance and chamber estimates (e.g. Van Gorsel *et al.* 2007), and when eddy covariance data are filtered to include only those periods when sufficient turbulence is observed, the total fluxes estimated from eddy covariance and scaled from chamber measurements may be comparable (Chambers *et al.* 2004). However, <5% of the data met the turbulence threshold in Chambers *et al.* (2004). Given the difficulty with obtaining reliable nocturnal ecosystem respiration estimates from eddy covariance data, partitioning the nocturnal ecosystem respiration flux into its component fluxes and estimating the leaf respiration flux remain challenging. By comparison, our multiple regression approach using leaf trait correlates of R_A offers a simple, cheaper and reasonably accurate alternative.

IMPLICATIONS FOR TRAIT-BASED MODELLING OF STAND-LEVEL RESPIRATION

Our results suggest that within a tropical forest canopy, estimating R from either N or photosynthesis alone results in a relatively large uncertainty. While across biomes these correlations may be strong, within a single biome or a forest, the utility of these bivariate correlations is limited. Uncertainty can be reduced by using multiple regression approaches as presented here. We found Q_{10} values to be significantly higher than 2·0 and much higher than the ecosystem-level respiration Q_{10} values from eddy covariance measurements, which exhibit global convergence to values around 1·4 (Mahecha *et al.* 2010). Much research remains to be done for mechanistic understanding of temperature sensitivity of respiration both at leaf and ecosystem levels. O'Sullivan *et al.* (2013) recently presently detailed analyses of Q_{10} in relation to temperature in *Eucalyptus pauciflora*. Such analyses for a large number of species from different biomes would help improve our ability to predictively model global leaf respiratory carbon fluxes. We examined whether it is feasible to link Q_{10} to other leaf traits and found that the concentration of TNC and growth form (trees, lianas) together explained 26% of the variance in Q_{10}. However, the physiological underpinning of this model is not clear and the explained variance is modest. Rather than trying to model Q_{10} from other leaf traits, it will be important to improve understanding of the dynamic nature and global patterns of the Q_{10}. Our estimates of stand-level leaf respiratory carbon flux based on R_A and Q_{10} from trait-based models were comparable to fluxes reported for other tropical forests, suggesting that trait-based modelling indeed has potential. The

parameterization of such trait-based models will, however, need to be done locally or regionally to assure accurate predictions.

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Data Accessibility

Temperature response data and leaf-to-canopy scaling data deposited in the Dryad repository: http://doi.org/10.5061/dryad.4541m.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Figure S1. Illustration of leaf-level respiration-temperature response curves. Log_{10}-transformed leaf respiration rates ($Log_{10}(R_A)$) plotted against leaf temperature (T_{Leaf}) for one leaf of each of the 14 tree species (top three rows) and 14 liana species (bottom three rows) used in the current study and the r^2 values of the curves. Species are indicated by their genus name only; see Table 1 for full species names. Solid black lines represent the linear least-square fits; solid grey lines indicate the 95% confidence intervals of the fits. The fits of 123 leaves used in this study were significant at $P < 0{\cdot}01$ with $r^2 > 0{\cdot}88$ for all (mean $r^2 = 0{\cdot}98$).

Figure S2. Relationship of area-based respiration (R_A) and total daily photosynthetic active radiation (PAR), based on *in situ* R_A measurements on leaves of *Ficus* (○), *Pittoniotis* (●), *Castilla* (□), *Anacardium* (■) and *Luehea* (Δ) for which leaf-level PAR at the natural leaf angle was monitored for 1 week with gallium-arsenide photodiodes calibrated against an LI-190SA PAR sensor (Licor) and logged every 5 min to a 21X datalogger (Campbell Scientific, Logan, UT). R_A was measured pre-dawn with an LI-6400 at 25 °C.