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Research paper

Canopy position affects the relationships between leaf respiration and associated traits in a tropical rainforest in Far North Queensland

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We explored the impact of canopy position on leaf respiration (R) and associated traits in tree and shrub species growing in a lowland tropical rainforest in Far North Queensland, Australia. The range of traits quantified included: leaf R in darkness (R_D) and in the light (R_L; estimated using the Kok method); the temperature (T)-sensitivity of R_D; light-saturated photosynthesis (A_{sat}); leaf dry mass per unit area (LMA); and concentrations of leaf nitrogen (N), phosphorus (P), soluble sugars and starch. We found that LMA, and area-based N, P, sugars and starch concentrations were all higher in sun-exposed/upper canopy leaves, compared with their shaded/lower canopy and deep-shade/understory counterparts; similarly, area-based rates of R_D, R_L and A_{sat} (at 28 °C) were all higher in the upper canopy leaves, indicating higher metabolic capacity in the upper canopy. The extent to which light inhibited R did not differ significantly between upper and lower canopy leaves, with the overall average inhibition being 32% across both canopy levels. Log–log R_D–A_{sat} relationships differed between upper and lower canopy leaves, with upper canopy leaves exhibiting higher rates of R_D for a given A_{sat} (both on an area and mass basis), as well as higher mass-based rates of R_D for a given [N] and [P]. Over the 25–45 °C range, the T-sensitivity of R_D was similar in upper and lower canopy leaves, with both canopy positions exhibiting Q_{10} values near 2.0 (i.e., doubling for every 10 °C rise in T) and T_{max} values near 60 °C (i.e., T where R_D reached maximal values). Thus, while rates of R_D at 28 °C decreased with increasing depth in the canopy, the T-dependence of R_D remained constant; these findings have important implications for vegetation-climate models that seek to predict carbon fluxes between tropical lowland rainforests and the atmosphere.

Keywords: functional traits, light, photosynthesis, Q_{10}, temperature.

Introduction

Tropical rainforests cover ~6–7% of the global land surface (Stork et al. 2007) but are one of the most productive vegetation types and hence contribute substantially to global terrestrial net primary productivity (NPP) (Malhi and Grace 2000). Crucial in determining rates of NPP of tropical rainforests is the rate of CO_2 release by leaf respiration (R) (Metcalfe et al. 2010, Huntingford et al. 2013). However, the role of leaf R in determining NPP of tropical forests remains poorly understood (Meir et al. 2001, Malhi et al. 2009, Slot et al. 2013), particularly with respect to canopy-dependent (i.e., sun-exposed vs shaded foliage) variations in the leaf R measured at a common

temperature (T). Moreover, while there is evidence that the T-dependence of leaf R varies between tropical early and late successional functional types (Slot et al. 2013), and upper and lower canopy leaves in temperate forests (Griffin et al. 2002, Turnbull et al. 2003), to date no study has adequately assessed whether the T-dependence of R varies with canopy position in tropical rainforests. Given these issues, canopy-dependent variations in the respiratory characteristics of tropical rainforests need to be more fully characterized.

Light is considered the most limiting resource for trees in tropical rainforest biomes, with light penetration in the vertical dimension of rainforests declining markedly with increasing canopy depth (Yoshimura and Yamashita 2012). As a result, within a closed canopy, the availability of light can be reduced by up to 50-fold from the top of canopy compared with foliage in the shaded understory (Baldocchi et al. 2002). Such variations in irradiance can have marked effects on leaf morphological, chemical and physiological traits (Valladares et al. 2000, Meir et al. 2002, Niinemets et al. 2006, Kosugi et al. 2012). Concomitant with higher dry leaf mass per unit leaf area (LMA) (Gutschick and Wiegel 1988, Rijkers et al. 2000, Rozendaal et al. 2006, Kosugi et al. 2012) and leaf nitrogen (N) on an area-basis (N_a) values (Mooney and Gulmon 1979, Hirose and Werger 1987, Werger and Hirose 1991, Rijkers et al. 2000, Meir et al. 2002, Markesteijn et al. 2007) exhibited by upper canopy leaves, rates of area-based light-saturated photosynthesis (A_{sat_a}) tend to be higher in upper canopy leaves (Pearcy et al. 1987, Meir et al. 2002, Crous and Ellsworth 2004, Rozendaal et al. 2006, Kosugi et al. 2012). Similarly, area-based rates of leaf R in darkness (R_{D_a}) of rainforest species tend to be greater in high-light grown/upper canopy leaves than their shaded/lower canopy counterparts (Valladares et al. 2000, Meir et al. 2002, Kosugi et al. 2012). Down regulation of R_{D_a} in shade has also been observed in the tropical rainforest species, *Alocasia macrorrhiza* (Sims and Pearcy 1994). However, it is not clear whether the same patterns occur when rates of R_D of tropical forests are assessed on a mass basis (R_{D_m}). Moreover, while there is evidence from cross-biome comparisons that variations in growth irradiance can alter bivariate relationships between R_{D_m} and associated leaf traits [e.g., N, phosphorus (P) and A_{sat} (Wright et al. 2006)], it is not known whether such relationships differ between upper and lower canopy leaves in tropical forests.

When we consider the role of R in determining NPP of tropical rainforests, it is important to acknowledge that leaf R continues in the light (R_L). R_L is typically lower than leaf R in darkness (R_D), with the degree of light inhibition ranging from 16 to 77% (Hurry et al. 2005). Substantial overestimation of daily leaf R and under estimation of NPP can occur if light inhibition of leaf R is not accounted for (Wohlfahrt et al. 2005, Wingate et al. 2007, Crous et al. 2012). While there is some evidence that the degree of light inhibition differs between high- and low-light grown leaves under controlled environmental conditions (Zaragoza-Castells et al. 2007), it is not known whether rates of R_L and the degree of light inhibition differ between upper and lower canopy positions in tropical rainforests.

To model daily rates of respiratory CO_2 release in tropical rainforests, further information is needed not only on the impact of canopy position on leaf R at a common T, but also on whether canopy position affects the T-sensitivity of leaf R (i.e., Q_{10}, the proportional increase in R per 10 °C rise in T). Dynamic vegetation models often model R_D using a Q_{10} approach, where R_D is assumed to increase exponentially with T with a constant Q_{10} of 2.0 (White et al. 2000, Cox 2001, Cramer et al. 2001). Based on this assumption, future global warming is predicted to increase R_D (Cox et al. 2000, Wythers et al. 2005, King et al. 2006), potentially resulting in carbon loss from tropical forests (Cox et al. 2000). However, several studies have shown that the Q_{10} often declines with increasing measuring T (James 1953, Forward 1960, Tjoelker et al. 2001, Atkin and Tjoelker 2003, Zaragoza-Castells et al. 2008, O'Sullivan et al. 2013). Reductions in Q_{10} with increasing T have been linked to substrate and/or adenylate limitations at high measuring Ts (Atkin and Tjoelker 2003). Given this, and the fact that the concentration of soluble sugars declines with increasing depth within the forest canopy (Mooney et al. 1995, Marenco et al. 2001), Q_{10}–T relationships may vary through the closed canopies of tropical rainforests. There is also a possibility that the T at which maximal rates of R_D occur (T_{max}) might also vary through closed canopies, given the reported link between T_{max} and foliar sugar concentrations (Hüve et al. 2012).

Our study sought to quantify the impact of canopy position on leaf R in several tree species growing in a lowland tropical rainforest in Far North Queensland, Australia. The specific aims of our study were to determine whether: (i) rates of leaf R at a common T (both in darkness and in the light) differ between sun-exposed and shaded leaves (including shaded lower canopy leaves of dominant trees and shrubs growing in the shaded understory); (ii) light inhibition of leaf R differs between upper and lower canopy leaves; (iii) bivariate relationships linking leaf R to associated traits (LMA, N concentration, P concentration and A_{sat}) differ between upper and lower canopy leaves; and (iv) Q_{10}–T relationships and T_{max} for leaf R_D differ between upper and lower canopy leaves. To our knowledge, this study is the first to quantify leaf R in both the light and darkness, and the impact of canopy position on the T-dependence of R_D, in a tropical rainforest.

Materials and methods

Study site

The study was carried out at the Daintree Rainforest Observatory (16°07′S, 145°27′E; 40 m above sea level) located in a lowland tropical wet forest ~140 km north of Cairns in Far North Queensland, Australia. A 48.5-m tall industrial crane (Liebherr 91

EC) established on the site provided access to the canopy of about one hectare of the rainforest. The annual precipitation is ~5440 mm; however, rainfall is highly seasonal with over 70% received between December and April. The mean annual temperature is 24.3 °C. The soil is an acidic, dystrophic, brown dermosol (Isbell 1996) with many (20–50%) cobbles and stones throughout the profile. The soil is developed in colluvium from the metamorphic and granitic mountains to the west, and supports a complex mesophyll vine forest (Tracey 1982) with irregular canopy height (25–35 m) in which dominant canopy trees belong to the members of the Proteaceae, Meliaceae, Sapindaceae, Apocynaceae, Lauraceae and Myrtaceae families. The canopy closure of the site is reasonably typical of seasonal tropical rainforests with a leaf area index of 3.9 (Kalácska et al. 2005).

Species selection

Twelve evergreen dominant canopy tree species (Table 1) were selected from a variety of families in order to make comparisons of upper and lower canopy foliage. In addition, 10 understory species were selected to compare with the overstory trees where possible. The understory species were made up of four shade-adapted species [*Atractocarpus hirtus* (F.Muell.) Puttock, *Bowenia spectabilis* Hook. ex Hook.f., *Cryptocarya laevigata* Blume and *Linospadix minor* (W.Hill) Burret], four 'suppressed' species that have the potential to become canopy dominants [*Cordyline cannifolia* R.Br., *Darlingia darlingiana* (F.Muell.) L.A.S.Johnson, *Licuala ramsayi* (F.Muell.) Domin and *Syzygium monospermum* Craven] and two other species, *Tetracera nordtiana* F.Muell.—a vine that is found near the ground as well having the potential to grow at the top of the canopy—thus, in our case the sampled plants might be considered suppressed, and *Pseuduvaria froggattii* (F.Muell.) Jessup—a small tree that can grow to 8 m, and as such will typically be found growing in the shade of taller trees—thus, it is difficult to conclude for this species if the individuals of this species at near ground level were 'suppressed'.

None of the trees sampled were bearing fruits during the sampling period. However, several of the overstory species were at various stages of flowering (Dr W. Edwards, James Cook Univ., Cairns, personal communication): *Acmena graveolens* (F.M.Bailey) L.S.Sm—late flowering; *Cardwellia sublimis* F.Muell.—mid-flowering; *Cryptocarya mackinnoniana* F.Muell.—early flowering; *Dysoxylum papuanum* (Merr. & L.M.Perry) Mabb.—early flowering; *Gillbeea adenopetala* F.Muell.—early flowering; *Myristica globosa* Warb.—nothing; and *Rockinghamia angustifolia* (Benth.) Airy Shaw—mid-flowering. There were no flowers in *Castanospermum australe* A.Cunn & C.Fraser ex Hook, *Elaeocarpus grandis* F.Muell., *Endiandra leptodendron* B.Hyland, *Xanthophyllum octandrum* (F.Muell.) Domin or *Ficus destruens* F.Muell. ex C.T.White. No data are available on *Gillbeea whypallana* Rozefelds & Pellow or *Ficus variegata* Blume or for most of the understory species, with the exception of *Licuala ramsayi* (early flowering), *P. froggattii* (no flowers) and *S. monospermum* (no flowers). In those species where flowering is likely, it is possible that the carbohydrate profile of sampled leaves may have been affected by the increased demand for photosynthate in the reproductive tissue.

Measurements were made on four replicate trees from each species, one replicate in each of the SE, NE, SW and NW quadrants of the area reached by the 55-m-long crane jib. Measurements of the overstory trees were made at two positions in the canopy: north-facing sun-exposed leaves at the top of the canopy (hereafter referred to as 'upper canopy' leaves) and south-facing leaves from deep in the canopy (hereafter referred to as 'lower canopy' leaves). For the lower canopy leaves these were sampled from as low in the canopy as possible (3–5 m above the ground surface), where average irradiance on a horizontal surface in the late morning was 117 μmol photons m^{-2} s^{-1} [measured using the external quantum sensor on an infra-red gas analysis (IRGA) system] (LICOR 6400XT, LI-COR, Inc., Lincoln, NE, USA). Equivalent average irradiance in the understory was 11 μmol photons m^{-2} s^{-1}, while at the top of the canopy the values were up to 2400 μmol photons m^{-2} s^{-1}.

In situ leaf gas exchange measurements

To assess canopy leaf gas exchange characteristics, a combination of in situ (using the canopy crane) and ex situ (using a laboratory at the base of the crane) gas exchange measurements were made. Leaf-level measurements were made with two IRGA systems incorporating CO_2 control and 6 cm^2 chambers, each with a red-blue light source (6400-02B). All measurements were made during the period of 2–24 September 2010. All in situ gas exchange measurements were made between 11 am and 1 pm at 28 °C using the crane facility. For both upper and lower canopy leaves, light-saturated photosynthesis (hereafter termed A_{sat}) was first measured with the following settings: 1800 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), relative humidity (RH) of 60–70%, 400 ppm CO_2; photosynthesis was measured when CO_2 concentrations in the sample IRGA had stabilized (typically within 10 min of exposure to 1800 μmol m^{-2} s^{-1} PPFD). Thereafter, R_D was measured after allowing at least 30 mins of darkness before measurements commenced.

Measurements were made using fully expanded, newly mature leaves still attached to the plant at both positions in the canopy; although we do not have definitive data on the age of the sampled leaves, all are likely to be <1 year. Note, we cannot rule out photoinhibition of photosynthesis in lower canopy and understory leaves; however, in no case did we observe declines in rates of A_{sat} during the stabilization period.

Ex situ gas exchange measurements—light response curves for canopy trees

To assess the impact of canopy position on R_L and the degree of light inhibition of leaf R, we used cut branches of individual trees sampled using the canopy crane, again using branches from the

Table 1. Effect of canopy location [overstory (upper and lower positions) and understory] on average (± se, $n = 3–4$ for individual species/canopy position combinations) values of leaf dry mass per unit leaf area (LMA), leaf fresh mass per unit leaf area (FMA), leaf dry matter content (DMC), mass- and area-based leaf N concentration (N_m, N_a), mass- and area-based leaf phosphorus concentration (P_m, P_a), ratio of N to P (N : P), mass- and area-based total soluble sugar (soluble sugars), starch (starch) and total non-structural carbohydrates (TNC) concentrations. Asterisks indicate the level of probability according to: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. See Table 3 for two-way ANOVA results for each trait. Cases where no data were available are indicated (ND).

Canopy type	Species	Canopy position	LMA (g_{DM} m^{-2})	FMA (g_{FM} m^{-2})	DMC (g_{DM} g_{FM}^{-1})	N_m (mg g^{-1})	N_a (g m^{-2})	P_m (mg g^{-1})	P_a (g m^{-2})	N : P	Soluble sugars (mg g^{-1})	Soluble sugars (g m^{-2})	Starch (mg g^{-1})	Starch (g m^{-2})	TNC (mg g^{-1})	TNC (g m^{-2})
Overstory	*Acmena graveolens*	Upper	152 ± 21	360 ± 55	0.43 ± 0.03	19.5 ± 0.5	2.97 ± 0.44	1.2 ± 0.2	0.19 ± 0.04**	16.7 ± 2.2	36.1 ± 0.7	5.46 ± 0.66	4.5 ± 1.0	0.64 ± 0.06	40.6 ± 1.6	6.10 ± 0.63
		Lower	112 ± 5	255 ± 14	0.44 ± 0.01	21.4 ± 0.5	2.39 ± 0.09	1.1 ± 0.0	0.12 ± 0.00	19.8 ± 0.7	36.6 ± 1.3	4.11 ± 0.27	6.1 ± 2.2	0.69 ± 0.26	42.7 ± 3.4	4.80 ± 0.46
	Cardwellia sublimis	Upper	124 ± 6*	ND	ND	24.1 ± 2.8	2.96 ± 0.30	0.8 ± 0.1	0.09 ± 0.00	32.1 ± 4.1	55.9 ± 2.1**	6.96 ± 0.54**	34.3 ± 5.1	4.34 ± 0.80	90.2 ± 6.9	11.30 ± 1.34
		Lower	100 ± 5	283 ± 7	0.41 ± 0.02	19.2 ± 2.5	1.94 ± 0.31	0.7 ± 0.0	0.07 ± 0.01	27.9 ± 3.6	40.7 ± 3.4	4.05 ± 0.31	32.7 ± 8.4	3.23 ± 0.81	73.4 ± 11.6	7.28 ± 1.09
	Castanospermum australe	Upper	94 ± 8	230 ± 22	0.41 ± 0.00	30.8 ± 1.6	2.92 ± 0.38	1.6 ± 0.1	0.15 ± 0.01	19.1 ± 1.4	67.8 ± 4.6**	6.32 ± 0.47**	36.8 ± 10.5**	3.44 ± 0.92**	104.6 ± 14.8**	9.75 ± 1.34**
		Lower	68 ± 4	169 ± 4	0.40 ± 0.02	30.8 ± 1.6	2.10 ± 0.21	1.5 ± 0.1	0.10 ± 0.00	20.5 ± 2.2	39.0 ± 4.1	2.61 ± 0.15	11.6 ± 3.3	0.75 ± 0.16	50.6 ± 7.3	3.35 ± 0.29
	Cryptocarya mackinnoniana	Upper	187 ± 10	367 ± 19	0.51 ± 0.01	18.8 ± 1.0	3.51 ± 0.18	0.9 ± 0.1	0.16 ± 0.01**	22.3 ± 1.1	46.7 ± 3.1	8.80 ± 1.00	6.5 ± 1.8	1.24 ± 0.36	53.2 ± 3.8	10.04 ± 1.20
		Lower	161 ± 22	301 ± 25	0.53 ± 0.03	16.3 ± 0.8	2.61 ± 0.33	0.7 ± 0.0	0.11 ± 0.01	23.3 ± 1.2	43.2 ± 5.7	7.31 ± 1.92	7.0 ± 2.2	1.09 ± 0.28	50.2 ± 5.2	8.40 ± 1.94
	Dysoxylum papuanum	Upper	90 ± 1	237 ± 5**	0.38 ± 0.01	29.4 ± 0.6	2.66 ± 0.11**	1.4 ± 0.1	0.12 ± 0.01**	21.1 ± 1.3	35.9 ± 2.4	3.40 ± 0.21	39.4 ± 11.6	4.53 ± 0.47**	75.3 ± 13.2	7.93 ± 0.36**
		Lower	57 ± 2	181 ± 6	0.32 ± 0.01	31.1 ± 0.8	1.78 ± 0.10	1.5 ± 0.0	0.08 ± 0.00	21.2 ± 0.6	29.1 ± 3.2	1.67 ± 0.22	20.2 ± 7.3	1.18 ± 0.45	49.3 ± 10.5	2.85 ± 0.66
	Elaeocarpus grandis	Upper	110 ± 4	247 ± 8**	0.44 ± 0.02	24.6 ± 2.1	2.70 ± 0.25	1.22 ± 0.1	0.13 ± 0.01	20.3 ± 1.8	155.2 ± 3.6	17.06 ± 0.87	30.0 ± 3.9	3.33 ± 0.50**	185.2 ± 7.2	20.38 ± 1.34
		Lower	94 ± 5	197 ± 10	0.48 ± 0.00	27.0 ± 1.2	2.54 ± 0.14	1.2 ± 0.1	0.11 ± 0.00	23.5 ± 1.0	161.1 ± 8.1	15.12 ± 1.00	15.9 ± 4.8	1.46 ± 0.42	177.1 ± 11.5	16.57 ± 1.13
	Endiandra leptodendron	Upper	88 ± 5	221 ± 2**	0.40 ± 0.02	25.0 ± 1.2	2.19 ± 0.09	1.1 ± 0.1	0.09 ± 0.00	23.7 ± 0.8	40.6 ± 0.8	3.59 ± 0.24	10.2 ± 2.2	0.90 ± 0.19	50.8 ± 1.7	4.49 ± 0.32
		Lower	73 ± 12	180 ± 13	0.40 ± 0.05	28.0 ± 3.0	1.95 ± 0.21	1.3 ± 0.3	0.09 ± 0.00	23.4 ± 1.9	30.2 ± 5.2	2.14 ± 0.46	15.7 ± 1.5	1.15 ± 0.21	45.9 ± 5.6	3.29 ± 0.61
	Ficus variegata	Upper	38 ± 2	182 ± 12	0.21 ± 0.02	37.5 ± 3.5	1.40 ± 0.08	3.8 ± 0.7*	0.14 ± 0.02	10.4 ± 1.4	ND	ND	ND	ND	ND	ND
		Lower	43 ± 1	164 ± 2	0.27 ± 0.01	29.4 ± 1.4	1.28 ± 0.07	1.8 ± 0.1	0.08 ± 0.01	16.3 ± 0.5	ND	ND	ND	ND	ND	ND
	Gillbeea whypallana	Upper	147 ± 10*	327 ± 14**	0.45 ± 0.02	15.5 ± 0.9	2.27 ± 0.12**	0.6 ± 0.0	0.09 ± 0.00**	24.3 ± 0.4	46.8 ± 3.5	6.95 ± 0.90	15.8 ± 5.5	2.41 ± 0.88	62.6 ± 8.2	9.36 ± 1.67
		Lower	97 ± 13	233 ± 30	0.42 ± 0.01	16.0 ± 0.5	1.56 ± 0.21	0.6 ± 0.0	0.06 ± 0.01	25.2 ± 0.9	50.1 ± 5.0	5.06 ± 1.18	12.9 ± 4.7	1.23 ± 0.41	63.0 ± 5.6	6.28 ± 1.23
	Myristica globosa	Upper	119 ± 6	277 ± 10	0.43 ± 0.02	21.5 ± 1.2	2.54 ± 0.06	1.1 ± 0.1	0.13 ± 0.01	20.3 ± 1.5	49.2 ± 1.3**	5.87 ± 0.44**	57.5 ± 16.5	7.08 ± 2.19**	106.7 ± 17.7**	12.94 ± 2.58**
		Lower	103 ± 6	282 ± 5	0.36 ± 0.02	22.1 ± 1.1	2.26 ± 0.16	1.3 ± 0.3	0.13 ± 0.02	19.2 ± 3.0	39.0 ± 1.3	3.99 ± 0.19	18.4 ± 4.2	1.90 ± 0.48	57.5 ± 4.9	5.89 ± 0.62
	Rockinghamia angustifolia	Upper	75 ± 4	180 ± 4*	0.42 ± 0.02	19.1 ± 0.7	1.44 ± 0.07**	1.0 ± 0.1	0.07 ± 0.00**	20.7 ± 1.7	84.8 ± 10.1	6.44 ± 0.99	4.3 ± 0.8	0.33 ± 0.07	89.1 ± 10.4	6.77 ± 1.04
		Lower	66 ± 2	153 ± 3	0.43 ± 0.01	16.9 ± 1.0	1.12 ± 0.06	0.7 ± 0.0	0.05 ± 0.00	24.1 ± 1.2	83.0 ± 5.6	5.52 ± 0.57	3.2 ± 0.3	0.21 ± 0.01	86.1 ± 5.4	5.73 ± 0.56
	Xanthophyllum octandrum	Upper	122 ± 1*	276 ± 5	0.44 ± 0.01	15.4 ± 0.8	1.88 ± 0.08**	0.8 ± 0.0	0.10 ± 0.00**	20.2 ± 0.9	80.8 ± 14.3	9.84 ± 1.79	9.2 ± 6.2	1.13 ± 0.76	90.1 ± 20.5	10.96 ± 2.55
		Lower	89 ± 8	247 ± 16	0.36 ± 0.01	28.0 ± 2.6	2.48 ± 0.21	0.8 ± 0.1	0.07 ± 0.01	35.9 ± 2.2	79.7 ± 2.6	7.10 ± 0.64	6.1 ± 1.5	0.54 ± 0.15	85.8 ± 1.2	7.65 ± 0.63
Understory	*Atractocarpus hirtus*	Understory	51 ± 3	185 ± 11	0.28 ± 0.01	15.4 ± 0.8	0.79 ± 0.07	0.8 ± 0.0	0.04 ± 0.00	19.2 ± 1.1	ND	ND	ND	ND	ND	ND
	Bowenia spectabilis	Understory	56 ± 2	204 ± 3	0.27 ± 0.01	30.5 ± 0.3	1.68 ± 0.04	1.2 ± 0.0	0.06 ± 0.00	26.5 ± 0.7	ND	ND	ND	ND	ND	ND
	Cordyline cannifolia	Understory	56 ± 3	237 ± 9	0.24 ± 0.01	18.2 ± 2.1	1.00 ± 0.08	1.3 ± 0.0	0.07 ± 0.00	14.2 ± 1.6	ND	ND	ND	ND	ND	ND
	Cryptocarya laevigata	Understory	60 ± 4	177 ± 10	0.34 ± 0.03	21.4 ± 1.2	1.27 ± 0.08	1.5 ± 0.4	0.08 ± 0.02	16.8 ± 3.2	ND	ND	ND	ND	ND	ND
	Darlingia darlingiana	Understory	60 ± 2	171 ± 7	0.35 ± 0.00	10.6 ± 0.6	0.64 ± 0.03	0.6 ± 0.0	0.03 ± 0.00	18.9 ± 1.1	ND	ND	ND	ND	ND	ND

(Continued)

Table 1. Continued

Canopy type	Species	Canopy position	LMA (g_{DM} m^{-2})	FMA (g_{FM} m^{-2})	DMC (g_{DM} g_{FM}^{-1})	N_m (mg g^{-1})	N_a (g m^{-2})	P_m (mg g^{-1})	P_a (g m^{-2})	N : P	Soluble sugars (mg g^{-1})	Starch (mg g^{-1})	Soluble sugars (g m^{-2})	Starch (g m^{-2})	TNC (mg g^{-1})	TNC (g m^{-2})
	Licuala ramsayi	Under story	48 ± 11	125 ± 32	0.38 ± 0.01	16.8 ± 1.3	0.76 ± 0.12	0.8 ± 0.0	0.04 ± 0.01	22.0 ± 1.6	ND	ND	ND	ND	ND	ND
	Linospadix minor	Under story	44 ± 4	152 ± 15	0.29 ± 0.01	17.7 ± 0.8	0.78 ± 0.10	1.0 ± 0.0	0.04 ± 0.00	17.6 ± 0.5	ND	ND	ND	ND	ND	ND
	Pseuduvaria froggattii	Under story	59 ± 5	272 ± 16	0.21 ± 0.02	11.5 ± 0.9	0.65 ± 0.02	0.9 ± 0.1	0.05 ± 0.00	14.3 ± 2.0	ND	ND	ND	ND	ND	ND
	Syzygium monospermum	Under story	70 ± 2	200 ± 5	0.35 ± 0.01	13.6 ± 0.8	0.95 ± 0.04	0.7 ± 0.1	0.05 ± 0.00	19.1 ± 0.4	ND	ND	ND	ND	ND	ND
	Tetracera nordtiana	Under story	36 ± 4	125 ± 8	0.30 ± 0.02	15.8 ± 1.3	0.57 ± 0.03	0.8 ± 0.1	0.03 ± 0.00	21.1 ± 3.8	ND	ND	ND	ND	ND	ND
	Mean ± se	Upper	114 ± 6	267 ± 11	0.42 ± 0.01	23.1 ± 1.0	2.37 ± 0.13	1.2 ± 0.1	0.12 ± 0.01	21.2 ± 0.8	63.2 ± 5.3	7.37 ± 0.60	22.9 ± 3.3	2.66 ± 0.39	86.1 ± 6.5	10.03 ± 0.75
		Lower	90 ± 5	218 ± 8	0.40 ± 0.01	23.6 ± 0.9	2.00 ± 0.08	1.1 ± 0.1	0.09 ± 0.00	23.2 ± 0.8	56.9 ± 5.8	5.29 ± 0.59	13.8 ± 1.67	1.23 ± 0.16	70.7 ± 6.0	6.53 ± 0.61
		Under story	54 ± 2	186 ± 8	0.30 ± 0.01	17.1 ± 0.9	0.93 ± 0.06	0.9 ± 0.1	0.05 ± 0.00	19.0 ± 0.8	ND	ND	ND	ND	ND	ND

north-facing upper canopy and the south-facing lower canopy. Branches were re-cut under water immediately after detachment. Thereafter, detached branches were transported to a nearby laboratory located for ex situ measurements of net CO_2 exchange (A_{net}) between 8:30–10:30 am and 1:00–2:30 pm for morning and afternoon sampled branches, respectively. A_{net}-irradiance curves were determined using two LI-COR 6400XT systems. Measurements started at 1800 µmol photon m^{-2} s^{-1} and decreased to 1500, 100 and then at 5 µmol photon m^{-2} s^{-1} intervals to darkness (air temperature 28 °C; RH 60–70%; atmospheric CO_2 concentration 400 ppm). An example of A_{net}–I curve is shown in Figure 1. An equilibrium period of 2 min was allowed at each irradiance level before A_{net} was measured. During measurements, CO_2 flow rates in the leaf cuvette were set to 500 µmol s^{-1} for the measurements made at high irradiance (1800 and 1500 µmol m^{-2} s^{-1}) and to 300 µmol s^{-1} for irradiances <100 µmol m^{-2} s^{-1}.

We employed the Kok (1948) method to estimate rates of R_L. The Kok method makes the assumption that the response of A_{net} to light is linear at low irradiance and the breakdown of this linear relationship occurs at an irradiance near the light compensation point (Yin et al. 2011). The linear section above the break (10–50 µmol m^{-2} s^{-1}) is extrapolated to the y-axis to

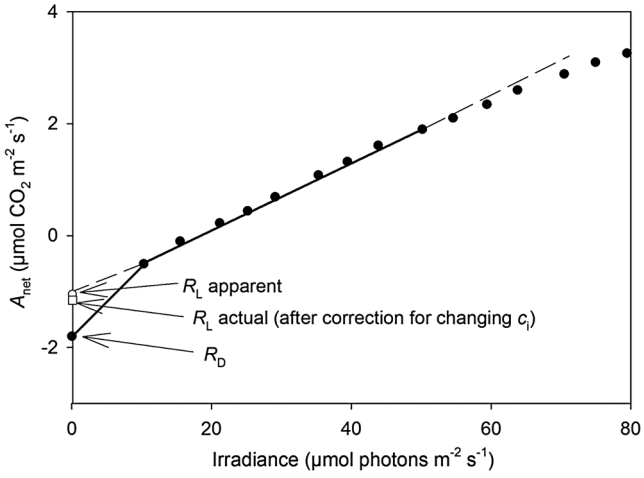

Figure 1. Representative plot of net CO_2 exchange rate (A_{net}, µmol CO_2 m^{-2} s^{-1}) versus irradiance (µmol photons m^{-2} s^{-1}) to illustrate the Kok effect. Solid symbols show measured rates of A_{net} over the 0–80 µmol photons m^{-2} s^{-1} range, with rates of leaf respiration in darkness (R_D = 1.8 µmol CO_2 m^{-2} s^{-1}) shown. The break from linearity at irradiances below 10 µmol photons m^{-2} s^{-1} (dashed line) is shown, with a linear regression fitted (r^2 = 0.99 for this replicate) to values between 10–50 µmol photons m^{-2} s^{-1} to estimate apparent rates of leaf R in the light (R_L 'apparent', ○ = 1.10 µmol CO_2 m^{-2} s^{-1}) at the y-axis intercept. Actual rates of R_L (□ = 1.13 µmol CO_2 m^{-2} s^{-1}) that take into account changes in internal CO_2 concentration (c_i) that occur as irradiance declined (Kirschbaum and Farquhar 1987) are also shown. Above 50 µmol photons m^{-2} s^{-1}, increases in A_{net} with irradiance were not linear (dotted line extension of linear regression from 10–50 µmol photons m^{-2} s^{-1} range data). Data are obtained from a single replicate plant of *C. mackinnoniana* from the upper canopy of Cape Tribulation tropical rainforest.

determine R_L whereas R_D is taken as the rate of A_{net} in darkness (Figure 1). To correct for increases in intercellular CO_2 concentrations (c_i) that tend to occur as irradiance is decreased (which decreases the slope of the A_{net}–I linear regression), we adjusted the rates of R_L (by iteration) to ensure that the intercepts of plots of photosynthetic electron transport (J) versus irradiance were minimized (Kirschbaum and Farquhar 1987, Atkin et al. 2013), assuming that $\Gamma_* = 36.9$ ppm at 25 °C (von Caemmerer and Farquhar 1981), and the temperature dependence of Γ_* is as reported in Brooks and Farquhar (1985).

Ex situ gas exchange measurements—short-term T-responses of R_D for canopy trees

The instantaneous T response of leaf R_D over a wide T range was quantified using cut branches of 12 species sampled from both upper and lower canopy positions. Instantaneous R_D–T curves were measured using the method outlined recently in O'Sullivan et al. (2013) and Hüve et al. (2011, 2012). To start the T-response curve experiment, a leaf was inserted into a 15.5 × 11.0 × 6.5-cm glass-topped, water-jacketed aluminium chamber at room T (near 28 °C) and kept in the darkness for 30 min. Then the chamber was cooled to a target air T of 15 °C before starting the T-response run. A programmable circulating water bath (model 32-HL, JULABO Labortechnik GmbH, Seelbach, Germany) was used to increase the air temperature in the chamber at a rate of 1 °C min^{-1} over the range of 15–70 °C and the rates of leaf R_D were recorded every 15 s, enabling high-resolution T-response curves to be generated. Past work has shown that respiratory CO_2 release often exhibits a 'burst' at leaf T_s >45 °C (O'Sullivan et al. 2013), coinciding with the onset of perturbation of photosynthesis (Hüve et al. 2011). Thus, when assessing the effect of canopy position on Q_{10} values, we focused on measurements <45 °C. An estimate for leaf R_D at any given measuring temperature was determined by plotting natural log-transformed values of R_D against leaf T over the 25–45 °C range and by fitting a second-order polynomial equation to $\log_e R_D$ vs T curves.

$$\log_e(R_D) = a + bT + cT^2. \quad (1)$$

Equation (2) was used to model rates of respiration in the temperature range 25–45 °C according to:

$$R_D = e^{a+bT+cT^2} \quad (2)$$

Using Eq. (3), a high-resolution modelled T-response curve was generated and the Q_{10} (i.e., proportional increase in respiration per 10 °C rise in T) was calculated by transformation of Eq. (2) to give the slope of the curve, k, at a given T.

$$k = b + 2cT. \quad (3)$$

Q_{10} values at any given T were then calculated using:

$$Q_{10} = e^{(10k)}. \quad (4)$$

When calculating average activation energies (E_a) of R_D over the 25–45 °C range, we constructed Arrhenius plots of $ln\ R$ against the inverse of leaf T (K); E_a values were calculated via multiplying the slope of each Arrhenius plot by the universal gas constant (8.314 J mol^{-1} K^{-1}).

Leaf mass per unit area and nutrient determination

After completion of the gas exchange measurements, leaves were harvested for the analysis of structure and chemical constituents. Initially, the fresh mass was measured (Mettler-Toledo Ltd, Port Melbourne, Victoria, Australia); thereafter, leaf area was determined (LI-3100 leaf area meter, LI-COR, Inc.). Subsequently, leaves were oven dried at 70 °C for 72 h, weighed and leaf dry mass per unit area (LMA) and dry matter content (DMC, ratio of leaf dry mass per unit fresh mass) were calculated. Previous studies (Dijkstra 1989, Vile et al. 2005) have shown that leaf fresh mass per unit area (FMA) is a good indicator of leaf thickness, with LMA being equal to FMA multiplied by DMC.

Concentrations of leaf N and P were determined with a LaChat QuikChem 8500 Series 2 Flow Injection Analysis System (Lachat Instruments, Milwaukee, WI, USA) using Kjeldahl acid digests (Allen 1974). For upper and lower canopy leaves only (i.e., not understory leaves), concentrations of soluble sugars, starch and total non-structural carbohydrates (TNC) were determined, as described previously (Loveys et al. 2003).

Data analysis

All statistical analysis was performed in SPSS v19 (SPSS Science, Birmingham, UK). Wherever necessary, variables were transformed ($\log_{10}$ transformations) to meet normality and homogeneity of variance requirements. Two-way analyses of variance (ANOVA) were carried out with least significant difference (LSD) post hoc testing to determine the impact of canopy position and species effect on different plant traits. Trait averages of the two canopy positions were compared using independent t-tests whenever necessary. We used individual tree measurements when assessing whether upper and lower canopy leaves differed in bivariate relationships, as has been done in earlier studies (Reich et al. 2008, Reich et al. 2009, Wyka et al. 2012, Xiang et al. 2013). Standardized major axis (SMA) regression analysis was performed using $\log_{10}$ transformed data to identify whether there were differences in slopes and/or elevation of log–log bivariate relationships. Differences in the elevation of regression slopes (i.e., y-axis intercept) and in shifts along the common slope were tested by ANOVA. Allometric equation parameters were calculated using SMATR [Version 2.0, http://www.bio.mq.edu.au/ecology/SMATR/ (Falster et al. 2006, Warton et al. 2006)].

Results

In situ comparison of traits in upper canopy, lower canopy and understory leaves

To gain an insight into overall patterns in leaf structural and chemical traits through the canopy, we first compared overall canopy position averages (comparing upper canopy, lower canopy and understory leaves) based on individual measurements (Tables 1 and 2). To gain further insights into the extent to which individual species exhibited similar or different responses to light availability, we also used a two-way ANOVA to compare traits of upper vs lower canopy leaves of the canopy tree species alone (i.e., without understory leaves; Table 3).

Figure 2 shows leaf structural trait values for upper and lower leaves of canopy trees, as well as values for understory shrubs. There were differences in LMA and FMA values, with a post hoc test ($P < 0.001$) revealing that the values decreased in order of: upper canopy > lower canopy > understory (Table 1, Figure 2). By contrast, there were no overall differences in DMC between the leaves in the upper and lower canopies (Table 1, Figure 2c). When considering upper and lower canopy leaves alone, the absence of a significant species × canopy position interaction term (Table 3) indicated that the canopy-dependent variations in LMA and FMA were largely consistent across species (i.e., the upper canopy leaves were thicker and contained more dry mass per unit leaf area than their lower canopy counterparts). For leaf DMC, the pattern was less clear, with the response to canopy position differing among the species (Tables 1 and 3).

When assessed on a leaf area basis, leaf N (N_a) decreased with increasing depth in the canopy, with upper canopy leaves exhibiting higher values than their shaded, lower canopy and deep-shaded understory counterparts (Table 1, Figure 3a). When assessed on a dry mass basis, no differences in leaf N concentration (N_m) were found between upper and lower canopy leaves (Table 3); however, N_m was significantly lower in understory leaves (Table 1, Figure 3b). For area-based leaf P (P_a), the values differed among canopy positions, with values in decreasing order being: upper canopy > lower canopy > understory (Figure 3c); by contrast, there was no difference in mass-based P (P_m) (Figure 3d). Finally, given the greater proportional change in leaf N than P when comparing upper/lower canopy leaves with leaves of understory leaves, average N : P ratios were 21.2 ± 0.8 and 23.2 ± 0.8 in the upper and lower canopies, respectively (with the effect of canopy position being significant when compared in a two-way ANOVA; Table 3), and 19.0 ± 0.8 in the understory (Table 1).

While there was no overall effect of within-tree canopy position on N_m (Table 3), not all species exhibited the same phenotypic response to light availability (as indicated by the significant species × canopy position interaction term). Leaf nitrogen on an area-basis was more consistently higher in upper canopy leaves (mean values 16% greater in the upper canopy compared with their lower canopy counterparts); however, the response of *X. octandrum* was of interest, as N_a values continued to be higher in the lower canopy leaves (thus contributing to the significant species × canopy position interaction term for N_a; Tables 1 and 3). With respect to canopy-dependent variations in P_m, Table 3 demonstrates that there was a significant overall effect of canopy position with P_m declining from the upper to lower canopy (mean values were 5% greater in the upper canopy). For P_a, the response was more consistent across species, with values in upper leaves being greater (mean values 22% higher) than those in lower leaves for several species (e.g., *A. gaveolens* and *C. mackinnoniana*), or with upper and lower canopy leaves exhibiting similar P_a values (e.g., *E. leptodendron* and *M. globosa*) (Tables 1 and 3). Collectively, these results point to markedly higher N_a and P_a (driven by variations in LMA) in the upper compared with lower canopy of the 12 tree species, whereas there are less consistent differences through the canopy in N_m and P_m concentrations.

Irrespective of whether expressed on a leaf area or mass basis and across all species, upper canopy leaves exhibited higher concentrations of total sugars, starch and TNC, with average mass-based values being 10, 40 and 18% higher, respectively, compared with lower canopy leaves (Tables 1 and 3).

When expressed on a leaf area basis, in situ measured rates of light-saturated photosynthesis (A_{sat_a}) and respiration in darkness (R_{D_a}) both declined significantly with increasing depth in the canopy (Tables 2 and 3, Figure 3e and g). At the top of the canopy, sun-exposed leaves exhibited significantly higher metabolic rates than their shaded, lower canopy and deep-shaded understory counterparts. Mean values of A_{sat_a} were 25% greater in the upper canopy leaves compared with their lower canopy counterparts (Tables 2 and 3). Importantly however, not all species exhibited the same A_{sat_a} response to canopy position (significant species × canopy position interaction; Table 3), with upper greater than lower leaves in some species (e.g., *C. australe* and *C. mackinnoniana*), and with upper equal to lower leaves (e.g., *A. gaveolens*) or upper less than lower leaves in other species (e.g., *F. variegata*). On a mass basis, no significant differences in A_{sat} (A_{sat_m}) were observed between upper and lower canopy leaves (Tables 2 and 3, Figure 3f); by contrast, A_{sat_m} was significantly lower in understory leaves (Tables 2 and 3, Figure 3f). Although there was no overall effect of canopy position on mass-, N- and P-based rates of A_{sat}, the significant interaction terms (Table 3) indicated that the effect of canopy position on each trait differed among the species.

In contrast to the variable A_{sat} responses among species to canopy position, in situ measured rates of leaf R_D in the upper canopy were generally higher than in lower canopy leaves, irrespective of whether rates were expressed on a leaf area, dry mass, N or P basis (Tables 2 and 3; Figure 3g

Table 2. Effect of canopy location [overstory (upper and lower positions) and understory] on in situ gas exchange: average (± se, $n = 3–4$ for individual species/canopy position combinations) values of area- and mass-based light-saturated photosynthetic rate at 1800 µmol photons m^{-2} s^{-1} PPFD (A_{sat_a} and A_{sat_m}, respectively), stomatal conductance (g_s) at saturating irradiance, intercellular [CO_2] (c_i) at saturating irradiance, leaf temperature (Leaf T), area- and mass-based rates of leaf respiration in darkness (R_{D_a} and R_{D_m}, respectively), ratio of R_D to gross photosynthesis (A_g; i.e., A_{400} plus R_D), PNUE, N-based rates of leaf dark respiration (R_D/N), PPUE, phosphorus-based rates of leaf dark respiration (R_D/P). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. See Table 3 for two-way ANOVA results for each trait.

Canopy type	Species	Canopy position	A_{sat_a} (µmol m^{-2} s^{-1})	c_i (ppm)	g_s (mol m^{-2} s^{-1})	Leaf T (°C)	R_{D_a} (µmol m^{-2} s^{-1})	R_D/A_g	A_{sat_m} (nmol g^{-1} s^{-1})	R_{D_m} (nmol g^{-1} s^{-1})	PNUE (A_{sat}/N)	R_D/N	PPUE (A_{sat}/P)	R_D/P
Overstory	*Acmena graveolens*	Upper	12.9 ± 1.8	270 ± 10	0.26 ± 0.05	30.0 ± 0.2***	1.8 ± 0.3**	0.13 ± 0.03	91.9 ± 17.8	13.2 ± 3.5	4.7 ± 0.9	0.68 ± 0.19	81.3 ± 20.6	10.6 ± 2.0
		Lower	12.6 ± 1.6	336 ± 7**	0.89 ± 0.23*	28.1 ± 0.0	0.8 ± 0.1	0.07 ± 0.01	111.6 ± 9.9	7.5 ± 0.7	5.3 ± 0.6	0.35 ± 0.03	104.4 ± 12.2	6.9 ± 0.6
	Cardwellia sublimis	Upper	12.3 ± 0.7	285 ± 2	0.27 ± 0.02	29.8 ± 0.3***	1.0 ± 0.2*	0.07 ± 0.01*	101.4 ± 12.3	8.3 ± 2.1	4.3 ± 0.5	0.34 ± 0.07	132.5 ± 6.0	10.5 ± 1.6
		Lower	11.2 ± 1.2	340 ± 4***	0.65 ± 0.07**	28.1 ± 0.0	0.6 ± 0.1	0.05 ± 0.00	111.1 ± 8.2	5.7 ± 0.6	5.9 ± 0.4	0.31 ± 0.04	161.8 ± 12.3	8.3 ± 0.8
	Castanos permum australe	Upper	13.3 ± 0.6**	247 ± 10	0.19 ± 0.013*	28.5 ± 0.1	1.3 ± 0.2*	0.09 ± 0.01	143.7 ± 9.6*	13.6 ± 1.8	4.8 ± 0.6	0.44 ± 0.05	88.7 ± 4.6	8.4 ± 1.0
		Lower	7.4 ± 0.9	256 ± 11	0.11 ± 0.02	30.0 ± 0.1***	0.6 ± 0.1	0.08 ± 0.00	108.4 ± 9.3	9.2 ± 1.2	3.5 ± 0.3	0.30 ± 0.04	72.3 ± 8.6	6.1 ± 0.9
	Cryptocarya mackinnoniana	Upper	15.2 ± 0.4**	271 ± 7	0.29 ± 0.02**	29.0 ± 0.1	1.1 ± 0.2*	0.07 ± 0.01	81.8 ± 5.3	5.7 ± 1.1	4.4 ± 0.2	0.31 ± 0.07	96.9 ± 7.7	6.8 ± 1.3
		Lower	6.0 ± 1.4	251 ± 20	0.10 ± 0.03	29.7 ± 0.2**	0.5 ± 0.1	0.09 ± 0.02	42.0 ± 13.3	3.7 ± 1.4	2.5 ± 0.7	0.23 ± 0.10	57.9 ± 16.8	5.1 ± 1.8
	Dysoxylum papuanum	Upper	14.3 ± 0.3***	321 ± 38	0.28 ± 0.04**	30.0 ± 0.1	1.8 ± 0.2**	0.12 ± 0.02	158.6 ± 3.3*	19.8 ± 2.1*	5.4 ± 0.2	0.67 ± 0.06***	109.4 ± 4.5	14.8 ± 2.3**
		Lower	4.1 ± 0.3	266 ± 5	0.06 ± 0.01	29.5 ± 0.2	0.5 ± 0.1	0.10 ± 0.02	72.4 ± 4.4	8.5 ± 1.7	2.3 ± 0.2	0.27 ± 0.05	49.7 ± 4.0	5.9 ± 1.3
	Elaeocarpus grandis	Upper	18.2 ± 0.4*	286 ± 5	0.47 ± 0.022**	28.7 ± 0.1	1.2 ± 0.1	0.06 ± 0.01	165.8 ± 3.4	10.6 ± 1.6	6.9 ± 0.6	0.44 ± 0.06	137.9 ± 8.7	8.6 ± 0.8
		Lower	16.6 ± 0.3	271 ± 7	0.33 ± 0.027	29.9 ± 0.1***	1.0 ± 0.1	0.06 ± 0.01	178.4 ± 11.8	11.1 ± 1.9	6.6 ± 0.5	0.41 ± 0.06	155.0 ± 7.4	9.5 ± 1.3
	Endiandra leptodendron	Upper	10.7 ± 0.6*	292 ± 7	0.24 ± 0.02	29.4 ± 0.1	0.8 ± 0.2**	0.07 ± 0.01*	122.1 ± 9.9	9.7 ± 2.6	4.9 ± 0.2	0.38 ± 0.08*	115.1 ± 6.5	8.8 ± 1.8**
		Lower	8.0 ± 1.4	293 ± 8	0.18 ± 0.05	29.6 ± 0.2	0.3 ± 0.1	0.04 ± 0.00	108.4 ± 4.9	4.4 ± 0.2	4.0 ± 0.4	0.16 ± 0.01	95.2 ± 14.9	3.8 ± 0.5
	Ficus variegata	Upper	8.9 ± 0.8	273 ± 11	0.15 ± 0.03	29.9 ± 0.2	2.0 ± 0.8	0.19 ± 0.07	236.6 ± 20.7	55.8 ± 21.6	6.5 ± 0.9	1.41 ± 0.44	68.4 ± 16.0	14.3 ± 3.9
		Lower	12.9 ± 0.5*	281 ± 18	0.29 ± 0.06	29.3 ± 0.1	1.1 ± 0.1	0.08 ± 0.01	295.9 ± 8.9	24.8 ± 3.5	10.1 ± 0.2	0.85 ± 0.14	164.1 ± 6.5	13.7 ± 1.9
	Gillbeea whypallana	Upper	11.9 ± 2.1	252 ± 16	0.22 ± 0.08	29.4 ± 0.3	1.3 ± 0.2**	0.11 ± 0.03*	82.0 ± 15.0	8.4 ± 0.7	5.2 ± 0.7	0.55 ± 0.07*	125.8 ± 17.6	13.5 ± 1.8**
		Lower	9.1 ± 1.2	273 ± 21	0.20 ± 0.08	29.8 ± 0.1	0.4 ± 0.1	0.04 ± 0.01	95.9 ± 10.5	4.4 ± 0.9	6.0 ± 0.5	0.27 ± 0.06	149.4 ± 13.2	7.0 ± 1.6
	Myristica globosa	Upper	8.8 ± 1.4	249 ± 12	0.13 ± 0.04	30.2 ± 0.0***	1.2 ± 0.4	0.11 ± 0.02*	76.1 ± 16.4	10.3 ± 3.8	3.4 ± 0.6	0.46 ± 0.14	68.8 ± 9.6	8.8 ± 2.1
		Lower	9.5 ± 1.1	326 ± 9**	0.42 ± 0.13	28.2 ± 0.1	0.6 ± 0.1	0.06 ± 0.01	92.1 ± 7.7	5.5 ± 1.0	4.2 ± 0.2	0.24 ± 0.03	81.5 ± 15.3	4.8 ± 1.2
	Rockinghamia angustifolia	Upper	5.6 ± 0.8*	324 ± 9*	0.17 ± 0.02**	29.2 ± 0.1	0.6 ± 0.1***	0.10 ± 0.02	74.9 ± 10.7	8.0 ± 1.3**	3.9 ± 0.6	0.42 ± 0.06**	80.0 ± 11.4	8.9 ± 2.0**
		Lower	2.5 ± 0.4	257 ± 18	0.03 ± 0.00	29.6 ± 0.0**	0.1 ± 0.0	0.05 ± 0.01	38.3 ± 6.5	1.8 ± 0.2	2.2 ± 0.3	0.11 ± 0.02	54.7 ± 9.0	2.5 ± 0.3
	Xanthophyllum octandrum	Upper	9.2 ± 1.6	262 ± 35	0.17 ± 0.05	29.5 ± 0.3	0.8 ± 0.1	0.08 ± 0.02	75.4 ± 12.6	6.4 ± 1.1	5.0 ± 1.2	0.43 ± 0.09	101.1 ± 20.6	8.5 ± 1.5
		Lower	8.1 ± 1.8	311 ± 37	0.35 ± 0.15	29.0 ± 0.7	0.7 ± 0.1	0.08 ± 0.03	95.2 ± 27.0	7.6 ± 1.6	3.3 ± 0.7	0.27 ± 0.05	115.2 ± 18.7	9.7 ± 1.8
Understory	*Atractocarpus hirtus*	Understory	3.4 ± 0.4	332 ± 8	0.11 ± 0.01	30.8 ± 0.1	0.2 ± 0.03	0.07 ± 0.00	65.4 ± 5.7	4.7 ± 0.4	4.3 ± 0.3	0.30 ± 0.02	80.9 ± 4.8	5.8 ± 0.4
	Bowenia spectabilis	Understory	3.3 ± 0.5	268 ± 6	0.05 ± 0.01	31.0 ± 0.1	0.24 ± 0.06	0.07 ± 0.01	58.9 ± 8.4	4.3 ± 1.0	1.9 ± 0.3	0.14 ± 0.03	50.6 ± 3.7	4.3 ± 0.6
	Cordyline cannifolia	Understory	2.7 ± 0.4	317 ± 28	0.09 ± 0.02	29.3 ± 0.7	0.39 ± 0.02	0.13 ± 0.01	48.3 ± 5.3	6.9 ± 0.4	2.9 ± 0.7	0.39 ± 0.04	37.7 ± 4.4	5.4 ± 0.2
	Cryptocarya laevigata	Understory	4.9 ± 1.1	252 ± 22	0.07 ± 0.02	30.5 ± 0.3	0.33 ± 0.10	0.08 ± 0.02	82.7 ± 17.5	6.1 ± 2.5	3.8 ± 0.6	0.28 ± 0.10	66.7 ± 22.9	4.4 ± 0.9
	Darlingia darlingiana	Understory	2.7 ± 0.2	304 ± 5	0.06 ± 0.01	30.7 ± 0.1	0.27 ± 0.08	0.09 ± 0.03	44.7 ± 3.5	4.6 ± 1.5	4.3 ± 0.4	0.42 ± 0.12	80.0 ± 5.4	7.8 ± 2.2
	Licuala ramsayi	Understory	2.1 ± 0.2	286 ± 23	0.05 ± 0.02	30.2 ± 0.7	0.12 ± 0.06	0.05 ± 0.02	53.4 ± 13.1	3.4 ± 1.6	3.1 ± 0.7	0.20 ± 0.09	70.0 ± 17.2	5.6 ± 0.8

(Continued)

Table 2. Continued

Canopy type	Species	Canopy position	A_{sat_a} (μmol m^{-2} s^{-1})	c_i (ppm)	g_s (mol m^{-2} s^{-1})	Leaf T (°C)	R_{D_a} (μmol m^{-2} s^{-1})	R_D/A_g	A_{sat_m} (nmol g^{-1} s^{-1})	R_{D_m} (nmol g^{-1} s^{-1})	PNUE (A_{sat}/N)	R_D/N	PPUE (A_{sat}/P)	R_D/P
	Linospadix minor	Under story	2.7 ± 0.1	321 ± 17	0.09 ± 0.04	30.0 ± 0.6	0.23 ± 0.04	0.08 ± 0.01	61.8 ± 5.5	5.3 ± 1.0	3.5 ± 0.3	0.30 ± 0.05	61.9 ± 5.5	5.7 ± 0.7
	Pseuduvaria froggattii	Under story	2.5 ± 0.6	262 ± 27	0.05 ± 0.02	30.9 ± 0.3	0.23 ± 0.05	0.09 ± 0.02	45.7 ± 14.0	3.9 ± 0.9	3.8 ± 0.9	0.34 ± 0.07	52.20 ± 9.3	5.0 ± 1.0
	Syzygium monospermum	Under story	2.9 ± 0.1	336 ± 5	0.09 ± 0.01	28.2 ± 0.0	0.29 ± 0.03	0.09 ± 0.01	41.5 ± 1.8	4.2 ± 0.3	3.1 ± 0.1	0.31 ± 0.03	58.6 ± 1.4	6.0 ± 0.6
	Tetracera nordtiana	Under story	2.7 ± 0.5	357 ± 6	0.14 ± 0.01	28.1 ± 0.0	0.28 ± 0.05	0.10 ± 0.02	79.5 ± 18.2	7.9 ± 1.8	5.0 ± 1.1	0.52 ± 0.14	91.4 ± 19.4	9.1 ± 1.3
	Mean ± se	Upper	11.8 ± 0.6	274 ± 4	0.24 ± 0.02	30.4 ± 0.1	1.2 ± 0.1	0.10 ± 0.01	114.2 ± 7.5	13.3 ± 2.2	4.9 ± 0.2	0.52 ± 0.05	101.0 ± 4.6	10.1 ± 0.6
		Lower	8.9 ± 0.6	288 ± 6	0.30 ± 0.04	30.1 ± 0.2	0.6 ± 0.0	0.06 ± 0.00	108.9 ± 9.3	7.5 ± 0.8	4.6 ± 0.3	0.30 ± 0.03	103.6 ± 6.9	6.7 ± 0.5
		Under story	3.0 ± 0.2	303 ± 7	0.08 ± 0.01	30.0 ± 0.2	0.3 ± 0.0	0.09 ± 0.00	58.2 ± 3.7	5.2 ± 0.4	3.6 ± 0.2	0.32 ± 0.03	64.6 ± 4.0	5.9 ± 0.4

Table 3. Effect of canopy position within the overstory on in situ gas exchange and leaf structure and chemistry: results of two-way ANOVA with species and overstory canopy position (upper and lower) as main effects, with the species × canopy position interaction term shown (significant interaction indicate that the effect of canopy position among species). LMA, leaf dry mass per unit leaf area; FMA, leaf fresh mass per unit leaf area; DMC, leaf dry matter content (ratio of dry mass to fresh mass); (N_a, N_m), area- and mass-based leaf N concentration respectively; (P_a, P_m), area- and mass-based leaf phosphorus concentration respectively; [N : P], nitrogen to phosphorus ratio; TNC, total non-structural carbohydrates; A_{sat}, light-saturated photosynthetic rate at 1800 μmol photons m^{-2} s^{-1} PPFD and 400 ppm [CO_2]; R_D, leaf respiration in the darkness; R_D/A_g, ratio of R_D to gross photosynthesis (A_g; i.e., A_{sat} plus R_D). ns, non-significant.

Leaf trait category	Parameter	*P*-values		
		Species	Canopy position	Interaction
Leaf structure	LMA	<0.001	<0.001	ns
	FMA	<0.001	<0.001	ns
	DMC	<0.001	ns	<0.050
Chemical composition	N_m (mg g^{-1})	<0.001	ns	<0.001
	N_a (g m^{-2})	<0.001	<0.001	<0.050
	P_m (mg g^{-1})	<0.001	<0.050	<0.010
	P_a (g m^{-2})	<0.001	<0.001	ns
	N : P ratio	<0.001	<0.010	<0.010
	Soluble sugars (mg g^{-1})	<0.001	<0.001	<0.010
	Soluble sugars (g m^{-2})	<0.001	<0.001	ns
	Starch (mg g^{-1})	<0.001	<0.050	ns
	Starch (g m^{-2})	<0.001	<0.001	ns
	TNC (mg g^{-1})	<0.001	<0.001	<0.050
	TNC (g m^{-2})	<0.001	<0.001	ns
Area-based gas exchange	A_{sat_a}	<0.001	<0.001	<0.001
	R_{D_a}	<0.010	<0.001	ns
Mass-based gas exchange	A_{sat_m}	<0.001	ns	<0.001
	R_{D_m}	<0.010	<0.050	ns
N-based gas exchange	A_{sat}/N	<0.001	ns	<0.001
	R_D/N	<0.001	<0.001	ns
P-based gas exchange	A_{sat}/P	<0.001	ns	<0.001
	R_D/P	<0.001	<0.001	ns
Ratio	R_D/A_g	<0.010	<0.001	<0.050

and h); no significant differences were observed between lower canopy and understory (Figure 3h). Mean values of R_{D_a} and R_{D_m} were 49 and 34% greater, respectively, in upper canopy leaves compared with the lower canopy counterparts. In no case was the interaction term significant (Table 3), indicating a more generic downward adjustment in leaf R_D to the contrasting light environments of the upper and lower canopies of the 12 tree species. When expressed on a per N basis, the average rates of R_D in the upper and

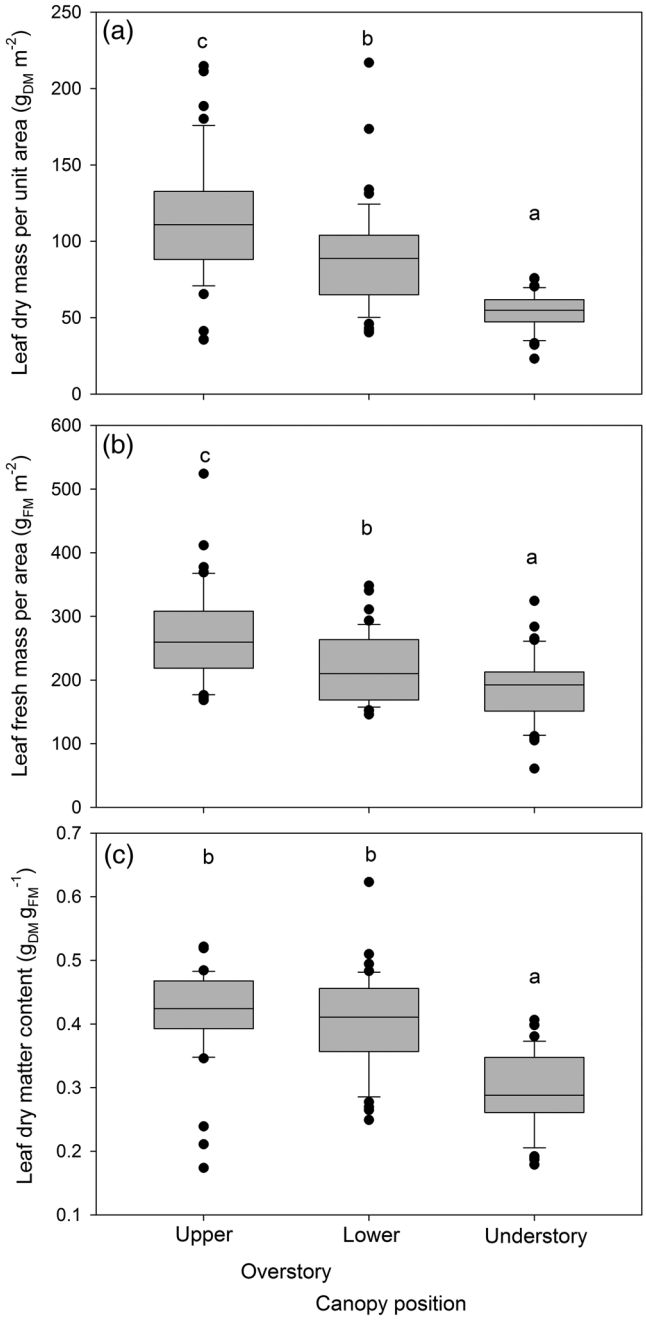

Figure 2. Box and whisker plots of LMA, FMA and leaf DMC are shown in relation to the upper overstory canopy, lower overstory canopy and understory. The upper and the lower edges of each box indicate the 75th and 25th percentiles, respectively. The horizontal line within each box is the median and the vertical bars indicate the 10th to the 90th percentile ranges. Letters indicate the result of a LSD post hoc test: boxplots with different letters are significantly different at $P < 0.05$.

lower canopy were 0.5 ± 0.1 and 0.3 ± 0.0 nmol CO_2 g^{-1} N s^{-1}, respectively.

To assess how the canopy position affected the potential balance between CO_2 release and light-saturated CO_2 uptake in leaves sampled in situ (using the canopy crane), we calculated the ratio of R_D to gross A measured at 400 ppm [CO_2] ($A_g = A_{sat} + R_D$), assuming for the moment that light did not inhibit leaf R (but see the later section on light inhibition). R_D/A_g values varied between 0.04 and 0.13 across all species (Table 2), with average R_D/A_g being significantly lower in the lower canopy leaves of the selected tree species, when compared with both leaves of understory shrubs and sun-exposed, upper canopy leaves (Figure 4).

Ex situ measurements of leaf respiration in the light for canopy trees

Estimates of leaf R_L were obtained for 10 tree species; however, due to time constraints and stomatal closure of some cut branches, it was not possible to obtain estimates of R_L for both canopy positions of all species; values of R_L were obtained for both canopy positions in eight of the 10 species (Table 4). A two-way ANOVA revealed that there were significant differences among species in the rate of R_L (when considering upper and lower canopy leaves collectively), irrespective of whether rates were assessed on an area- or dry-mass basis ($P < 0.01$). Moreover, when assessed across species, rates of R_L of the upper canopy were significantly higher in comparison to the lower canopy, both when assessed on an area- ($P < 0.001$) or mass-basis ($P < 0.01$) (Table 4). For both area- and mass-based comparisons, the species × canopy position interaction term for R_L was not significant, indicating that rates were consistently higher in upper canopy leaves (Table 4).

For most species, $R_L < R_D$ (Figure 5); however, $R_L : R_D$ ratios did not differ significantly between upper and lower canopies. For the eight species with both upper and lower canopy leaves, $R_L : R_D$ ratios were 0.75 ± 0.05 in the upper canopy and 0.62 ± 0.07 in the lower canopy (Table 4; canopy effect in two-way ANOVA: $P = 0.274$). Averaged across the two canopy positions, $R_L : R_D$ was 0.68 ± 0.05 (i.e., 32% inhibition). Canopy position also had a significant impact on the ratio of R_L to gross light-saturated net CO_2 assimilation (i.e., R_L/A_g), measured ex situ using cut branches (Table 4). Averaged across all species, the ratio of R_L/A_g decreased from 0.10 ± 0.01 to 0.05 ± 0.01 from the upper canopy to the lower canopy. Importantly, these estimates of R_L/A_g were markedly lower than the corresponding R_D/A_g values obtained from the same detached branches, which were 0.13 ± 0.01 to 0.10 ± 0.02 for the upper and lower canopy leaves.

Relationships between R_D and other leaf traits

Figure 6 shows log–log plots of relationships between in situ measured rates of R_D (on an area- and mass-basis) and a range of leaf traits for both upper and lower canopy positions (see Table S1 available as Supplementary Data at *Tree Physiology* Online for r^2 values, P-values, slopes and intercept of each SMA relationship). Across all species, SMA tests revealed no difference in slope in the R_D–A_{sat} relationship between the upper and lower canopy leaves; however, there was a shift in the

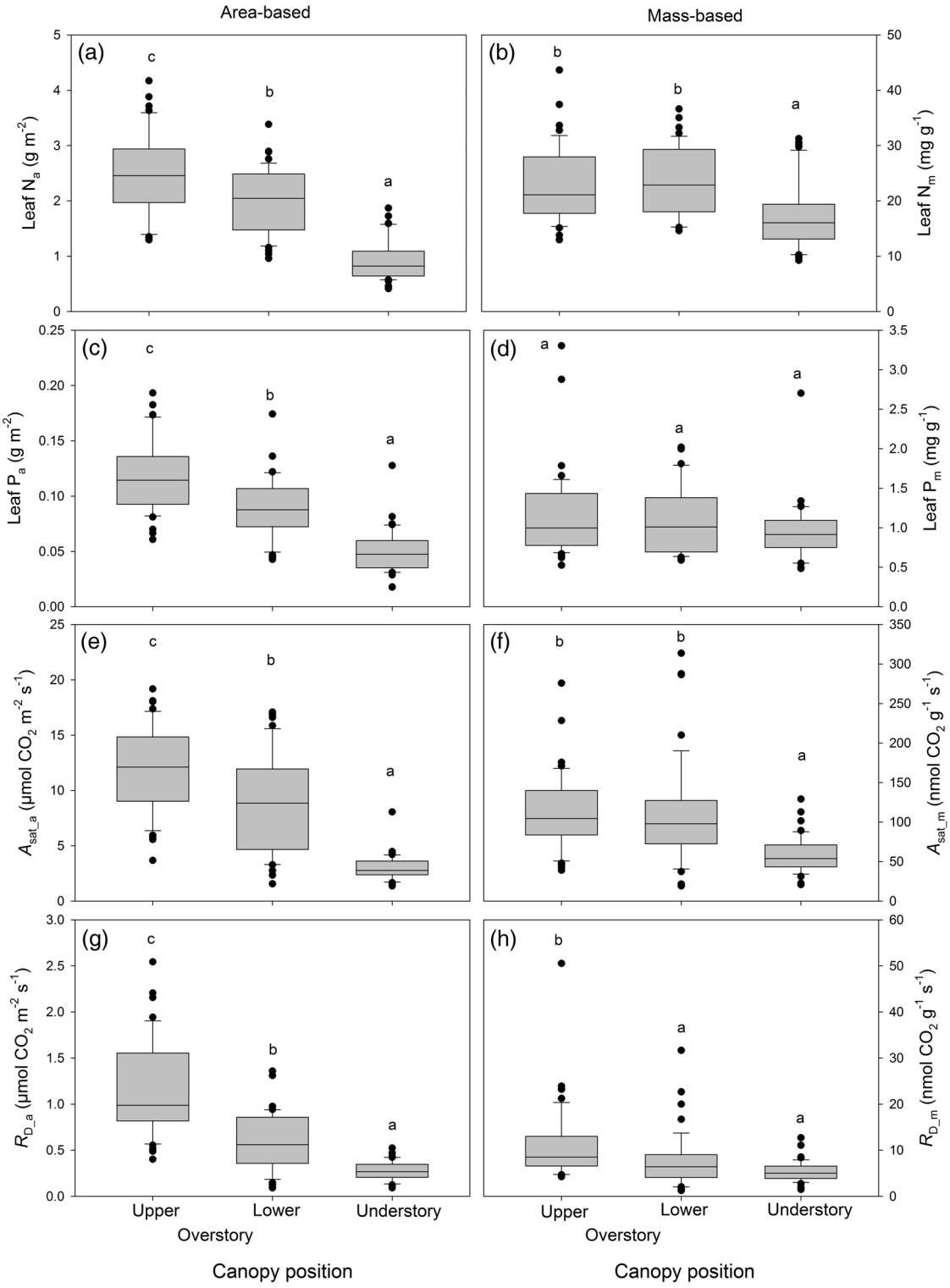

Figure 3. Box and whisker plots of area- and mass-based leaf N (N_a, N_m), phosphorus (P_a, P_m), light-saturated photosynthesis measured at 400 ppm [CO_2] and 1800 µmol photons m^{-2} s^{-1} (A_{sat_a}, A_{sat_m}) and rates of leaf respiration measured in darkness (R_{D_a}, R_{D_m}), shown in relation to upper overstory canopy, lower overstory canopy and understory. The upper and the lower edges of each box indicate the 75th and 25th percentiles, respectively. The horizontal line within each box is the median and the vertical bars indicate the 10th to the 90th percentile ranges. Letters indicate the result of a LSD post hoc test: boxplots with different letters are significantly different at $P < 0.05$.

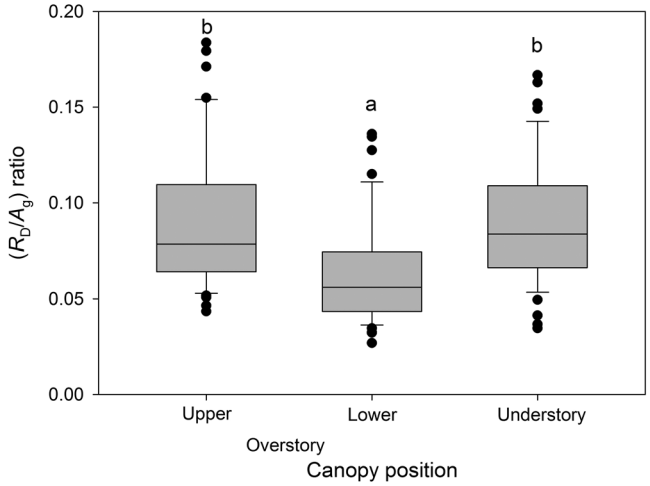

Figure 4. Impact of overstory canopy position on the ratio of rates of leaf respiration measured in darkness (R_D) to gross photosynthesis (A_g; i.e., net CO_2 assimilation in the light plus R_D). Values shown are for measurements made at each respective canopy position. Values represent the mean ± SE for 45 replicate individuals (12 species) of the upper canopy, 46 replicate individuals (12 species) of the lower canopy and 44 replicate individuals (10 species) of the understory. Letters indicate the result of a LSD post hoc test; among canopy positions, bars with different letters are significantly different at $P < 0.05$.

elevation of the relationship (i.e., *y*-axis intercept), with upper canopy leaves exhibiting higher area- and mass-based R_D at a given A_{sat} value (Figure 6a and f). Furthermore, upper canopy leaves exhibited a significant shift along the common slope when compared with lower canopy leaves (Figure 6a and f). By contrast, neither canopy layer exhibited significant log–log area-based R_D-LMA relationships (Figure 6b). There was a significant relationship between mass-based R_D and SLA, with the upper and lower canopies exhibiting a common slope and with upper canopy leaves exhibiting higher mass-based R_D at a given SLA (Figure 6g). Only the lower canopy leaves exhibited a significant R_D–N_a relationship (Figure 6c). By contrast, both upper and lower canopy leaves exhibited significant mass-based R_D–N_m bivariate relationships (with a common slope), with upper canopy leaves exhibiting higher mass-based R_D rates at a given N_m than their lower canopy counterparts. There was a common slope and intercept for upper and lower canopy area-based R_D–P_a relationships. By contrast, upper canopy leaves exhibited significantly higher rates of mass-based R_D at a given P_m compared with their lower canopy counterparts (Figure 6i). Finally, no relationship was found between leaf R_D and total soluble sugars in the upper or lower canopy leaves, irrespectively of whether the rates were assessed on a leaf area- or mass-basis (Figure 6e and j).

Canopy-dependent variations in the temperature dependence of R_D

Leaf *R* in darkness increased with increasing leaf *T* in upper and lower canopy leaves of all 12 selected canopy tree species, and area- and mass-based rates R_D at T_{max} were significantly higher in the upper canopy (see Table S2 available as Supplementary Data at *Tree Physiology* Online). When averaged across all 12 species, ex situ measurements using cut branches revealed that area-based rates of R_D were consistently higher in the upper canopy (Figure 7) as found in the in situ measurements. Canopy position had little effect on the leaf *T* where R_D reached its maximum (i.e., T_{max}), with upper and lower canopy leaves exhibiting T_{max} values of 59.4 ± 0.6 °C and 60.2 ± 0.8 °C, respectively. To assess whether canopy position altered the temperature coefficient of R_D, we calculated Q_{10} values at 1 °C intervals using coefficients of a second-order polynomial fitted to log R_D vs *T* over the 25–45 °C range (see Table S2 available as Supplementary Data at *Tree Physiology* Online; Eq. (1–4)). The inset of Figure 7 shows the average Q_{10} values (mean of all 12 species) for upper and lower canopy positions plotted against *T* over the 25–45 °C range. At a measuring *T* of 25 °C, Q_{10} values were 2.04 ± 0.08 in the upper canopy and 1.70 ± 0.06 in the lower canopy (see Table S2 available as Supplementary Data at *Tree Physiology* Online). The equivalent activation energy (E_a) values were 46.2 ± 1.6 and 50.2 ± 1.6 J mol^{-1} K^{-1} for upper and lower canopy leaves, with no significant differences being found in E_a among species or canopy positions. Above 25 °C, the Q_{10} value of upper canopy leaves exhibited a slight decrease with increasing leaf *T* up to 45 °C, whereas the Q_{10} of lower canopy leaves increased slightly. However, overall there was no significant difference in Q_{10}–*T* relationships between upper and lower canopy leaves, with R_D near doubling for every 10 °C increase over the 25–45 °C range.

Discussion

In recent decades, increasing attention has been focused on how leaf chemistry, structure and photosynthetic CO_2 uptake (*A*) vary through vertical canopy profiles of tropical rainforests (Carswell et al. 2000, Rijkers et al. 2000, Meir et al. 2002, Kosugi et al. 2012). By contrast, our understanding of how leaf respiration (*R*) varies through tropical rainforest canopies remains limited (Kosugi et al. 2012). Using a canopy crane to gain access to multiple positions within the canopy of a lowland tropical wet forest, we addressed this knowledge gap via characterization of leaf *R* and associated traits in sun-exposed and shaded leaves. Our study is the first to investigate the effect of canopy position on leaf *R* in darkness (R_D) and the light (R_L) in a tropical wet forest, as well as the first to quantify the effect of canopy position on the short-term temperature dependence of R_D.

Leaf photosynthesis in sun-exposed and shaded leaves

Figure 3e shows that in situ area-based rates of A_{sat} (A_{sat_a}) were highest in the upper canopy, and declined in the lower

Table 4. Effect of canopy position within the overstory on ex situ Kok effect measurements: average (± se, $n = 2–4$ for individual species/canopy position combinations) values of area- and mass-based rates of leaf respiration in the light (R_{L_a} and R_{L_m}) and in the darkness (R_{D_a} and R_{D_m}), ratio of R_L to R_D, and ratios of leaf R to A_g {[i.e., A_n plus R (either R_L or R_D, where relevant)]} for upper and lower canopy of each species. Also significance levels of t-test performed comparing upper and lower canopies are shown for each species. Finally, results of two-way ANOVA with species and canopy position (upper and lower) as main effects, with the species × canopy position interaction term shown (significant interaction indicates the effect of canopy position among species). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. ns, non-significant.

Species	Canopy position	R_{L_a} (μmol m^{-2} s^{-1})	R_{L_m} (nmol g^{-1} s^{-1})	R_{D_a} (μmol m^{-2} s^{-1})	R_{D_m} (nmol g^{-1} s^{-1})	R_L/R_D ratio	R_L/A_g ratio	R_D/A_g ratio
Argyrodendron peralatum	Upper	0.75 ± 0.15*	4.45 ± 0.75*	0.88 ± 0.16*	5.17 ± 0.78*	0.85 ± 0.02	0.10 ± 0.02	0.11 ± 0.02
	Lower	0.37 ± 0.03	2.64 ± 0.38	0.44 ± 0.04	3.04 ± 0.21	0.87 ± 0.10	0.06 ± 0.01	0.08 ± 0.01
Cardwellia sublimis	Upper	0.72 ± 0.12	5.50 ± 0.92	0.91 ± 0.13	6.98 ± 0.92	0.80 ± 0.11	0.08 ± 0.01	0.10 ± 0.01
	Lower	0.20	1.66	0.48	4.59	0.41	0.06	0.15
Cryptocarya mackinnoniana	Upper	0.78 ± 0.21	3.96 ± 1.09	0.81 ± 0.14	4.14 ± 0.72	0.89 ± 0.16	0.06 ± 0.01	0.06 ± 0.01
	Lower	0.28	2.10	0.34	2.65	0.73	0.04	0.05
Dysoxylum papuanum	Upper	1.10 ± 0.37	12.72 ± 4.04	1.40 ± 0.32	16.25 ± 3.42	0.75 ± 0.11	0.11 ± 0.01	0.14 ± 0.02
	Lower	0.58 ± 0.20	9.70 ± 3.12	0.73 ± 0.17	12.26 ± 2.47	0.74 ± 0.13	0.09 ± 0.03	0.11 ± 0.02
Endiandra leptodendron	Upper	0.52	7.26	0.63	8.63	0.85	0.06	0.07
	Lower	0.32	3.66	0.41	4.68	0.77	0.05	0.06
Myristica globosa	Upper	0.41 ± 0.08	3.80 ± 0.70	0.62 ± 0.15	5.83 ± 1.30	0.69 ± 0.14	0.08 ± 0.03	0.14 ± 0.07
	Lower	0.29	3.27	0.47	5.27	0.64	0.05	0.07
Syzygium sayeri	Upper	0.42 ± 0.11	3.21 ± 1.18	0.80 ± 0.12	6.06 ± 1.53	0.51 ± 0.07	0.15 ± 0.05	0.27 ± 0.07
	Lower	0.10	1.04	0.45	5.25	0.23	0.03	0.16
Xanthophyllum octandrum	Upper	0.64 ± 0.32	12.44 ± 7.83	0.79 ± 0.30	15.23 ± 8.04	0.67 ± 0.22	0.15 ± 0.08	0.18 ± 0.08
	Lower	0.40 ± 0.16	4.24 ± 1.52	0.64 ± 0.11	6.86 ± 0.92	0.58 ± 0.16	0.07 ± 0.02	0.12 ± 0.00
Total	Upper	0.67 ± 0.08	6.70 ± 1.35	0.84 ± 0.09	8.44 ± 1.67	0.75 ± 0.05	0.10 ± 0.01	0.13 ± 0.02
	Lower	0.32 ± 0.05	3.54 ± 0.96	0.49 ± 0.04	5.57 ± 1.06	0.62 ± 0.07	0.05 ± 0.01	0.10 ± 0.02
P-values	Species	**	**	*	**	*	ns	**
	Canopy position	***	**	***	*	ns	*	ns
	Interaction	ns	ns	ns	ns	ns	ns	ns

canopy and understory, mirroring declines in LMA (Figure 2a), N_a and P_a (Figure 3a and c). Past studies have reported similar impacts of canopy position on A_{sat_a} (Pearcy et al. 1987, Niinemets and Tenhunen 1997, Meir et al. 2002, Crous and Ellsworth 2004, Rozendaal et al. 2006, Kosugi et al. 2012). When expressed on a mass basis, little difference in A_{sat_m} was observed between upper and lower canopy leaves of the selected tree species; thus, differences in the photosynthetic capacity of canopy trees result largely from differences in leaf thickness rather than differences in photosynthetic capacity per unit mass, N or P (Table 3). By contrast, A_{sat} was markedly lower in the understory plants growing in deep shade, irrespective of whether rates were expressed on a leaf area- or mass-basis (Figure 3e and f). Thus, it appears that photosynthetic capacity was fundamentally different (as were N_m values; Figure 3b) in leaves of plants growing in the deep shade of the forest understory.

When assessing the impact of canopy position on A_{sat}, consideration needs to be given to the fact that lower canopy and understory leaves are rarely exposed to saturating irradiance. We used data from our ex situ light response curves to gain some insight into potential rates of A_{sat} at a light intensity near that experienced by lower canopy leaves (100 μmol photons m^{-2} s^{-1}). (Note, due to time constraints light response curves were not measured for the understory shrubs.) When averaged across the eight species for which A_{net}–I data were available in both canopy positions, rates of area- and mass-based A_{sat} at 100 μmol photons m^{-2} s^{-1} were 2.3 ± 0.1 μmol CO_2 m^{-2} s^{-1} and 24.8 ± 2.4 nmol CO_2 g^{-1} s^{-1}, respectively, for the lower canopy leaves. This compares with the light-saturated rates of area- and mass-based A_{sat} of 5.5 ± 0.4 μmol CO_2 m^{-2} s^{-1} and 59.2 ± 6.3 nmol CO_2 g^{-1} s^{-1}, respectively. Thus, light limitations in the shaded lower canopy are likely to have markedly reduced the prevailing rate of CO_2 uptake by the selected species.

In situ leaf respiration in darkness in sun-exposed and shaded leaves

In a similar manner to A_{sat_a}, in situ leaf R_{D_a} also exhibited a decreasing trend from the sun-exposed upper canopy to the shaded lower canopy and deep-shaded environment of the

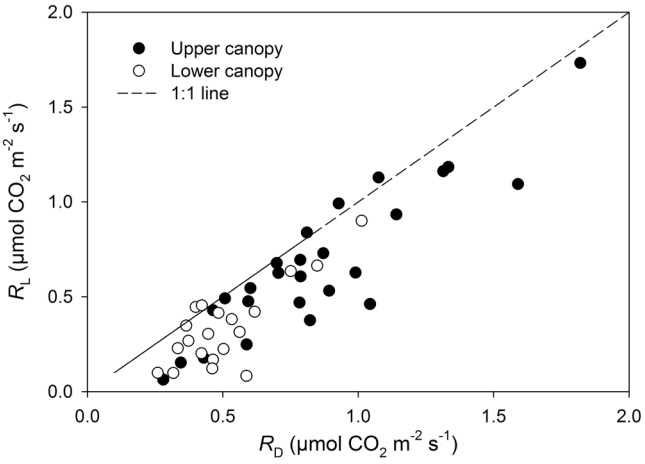

Figure 5. Area-based rates of leaf R_L plotted against corresponding rates of R_D. Data shown are for measurements made at each respective overstory canopy position across all species. The dashed line shows the 1 : 1 relationship. See Table 4 for two-way ANOVA results.

understory (Figure 3g, Tables 2 and 3). However, in contrast to the trends in A_{sat_m} (where upper and lower canopy foliage exhibited similar A_{sat}; Figure 3f), R_{D_m} of upper canopy leaves were significantly higher than for the lower canopy and understory leaves (Figure 3h). Thus, changes in R_D through the canopy were proportionally greater than A_{sat}. This finding contrasts with comparisons of tropical rainforest evergreen tree species growing in sun-exposed gaps versus plants growing under a closed canopy, where no differences in R_{D_m} were reported (Rijkers et al. 2000, Poorter et al. 2006). However, our results are similar to those of past studies on temperate forest systems, where canopy-dependent variations in R_{D_m} occurred (Griffin et al. 2001, Tissue et al. 2002), with the higher rates of R_{D_m} in upper canopy leaves being underpinned by a greater density of mitochondria per cell area (Tissue et al. 2002) to support the higher metabolic demands in the upper canopy.

Given that R_{D_m} often scales with A_{sat_m} and leaf N_m (Givnish 1988, Gifford 1995, 2003, Reich et al. 1998, 2006, Loveys et al. 2003, Wright et al. 2004, Atkin et al. 2006), one might have expected R_{D_m} to follow the same canopy pattern as A_{sat_m} and N_m. That is, there would be little difference in R_{D_m} between upper and lower canopy leaves, but with markedly lower rates in understory shrubs. However, as shown in Figure 3h, R_{D_m} were higher in upper canopy leaves, while there was no significant difference in R_{D_m} between lower canopy and understory leaves. What factors might account for these observations? In addition to the abundance of mitochondria, respiratory rates are controlled by the supply of respiratory substrate as well as by the demand for respiratory energy (Lambers et al. 2008). Although we have no data on the flux of substrates available to the respiratory system, our analysis of carbohydrate concentrations revealed that, despite similar rates of A_{sat_m} in upper and lower canopy leaves, substrates were in greater abundance in upper canopy leaves (compared with their lower canopy counterparts). Although this may seem surprising, in reality leaves in the shaded lower canopy exhibit markedly lower daily rates of carbon gain (compared with their upper canopy counterparts) due to photosynthesis being light-limited. Accumulation of soluble sugars in the upper canopy might explain, in part, why upper canopy leaves exhibited higher rates of R_{D_m} (Figure 3h) if variations in rates of R_{D_m} are dependent on substrate availability as reported in some studies (Azcón-Bieto and Osmond 1983, Tissue et al. 2002). Further, higher rates of R_{D_m} in the upper canopy might reflect the higher energy demand for protein repair (e.g., from photodamage in sun-exposed leaves), maintenance of solute gradients and loading of sugars into the phloem (Ryan 1991, Amthor 2000, Bouma 2005). Thus, higher rates of daily photosynthesis (and consequent increased substrate supply and demand for respiratory energy) are likely to have contributed to the higher average rates of R_{D_m} exhibited by the upper canopy leaves, compared with their lower canopy counterparts (Figure 3h) (Van Der Werf et al. 1992, Reich et al. 2006).

Given the explanations above for why rates of R_{D_m} were higher in upper canopy than in lower canopy leaves, one might have expected rates of R_{D_m} to be greater in lower canopy leaves (of overstory trees) than understory leaves, as A_{sat_m} and leaf N_m of lower canopy leaves is greater than understory leaves. Yet, rates of R_{D_m} were similar in the lower canopy and understory leaves. Why was this? One possibility is that, despite exhibiting lower A_{sat_m} and leaf N_m, understory species may have in fact achieved relatively high daily rates of net photosynthesis in the deep shade at the forest floor (e.g., via greater efficiency of light capture and/or use, underpinned by improvements in leaf architecture, anatomy, allocation of leaf N within leaves and photosynthetic N-use efficiency) (Björkman 1981, Seemann et al. 1987, Evans and Seeman 1989, Evans and Poorter 2001, Lambers et al. 2008). Alternatively, if actual rates of daily photosynthesis were lower in the understory shrubs, the maintenance of R_{D_m} might reflect a lower efficiency of energy production and/or use compared to their lower canopy counterparts. While little is known about the efficiency of ATP use in shade-adapted plants, there is some evidence that shade-adapted species exhibit low rates of the non-phosphorylating alternative oxidase, thus increasing their efficiency of ATP synthesis (Noguchi et al. 2001). Thus, it seems unlikely that the relatively high rates of R_{D_m} in the understory plants (several of which could be considered shade-adapted) were due to lower efficiency of ATP synthesis; rather, some aspect of energy use (e.g., specific ATP costs of cellular maintenance) might have been greater.

A further factor that might contribute to the maintenance of R_{D_m} in the understory shrubs relative to shaded lower canopy leaves of overstory trees is the fact that the understory plants were much smaller than their overstory dominants. As noted by

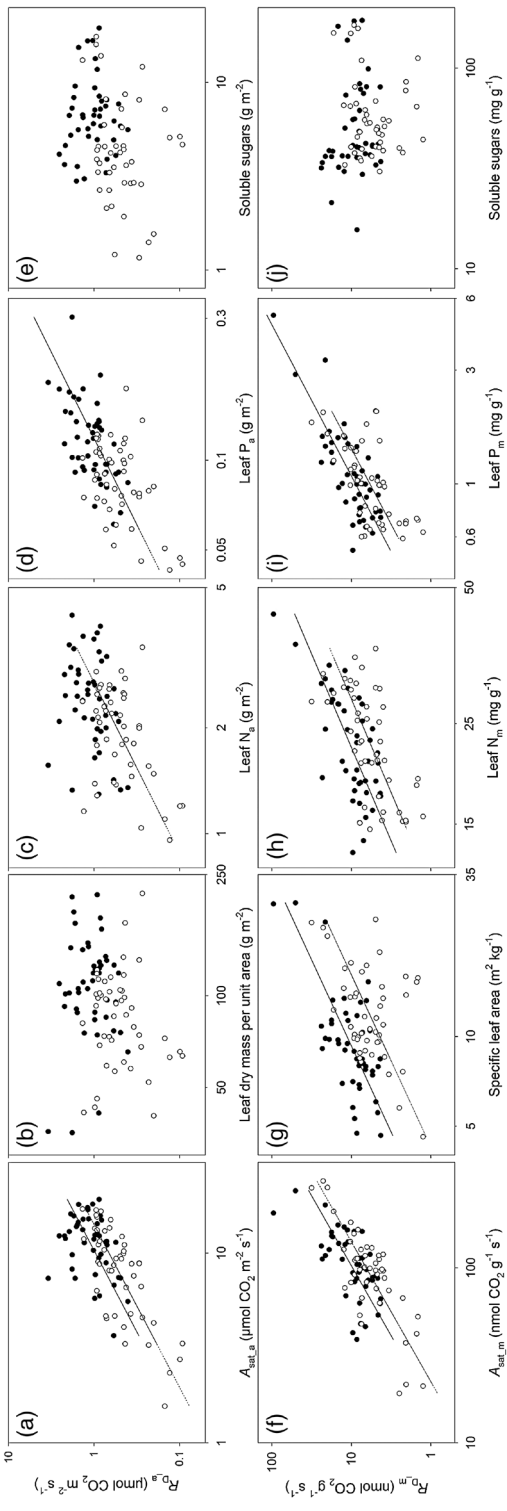

Figure 6. Log–log plots of area- and mass-based rates of in situ measured rates of leaf R_D in relation to light-saturated photosynthesis (A_{sat}) (a, f), LMA (b) and SLA (g), leaf N (c, h), leaf P (d, i) and total soluble sugars (e, j) for 12 tropical rainforest tree species, with upper and lower overstory canopy positions are shown. Data points represent individual plant values (upper canopy, 45 individuals; lower canopy, 46 individuals).

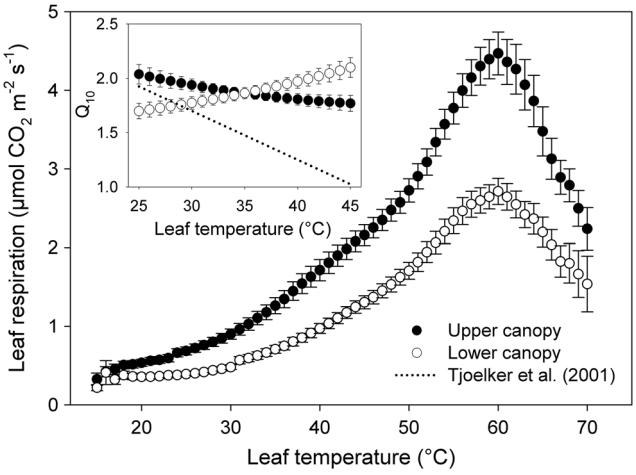

Figure 7. Comparison of mean upper and lower overstory canopy high-resolution temperature (T) response curves of leaf dark respiration (R_D) of 12 tree species growing in Daintree rain forest, Far North Queensland. Symbols show the actual measured T-response of leaf R over the 15–70 °C leaf T range (closed symbols—upper canopy, open symbols—lower canopy). The inset figure compares the temperature sensitivity of leaf respiration (Q_{10}) of upper and lower canopy tree species. The values shown are the modelled Q_{10} calculated over the 25–45 °C range using Eq. (1) and (4). The dashed line shows the Q_{10} relationship proposed by Tjoelker et al. (2001) utilizing literature data across different biomes. Values are means ± se (upper canopy: $n = 38$; lower canopy: $n = 38$). See Table S2 available as Supplementary Data at *Tree Physiology* Online for individual species data.

Steppe et al. (2011), several leaf traits crucial to metabolic performance decrease in value as trees increase in size, including LMA, N_m and A_{sat_m}. Thus, comparison of lower canopy leaves (of large dominant overstory trees) with leaves of smaller understory plants might be influenced by the relative difference in plant size and age of the overstory and understory plants. Finally, exposure to full shade would have affected the performance of understory plants, compared with shaded lower canopy leaves of overstory trees whose upper canopy leaves experience full sunlight. The growth (and presumably physiological performance) of a given shaded branch depends on whether other branches on the same tree are also shaded or exposed to full sun, with the growth (and life span) of shaded branches being better on 'suppressed' plants where all branches are shaded (Sprugel 2002). Although some of our understory species are adapted to shade, four or five understory species could be considered to be 'supressed' (see Materials and methods). If rates of R_{D_m} of shaded leaves are indeed relatively higher in fully shaded plants (compared with shaded leaves in plants whose upper leaves are illuminated), then this might explain the maintenance of R_{D_m} in the understory leaves (relative to lower canopy leaves). It would not, however, explain the relatively low rates of lower A_{sat_m} and leaf N_m in the understorey leaves. Clearly, further work is needed to establish which of these above factors account for the maintenance of R_{D_m} in the understory shrubs, despite their lower A_{sat_m} and leaf N_m values.

Light inhibition of leaf respiration—impact of canopy position

One of the objectives of our study was to assess whether upper and lower canopy leaves differed in the degree of light inhibition of leaf *R*. We found that R_L was lower than R_D (Figure 5). Similar to canopy-dependent differences in growth irradiance, leaf structure/chemistry (Figures 2 and 3) and metabolic activity (Figure 3), there were some differences in the average degrees of light inhibition in the upper and lower canopy leaves (i.e., 25 and 38% inhibition for upper and lower canopy leaves, respectively); however, the differences were not statistically significant (Table 4), with the average across both canopy positions, light inhibited leaf *R* by ~32%.

Given the marked differences in growth irradiance within the canopy, the question arises as to whether irradiance in the lower canopy (nominally near 100 μmol photons m^{-2} s^{-1}) was sufficiently low enough to result in leaf *R* not being fully inhibited. Past work on a temperate evergreen tree species (Atkin et al. 2000) has shown that maximal light inhibition occurs at irradiances less than experienced by our lower canopy leaves. Moreover, we observed the Kok effect occurring at irradiance values <10 μmol photons m^{-2} s^{-1} (Figure 1), suggesting that light inhibition was likely to be maximal in situ, both in upper and lower canopy leaves. Such inhibition is likely to have reflected light-mediated changes in cellular energy status, photorespiration-dependent inactivation of the pyruvate dehydrogenase complex (Budde and Randall 1990, Gemel and Randall 1992), demand for TCA cycle intermediates (Igamberdiev et al. 2001, Hurry et al. 2005, Tcherkez et al. 2005, Tcherkez et al. 2008, 2012, Gauthier et al. 2010) and/or light suppression of CO_2 release by the oxidative pentose phosphate pathway (Buckley and Adams 2011). Although it is not clear which of these factors account for within-canopy variation in light inhibition, it seems that exposure to higher growth irradiance in the upper canopy would have resulted in greater demand for respiratory products in the light (e.g., ATP, NADH and carbon-skeletons), not only to support higher daily rates of photosynthesis and carbon-export, but also to support higher rates of light-dependent N assimilation. Whatever the mechanism(s) underpinning within-canopy variation in light inhibition, our results suggest that ecosystem carbon exchange models can assume a single degree of light inhibition when predicting daytime carbon fluxes in trees of this tropical lowland wet forest, irrespective of canopy position.

Unlike many other ecosystems, tropical rainforests often experience relatively little diurnal or seasonal variation in temperature, or seasonal variation in daily photoperiod (which is ~12 h near the equator). Given these factors, one might make an approximate estimate of daily leaf *R* simply by taking the average of R_D and R_L, integrated over a 24 h period. However, in cases where there are diurnal and/or seasonal changes in temperature, consideration would need to be given to the sensitivity of R_D and R_L to short- and long-term changes in temperature.

Balance between leaf respiration and photosynthesis

In models that seek to predict rates of carbon exchange in terrestrial ecosystems, it is often assumed that there is a near constant balance between leaf *R* and *A*. Some studies have reported near constant $R : A$ ratios when comparing contrasting species and/or plants grown in different environments (Ziska and Bunce 1998, Loveys et al. 2002, 2003, Gifford 2003, Lambers et al. 2008, Van Oijen et al. 2010). By contrast, variability in $R : A$ ratios has also been reported, particularly in studies comparing leaves developed in contrasting environments (Tjoelker et al. 1999, Atkin et al. 2006, Campbell et al. 2007, Way and Sage 2008, Zaragoza-Castells et al. 2008). Whether $R : A$ ratios are constant or not can have important impacts on the predicted carbon budget of individual plants, as well as predicted rates of ecosystem net CO_2 exchange and the global carbon balance (Gifford 2003). Given this, and the lack of data on how canopy position affects $R : A$ ratios in tropical wet forests, we investigated whether the ratio of leaf *R* to gross photosynthesis (A_g) differed between sun-exposed and shaded leaves (Figure 4). Our results, using light-saturated A_g, pointed to a higher $R_D : A_g$ ratio in upper versus lower canopy leaves, but with the $R_D : A_g$ ratio being similar in upper canopy and understory leaves. On first inspection, these findings question the validity of using a constant $R_D : A_g$ ratio for modelling carbon fluxes in such tropical wet rainforest canopies.

However, to properly assess the balance between respiratory CO_2 release and photosynthetic CO_2 uptake, several factors need to be considered, including the extent to which leaf *R* continues in the light and the how light gradients in the canopy impact on actual rates of A_g. Our study shows that light inhibited leaf *R* by an average of 32% across the two canopy positions, so $R_L : A_g$ ratios are $< R_D : A_g$. Furthermore, the fact that light is limiting in the shaded lower canopy means that such ratios need to compare upper canopy leaves using light-saturated rates of A_g with ratios for lower canopy leaves using light-limited rates of A_g. Comparing $R_D : A_g$ ratios using rates of A_g at light saturation and 100 μmol photons m^{-2} s^{-1} (using ex situ gas exchange measurements) revealed that for leaves in the lower canopy, $R_D : A_g$ increased from 0.10 ± 0.01 (A_g at light saturation) to 0.20 ± 0.02 (A_g at 100 μmol photons m^{-2} s^{-1}); this compares with an $R_D : A_g$ ratio of 0.13 ± 0.02 for the upper canopy at light saturation (Table 4)—thus, when compared at their respective growth irradiances, $R_D : A_g$ ratios are likely to have been markedly higher in the shaded lower canopy (0.2) than the sun-lit upper canopy (0.13). When we consider rates of leaf *R* in the light, $R_L : A_g$ ratios in the lower canopy increased from 0.05 ± 0.01 (A_g at light saturation) to 0.12 ± 0.01 (A_g at 100 μmol photons m^{-2} s^{-1}); this compares with an $R_L : A_g$ ratio of 0.10 ± 0.02 for the upper canopy at light saturation (Table 4), suggesting that there is

relatively little difference in $R_L : A_g$ ratios between upper and lower canopy leaves of forest trees when each are compared at their prevailing growth irradiance. For understory shrubs, it is likely that actual in situ $R_D : A_g$ ratios are higher than indicated in Figure 4, because such leaves are likely to operate with very low prevailing rates of A_g. Taken together, we conclude therefore that while $R_L : A_g$ ratios are likely to be relatively homoeostatic within upper and lower canopy leaves of the selected tree species, whereas respiratory CO_2 release in darkness is likely to represent a greater fraction of A_g in the shaded lower canopy and deep-shaded understory.

Bivariate relationships in upper and lower canopy leaves

In a global analysis of scaling between leaf R_D and related traits, Wright et al. (2006) found that species at higher-irradiance sites exhibited higher R_{D_m} at a given leaf N_m, SLA and A_{sat_m}. Our results indicate that bivariate relationships linking R_{D_m}-A_{sat_m}-SLA-N_m-P_m were canopy position dependent (Figure 6). For example, upper canopy leaves exhibited higher average R_{D_m} for any given A_{sat_m} (or SLA/N_m/P_m) when compared with lower canopy leaves. Thus, canopy position appears to systematically alter the *y*-axis intercept (but not slope) of the log–log scaling relationships linking R_{D_m} with other associated leaf structural, chemical and metabolic traits. Underpinning this shift in the elevation of the bivariate relationships was the greater proportional change in average rates of R_{D_m} when compared with changes in A_{sat_m}, SLA, N_m and P_m. If this finding holds more widely it would suggest that failure to account for the effect of canopy position on R_{D_m}-A_{sat_m}-SLA-N_m-P_m relationships could lead to discrepancies in models of CO_2 exchange in tropical forest ecosystems.

We also observed no relationship between R_D and sugar concentrations, either in upper or in lower canopy leaves (Figure 6). Our data do thus not support the hypothesis that rates of R_D will scale positively with substrate concentrations (Azcón-Bieto and Osmond 1983, Lambers et al. 2008, Lewis et al. 2011) but rather that higher rates of R_D in upper canopy rainforest leaves reflect differences in the demand for respiratory energy.

While significant relationships were observed between R_{D_m} and other mass-based traits, relationships were less consistent when assessed on a leaf area-basis (Figure 6). Why was this? Past studies have highlighted the fact that area-based rates of *R* are often poorly correlated with their other area-based leaf traits (Field and Mooney 1983, Wright et al. 2004). More recently, both Osnas et al. (2013) and Lloyd et al. (2013) have drawn attention to the statistical implications of using a common parameter (mass or area) as the main factor for normalization. In response, Westoby et al. (2013) and Poorter et al. (2014) both highlight the appropriateness and utility of mass-based expressions when considering the carbon economy of individual leaves and whole plants. If one assumes that mass-based relationships are valid when assessing relationships between respiration and other traits, then a clear picture emerges of upper canopy leaves having a higher elevation but a similar bivariate slope to their lower canopy counterparts.

Temperature sensitivity of leaf respiration

In past studies on non-tropical forests, canopy position was found to influence the apparent E_a or Q_{10} values calculated across a wide range of measuring *T*s (Bolstad et al. 1999, Griffin et al. 2002, Turnbull et al. 2003). However, to our knowledge no such comparisons have been made for tropical lowland wet forests—hence, our study represents the first assessment of how canopy position affects the *T* dependence of R_D. Moreover, unlike past studies that relied on temperature response curves fitted to relatively few data points collected at broad *T* intervals (e.g., every 5 °C), our study employed a high resolution protocol (Hüve et al. 2011, 2012, O'Sullivan et al. 2013) to assess the impact of canopy position on Q_{10}–*T* relationships. We found that the differences were modest and overall it appears that both upper and lower canopy leaves exhibit a similar *T*-dependency of leaf R_D, with rates near doubling per 10 °C rise in *T* over the 25–45 °C range (i.e., Q_{10} values were constant and near 2.0). Our study is also the first to quantify the high *T* tolerance of R_D. Irrespective of canopy position, tropical rainforest species exhibit tolerance of temperatures near 60 °C (Figure 7). This finding suggests that tropical tree species in this forest could exhibit considerable resilience when exposed to heat wave events; if true, this finding would have marked significance for our understanding of how a warmer world could impact on metabolic functioning of tropical rainforest ecosystems.

The absence of a marked decline in Q_{10} values with increasing measuring *T* was surprising, given previous findings of this nature in temperate ecosystems (Tjoelker et al. 2001, Atkin and Tjoelker 2003). Thus, contrary to other ecosystems, it appears that we can assume a constant Q_{10} value of near 2.0 for both upper and lower canopy leaves in our tropical lowland rainforest ecosystem. This finding may have important implications for modelling carbon fluxes in tropical lowland forests, as variations in the *T*-sensitivity of R_D can markedly alter the extent of net carbon uptake by tropical forests (Huntingford et al. 2013).

Conclusions

In conclusion, we found in a tropical lowland rainforest of North Queensland that area-based A_{sat}, R_D, R_L and LMA were all highest in the upper canopy compared with shaded lower canopy and understory leaves, with $R_L < R_D$. The effect of light on leaf *R* differed between upper and lower canopy leaves, being greatest in the lower canopy. The impact of canopy position on photosynthetic and respiratory rates differed when comparisons were made on a dry mass-basis; for example, while there was no significant difference in rates of A_{sat_m} (or leaf N_m) between upper

and lower canopy leaves of the dominant trees, upper canopy leaves exhibited significantly higher mass-based R_D and R_L when compared with their lower canopy counterparts. This asynchronous effect of canopy position on photosynthetic and respiratory metabolism on a mass-basis resulted in a canopy-dependent change in the balance between light-saturated CO_2 uptake and respiratory CO_2 release. Importantly, our results provide strong evidence that the temperature dependence of leaf R_D does not differ in upper and lower canopy leaves, both in terms of Q_{10} values and high temperature tolerance (T_{max}). Collectively, these findings enhance our understanding of how canopy position impacts on respiratory CO_2 release and associated traits in tropical wet forests, with the results having implications for vegetation–climate models that seek to predict carbon fluxes between tropical rainforests and the atmosphere.

Supplementary data

Supplementary material is available at *Tree Physiology* online.

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Conflict of interest

None declared.

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