

Drought stress and tree size determine stem CO₂ efflux in a tropical forest

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Summary

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- CO₂ efflux from stems (CO_{2,stem}) accounts for a substantial fraction of tropical forest gross primary productivity, but the climate sensitivity of this flux remains poorly understood.
- We present a study of tropical forest CO_{2,stem} from 215 trees across wet and dry seasons, at the world's longest running tropical forest drought experiment site.
- We show a 27% increase in wet season CO_{2,stem} in the droughted forest relative to a control forest. This was driven by increasing CO_{2,stem} in trees 10–40 cm diameter. Furthermore, we show that drought increases the proportion of maintenance to growth respiration in trees > 20 cm diameter, including large increases in maintenance respiration in the largest droughted trees, > 40 cm diameter. However, we found no clear taxonomic influence on CO_{2,stem} and were unable to accurately predict how drought sensitivity altered ecosystem scale CO_{2,stem}, due to substantial uncertainty introduced by contrasting methods previously employed to scale CO_{2,stem} fluxes.
- Our findings indicate that under future scenarios of elevated drought, increases in CO_{2,stem} may augment carbon losses, weakening or potentially reversing the tropical forest carbon sink. However, due to substantial uncertainties in scaling CO_{2,stem} fluxes, stand-scale future estimates of changes in stem CO₂ emissions remain highly uncertain.

Introduction

Aboveground woody biomass is the largest store of carbon in tropical rainforests. The respiration from the stem and branch material within this woody pool has been estimated to account for 13–25% of total ecosystem respiration (Chambers *et al.*, 2004; Cavaleri *et al.*, 2006; Malhi *et al.*, 2009) and 12–27% of gross primary productivity (Ryan *et al.*, 1994; Chambers *et al.*, 2004; Malhi *et al.*, 2009; Doughty *et al.*, 2015). However estimates of stem CO₂ efflux (CO_{2,stem}) remain highly uncertain in tropical forests, as only a handful of studies of CO_{2,stem} exist (Ryan *et al.*, 1994; Meir & Grace, 2002; Malhi *et al.*, 2009, 2013; Robertson *et al.*, 2010; Angert *et al.*, 2012; Katayama *et al.*, 2014, 2016). Consequently, substantial inconsistency exists amongst studies concerning how CO_{2,stem} in tropical forests changes with tree height (Cavaleri *et al.*, 2006; Katayama *et al.*, 2014, 2016), with season (Cavaleri *et al.*, 2006; Stahl *et al.*, 2011) and across environmental gradients (Robertson *et al.*, 2010), and how CO_{2,stem} scales with tree size and growth rate (Meir & Grace, 2002; Cavaleri *et al.*, 2006; Katayama *et al.*, 2016). Given

the concern over tropical forests shifting from a global sink to a source of carbon as the climate changes (Lenton, 2011; Davidson *et al.*, 2012; Brienen *et al.*, 2015; Doughty *et al.*, 2015), understanding how CO_{2,stem} varies with environmental change and how we calculate fluxes at ecosystem scales is becoming increasingly important.

The CO₂ efflux from tree stems is likely to be mostly comprised of respiration derived from growth of new tissue (R_g) and maintenance (R_m) of existing tissues (McCree, 1970; Thornley, 1970; Ryan, 1990; Damesin *et al.*, 2002; Meir & Grace, 2002). However CO₂ efflux measured on trees may under- or overestimate stem respiration from the immediately underlying woody tissue due to other processes occurring within the trees, for example: high concentrations of CO₂ in the soil, most likely from root respired CO₂, being transported up to the site of measurement in sap (McCree, 1970; Levy *et al.*, 1999; McGuire *et al.*, 2007; Saveyn *et al.*, 2008; Teskey *et al.*, 2008; Aubrey & Teskey, 2009; Trumbore *et al.*, 2013; Hillman & Angert, 2016); the transport of CO₂ from below the point of measurement upwards in sap (Angert *et al.*, 2012; Trumbore *et al.*, 2013; Hilman & Angert,

2016); and nonphotosynthetic CO₂ fixation by phosphoenolpyruvate carboxylase (PEPC) (Berveiller & Damesin, 2008). These processes can change over time with changes in sap pH, stem temperature, sap flow velocity or changes in gas diffusivity in the stem over time, which may arise from an increase in air-filled spaces or even cracks in the bark (Cherubini *et al.*, 1997; Levy *et al.*, 1999; Sorz & Hietz, 2006; Teskey *et al.*, 2008; Trumbore *et al.*, 2013). Within tropical trees these processes have been relatively sparsely studied, in part due to the complexities of measuring such processes (Trumbore *et al.*, 2013), particularly in what are often remote, challenging field locations. However, a new approach recently used in tropical forests combined oxygen consumption and CO₂ efflux measurements to show that the apparent respiratory quotient of O₂ to CO₂ (ARQ) of tropical trees was less than the expected value of 1 (0.66 ± 0.18), suggesting that up to a third of CO₂ was being transported away from the site of measurement causing underestimations of stem respiration (e.g. Angert *et al.*, 2012). These results underline the notion that stem CO₂ efflux measurements are likely to comprise signals from growth and maintenance respiration in combination with other stem processes, thus requiring caution when interpreting results.

Tropical forest growth and maintenance respiration (R_g and R_m) components have generally been derived from linear regressions of CO_{2,stem} on growth rate (McCree, 1970; Thornley, 1970; Meir & Grace, 2002), with the intercept interpreted to give the maintenance respiration flux at zero growth rate. Due to the potential loss or gain of CO₂ from other within-stem processes, it is unlikely that these calculations give an entirely accurate representation of R_g or R_m . If, however, we assume that CO₂ is gained or lost equally from the CO₂ produced by R_g or R_m , such methods may still provide a good representation of the proportion of CO₂ derived from growth and respiration, even if the quantitative values are not certain. Knowing these proportions is important because as trees experience climate stress it is likely that growth rates will decline (da Costa *et al.*, 2010; Brien *et al.*, 2015; Korner, 2015), whilst simultaneous investment into maintaining existing tissues may rise (Metcalf *et al.*, 2010; Rowland *et al.*, 2015b). Nonetheless, no studies in tropical forest have determined how growth and maintenance respiration change as mature tropical trees experience climate-related stress, and how this is likely to influence stand-scale CO₂ efflux from woody tissue.

One of the key future climate changes which tropical forests are expected to experience in the coming decades is water stress caused by increased seasonal, interannual and decadal-scale drought (Fu *et al.*, 2013; Boisier *et al.*, 2015; Duffy *et al.*, 2015). Relative to photosynthetic fluxes, how respiration fluxes will respond to drought stress remains poorly constrained (Meir *et al.*, 2008; Atkin & Macherel, 2009; Rowland *et al.*, 2014). Limited data on temperate species suggest that stem CO₂ efflux declines with water stress (Saveyn *et al.*, 2007; Rodríguez-Calcerrada *et al.*, 2014). These studies agree with a number of studies on leaves, which find that leaf respiration is downregulated during short-term drought stress, due to declining substrate availability (Ayub *et al.*, 2011; Catoni & Gratani, 2014; Chastain *et al.*, 2014; O'Brien *et al.*, 2015). By contrast, some studies have

shown increased leaf respiration with drought stress, particularly when drought occurs over extended periods (Miranda *et al.*, 2005; Atkin & Macherel, 2009; Metcalfe *et al.*, 2010; Rowland *et al.*, 2015b; Varone & Gratani, 2015). Increased respiration during drought conditions may be expected if a greater amount of substrate is required for hydraulic repair and maintenance (Brodersen & McElrone, 2013), phloem transport regulation (Mencuccini & Hölttä, 2010) or oxidation of reactive oxygen species (Atkin & Macherel, 2009). Consequently, changes in respiration following drought are likely to be controlled by tree size and genera because trees of different sizes and genera have been shown to experience different hydraulic and metabolic costs as a consequence of drought stress (Rowland *et al.*, 2015a,b), alongside having differing stem growth and maintenance costs.

However, a paucity of studies in tropical ecosystems, and globally, means that our current understanding of how CO_{2,stem}, one of the largest components of autotrophic respiration, will respond to future increases in water stress still remains highly uncertain. This uncertainty is amplified by the existence of various methods for scaling these fluxes to the ecosystem, including according to total stem area or sapwood volume (e.g. Levy & Jarvis 1998; Cavaleri *et al.*, 2006; Katayama *et al.*, 2014), which result in large differences in ecosystem-scale estimates of stem CO₂ release. In the present study, we report the results from a study of CO_{2,stem} on 215 trees in dry and wet seasons, in a forest that has experienced 15 yr of experimental drought and in adjacent corresponding control forest. Using these data we test the following hypotheses: drought causes an increase in CO_{2,stem}, due to increasing maintenance costs associated with low moisture availability; CO_{2,stem} will be significantly different among genera, as metabolic processes and responses to drought are taxonomically conserved; long-term drought increases the proportion of maintenance to growth respiration, as a consequence of increasing maintenance costs and reducing growth; and the effect of long-term drought on stand-scale estimates of CO_{2,stem} changes according to whether CO_{2,stem} rates are scaled using estimates of total stem area or of sapwood volume.

Materials and Methods

Site

The study was performed at a through-fall exclusion (TFE) experiment in the Caxiuanã National Forest reserve in eastern Amazonia (1°43' S, 51°27' W). The site is 15 m above sea level, located within *terra firme* forest on yellow oxisol soils (Ruivo & Cunha, 2003). It experiences a mean annual rainfall of 2000–2500 mm and a pronounced dry season in the later 6 months of the year.

The experiment comprised two 1-ha plots, a control plot with no drought infrastructure and a TFE where plastic panels and guttering at 1–2 m in height are used to exclude 50% of the canopy through-fall from reaching the forest floor (da Costa *et al.*, 2010). Both plots were trenched to 1–2 m to prevent lateral flow of water in the soil. To maintain biogeochemical inputs into the soil, leaf litter on the TFE panels is relocated to the forest

floor every few days. The TFE treatment has been maintained since January 2002, and therefore before this study all trees on the TFE had experienced 15–16 yr of a 50% reduction in canopy through-fall. Further details on the experiment can be found in da Costa *et al.* (2010) and Meir *et al.* (2015).

Sample selection

Measurements were performed on 215 trees in total, 105 from the control plot and 110 from the TFE during October 2016 (mid dry season) and April 2017 (mid wet season). First, we selected trees from 12 of the most common genera found on both the control plot and the TFE (*Aspidosperma*, *Eschweilera*, *Inga*, *Licania*, *Micropholis*, *Minquertia*, *Pouteria*, *Protium*, *Swartzia*, *Syzygiopsis*, *Virola* and *Vouacapoua*), totalling 87 and 77 trees on the control and the TFE plots, respectively. The remainder of the trees – 18 on the control plot and 33 on the TFE plot – comprised trees with a diameter at breast height (dbh) > 30 cm on the TFE and > 40 cm on the control, measured to ensure more equal division of trees amongst size classes. From October 2013 to January 2016, seven measurement campaigns were also carried out on 16–18 trees on the control and 19–20 trees on the TFE, of the genera (*Eschweilera*, *Licania*, *Manilkara*, *Pouteria*, *Protium* and *Swartzia*) previously sampled for photosynthesis measurements by Rowland *et al.* (2015b). A list of all of the species samples in each measurement campaign from 2013 to 2017 is presented in Supporting Information Table S1.

CO₂_{stem} measurements

CO₂_{stem} was measured using a transparent acrylic chamber, temporarily sealed onto the stem surface using a closed cell non-CO₂ adsorbent foam gasket and two ratcheting straps. The chamber was sealed to the stem at a constant gasket thickness and had a volume of 213 cm³ (including tubing and foam) and a surface area of 75 cm² of the bark surface. The chamber size and construction were similar to those used for other measurements of CO₂ efflux in tropical forests (Stahl *et al.*, 2011; Rowland *et al.*, 2013). The chamber was connected to an infrared gas analyser (EGM4, EGM5; PPSystems, Hitchin, UK) for 220 s and was used to detect an increase in CO₂ concentration inside the chamber. Following Rayment & Jarvis (2001), to promote air mixing in the chamber without creating vortex effects from the operation of a fan, the chamber also contained a 15-cm length of tube perforated with 0.5 mm diameter holes, connected to the inlet. During each measurement we tested for leaks by exposing the edges of the chamber to very high CO₂ concentrations. If any increase in CO₂ concentration inside the chamber was detected, the measurement was aborted. Wood temperature (*T_w*) was measured using a type T thermocouple placed into the bark, or where this was not possible, on the bark surface. All measurements were made between 08:00 h and 14:00 h.

Measurements of the increase in CO₂ concentration between 120 and 220 s were used for analysis, leaving 2 min for the chamber to stabilize post-installation. The slope of the linear regression

between time and CO₂ was extracted to calculate CO₂_{stem} (stem CO₂ efflux, μmol m⁻² s⁻¹) using Eqn 1

$$\text{CO}_{2_stem} = \frac{\Delta\text{CO}_2}{\Delta t} \times \frac{V_c}{S_c} \times a \times \frac{273.15}{273.15 + T_w}, \quad \text{Eqn 1}$$

(ΔCO₂/Δ*t*, slope of the CO₂–time relationship; *V_c* volume (cm³) and *S_c* the surface area (cm²) of the chamber; *a*, volume of a mole of CO₂ (mol cm³); *T_w*, measured wood temperature (°C)). Linear slope values with a correlation coefficient < 0.98 were discarded from the analysis and the data were temperature-corrected to 25°C using a *Q₁₀* of 2.0 (Cavaleri *et al.*, 2006). After excluding measurements with leaks or with a correlation coefficient < 0.98, 97 measurements were included on the control plot and 108 on the TFE plot from the dry season; and 97 from the control and 99 from the TFE plots, respectively, were included from the wet season.

Diurnal tests

In order to test for daytime increases in stand-scale CO₂_{stem} (*S*_{CO₂_{stem}), which could result in biases according to the time CO₂_{stem} was measured or indicate other forms of CO₂ transport or consumption (Teskey *et al.*, 2008; Angert *et al.*, 2012), we measured CO₂_{stem} every 15 s for 24 h on 20 trees from the control and the TFE in October 2013, using an open path respiration system similar to that used elsewhere (Rayment & Jarvis, 2001; Meir & Grace, 2002) and a CIRAS 1 IRGA (PPSystems); for further details see Methods S1). We found very limited diurnal variation in CO₂_{stem}, indicating limited bias concerning the time of day the measurements were taken (see Fig. S1 and Methods S1).}

Growth data

Quarterly mean tree-level stem diameter increment per plot from 2010 to 2015 were taken from dendrometer measurements presented in Rowland *et al.* (2015a), and updated to the end of 2016 following the same methodology and converted to units of cm d⁻¹. A long-term annual increment then was calculated for each tree based on the 2010–2016 dataset. This interval (2010–2016) was chosen as it represented the period after which the growth rates of the small and medium trees on the TFE (10–40 cm dbh) had re-stabilized following increased growth rates in response to elevated light intensities (see Rowland *et al.*, 2015a for further details). We note that accurate growth measurements were not available for some of the larger trees in this study, as it was not feasible to monitor these trees on a three-monthly basis due to their size or because a dendrometer could not be accurately fitted on the tree due to substantial trunk-shape irregularities.

Scaling

Scaling was performed using three methods, which are described in detail in Methods S1. The three methods were used to assess the effect of different scaling assumptions on *S*_{CO₂_{stem}}

estimates. Method one (M1) involved scaling according to total stem surface area. Method two (M2) used estimated total sapwood volume as the scalar. Initially we estimated total sapwood volume to be 34% of total tree volume (an estimate of the sapwood area: basal area ratio at 1.3 m above ground level; see Methods S1 and Fig. S2) and then, given that 34% is likely to underestimate the greater percentage sapwood area in smaller diameter branches, we assessed how this calculation changed using an estimate of 50% and 80% sapwood volume. We assumed constant live-cell fraction in all sapwood volume estimates. Method 3 (M3) involved a combination of the two scaling methods above. Following Cavaleri *et al.* (2006), but taking a total sapwood volume approach, we assumed that for any part of the canopy < 10 cm in diameter CO_{2_stem} scaled with total stem surface area, and for sections > 10 cm CO_{2_stem} was scaled with total sapwood volume. For all methods trees within 10 m of the edge of the plots were excluded from our calculations to eliminate possible long-term effects of the trenching on the community structure and tree physiology (da Costa *et al.*, 2010). Wet and dry season S_CO_{2_stem} estimates from all scaling methods were averaged and converted to units of $\text{Mg C ha}^{-1} \text{ yr}^{-1}$.

Analysis

All statistical analyses were performed in the statistical package R (v.3.4.0; R Core Team, 2017) and all errors are shown as standard errors on the mean, but do not account for the sampling error of the calibration of the gas analyser (< 1% in EGM). Following Damesin *et al.* (2002) and Meir & Grace (2002), we calculated averaged plot-level maintenance respiration as the intercept of the relationship between growth and CO_{2_stem} , but using a bootstrapping technique, to avoid assumptions about normality of distribution and to facilitate the calculation of errors. First we randomly sampled our study trees, with replacement, to create 1000 samples of the trees which had growth and CO_2 efflux data on each plot (75 control, 87 TFE). Following this, we calculated 1000 estimates of: mean total CO_{2_stem} per tree, for each plot; the y -intercept of the woody increment– CO_{2_stem} relationship (R_m); and R_g , calculated as mean CO_{2_stem} minus R_m . Mean and SE values of CO_{2_stem} , R_m and R_g per tree for each plot were then calculated from the mean and SD of the bootstrapped samples. Data comparisons of the proportions of R_m and R_g between plots, seasons and tree size classes (small: 10–20 cm dbh, medium: 20–40 cm dbh and large: > 40 cm dbh) were then made. Given that the bootstrapping created a normal distribution, statistical comparisons of CO_{2_stem} , R_m and R_g were made using a parametric paired t -test and only percentage values of R_m and R_g are presented, acknowledging that absolute values are uncertain because of uncertainties in estimating woody respiration from CO_{2_stem} (Teskey *et al.*, 2008; Trumbore *et al.*, 2013).

Analysis of whether CO_{2_stem} scales with surface area or sapwood volume was performed following Levy & Jarvis (1998). Log-transformed linear relationships were created for CO_{2_stem} ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) against dbh and CO_{2_stem} ($\mu\text{mol m}^{-3} \text{ s}^{-1}$) against $1/\text{dbh}$. A significant relationship between area-based CO_{2_stem} and dbh indicates that a scaling relationship with

volume exists, and a significant relationship between volume-based CO_{2_stem} and $1/\text{dbh}$ indicates that a scaling relationship with area exists (Levy & Jarvis, 1998). Consequently the slopes of these relationships indicate the proportional scaling with volume or area (respectively) as, for example, a slope of 1 between dbh and CO_{2_stem} ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) would indicate perfect volume scaling, whilst a slope of 0 would indicate perfect area scaling (see Levy & Jarvis, 1998).

Results

Drought response of CO_{2_stem}

The CO_{2_stem} rates of trees on the control plot averaged $1.00 \pm 0.10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ across both seasons, showing significantly higher CO_{2_stem} values in the dry season (dry = $1.01 \pm 0.08 \mu\text{mol m}^{-2} \text{ s}^{-1}$, wet = $0.87 \pm 0.07 \mu\text{mol m}^{-2} \text{ s}^{-1}$; $P < 0.01$; Fig. 1a). By contrast, on the TFE plot there was a significant increase in CO_{2_stem} during the wet season relative to the control plot and the dry season ($P < 0.01$, dry = $0.99 \pm 0.06 \mu\text{mol m}^{-2} \text{ s}^{-1}$, wet = $1.23 \pm 0.08 \mu\text{mol m}^{-2} \text{ s}^{-1}$). This represented a 27% increase in CO_{2_stem} on the TFE during the wet season relative to the control plot, a seasonal increase on the TFE plot itself of 24% relative to the dry season, and therefore an overall 11% increase in the mean wet and dry season CO_{2_stem} on the TFE relative to the control plot (Fig. 1a). Data from a previous analysis of 21 trees (see the Materials and Methods section, Methods S1 and Table S1) per plot measured six times between October 2013 and February 2016, also confirmed that the TFE tended to have consistently higher fluxes than the control plot during the wet season and more equal fluxes during the dry season (Fig. 1b). However, we note that the magnitude and plot differences in these latter flux values are likely to be less reliable due to a lower sample size.

The increase in CO_{2_stem} on the TFE was controlled predominantly by significant increases in CO_{2_stem} from trees smaller than 40 cm dbh, which occurred in the wet, but not the dry season (Fig. 2). Interestingly, CO_{2_stem} increased with tree size on both plots, and this increase was more pronounced in the wet season and on the control plot (Fig. 2), where CO_{2_stem} of the largest tree size class (> 60 cm dbh) was 3.4-fold greater than that for the smallest (< 15 cm dbh; Fig. 2b). On the TFE, due to the elevated CO_{2_stem} in the smallest trees, this increase in CO_{2_stem} from the smallest to the largest trees was reduced to 2.6-fold.

Taxonomic patterns in CO_{2_stem}

Strong changes in CO_{2_stem} with tree size resulted in high variation in CO_{2_stem} within each genus (Fig. 3). Consequently, no significant differences were found among genera on each plot in dry season (Fig. 3). *Protium* was found to have significantly elevated CO_{2_stem} on the TFE, relative to the control during the wet season, although it did not demonstrate a significant increase from dry to wet season on the TFE (Fig. 3b,d). It is also noteworthy that, excluding *Protium* on the control and *Aspidosperma* and *Inga* on the TFE, the mean values per genus are largely similar

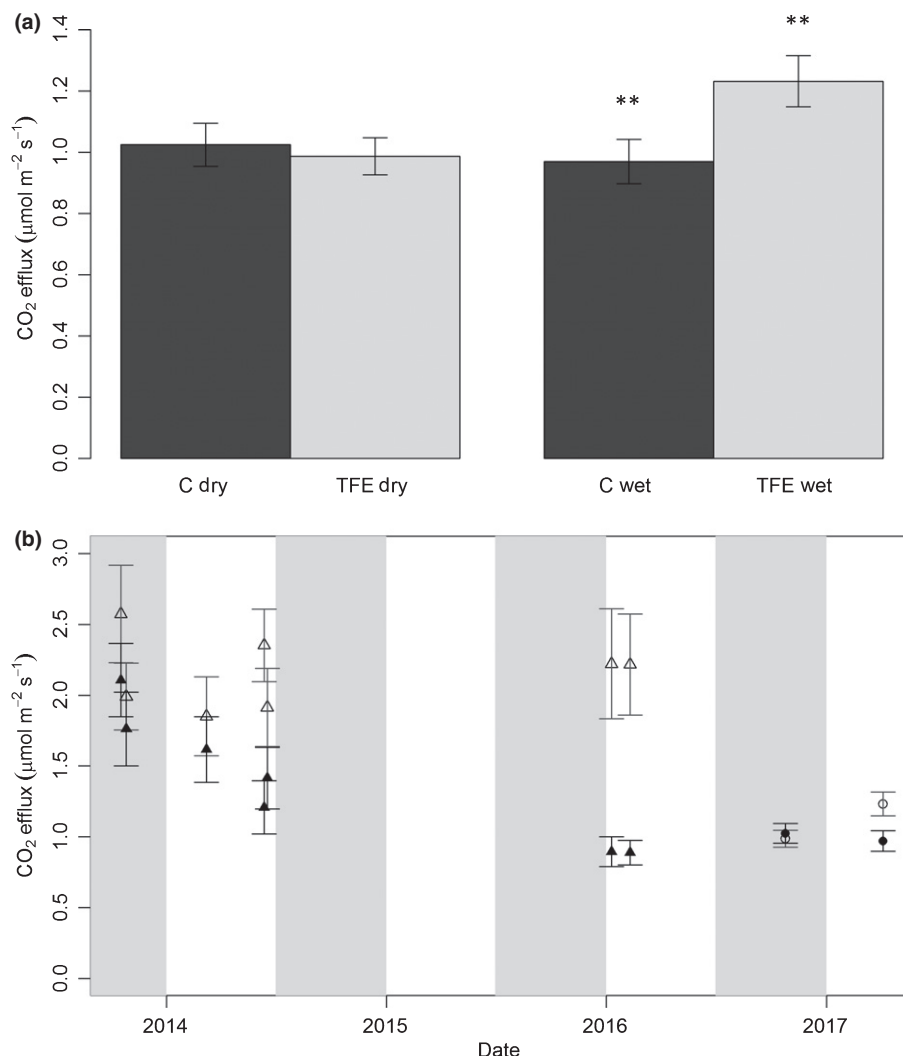


Fig. 1 (a) Stem CO₂ efflux (μmol m⁻² s⁻¹) in mid dry season (October 2016) and mid wet season (April 2017) on the control (C, black) and through-fall exclusion (TFE) plot (grey). Asterisks indicate significant increase at *P* < 0.01 between columns. (b) Stem CO₂ efflux (μmol m⁻² s⁻¹) on a time series of data from the control (closed symbols) and the TFE (open symbols) plot. Grey shaded areas show dry season months (July–December), triangles indicate measurements taken with *n* = 21 individuals per plot, and circles indicate measurements taken with *n* = 105 on the control and *n* = 110 on the TFE (see the Materials and Methods section and Supporting Information Table S1). Error bars indicate ± SE.

within and between plots, as well as between seasons. These results suggest that CO_{2,stem} drought responses are not strongly taxonomically conserved.

Growth and maintenance respiration

Relationships between CO_{2,stem} and mean woody increment for 2010–2016 were performed on a *per* tree basis separately for mean annual total (wet and dry season), wet season and dry season CO_{2,stem}, and for mean annual CO_{2,stem} divided into small, medium and large size classes. On both plots CO_{2,stem} by season or size class always had a positive and significant (at least *P* < 0.01) relationship with mean wood increment (Fig. 4; Table 1). These relationships had a larger *r*² values on the control plot (e.g. *r*² control plot annual mean = 0.61, TFE plot annual mean = 0.37; Table 1); however, there were also consistently greater *r*² values in larger trees compared to small trees on both plots (Fig. 4; Table 1). When the percentage *R*_m and *R*_g values were estimated from these relationships, we find that on an annual basis the CO₂ efflux associated with *R*_m accounts for 58 ± 10% and 67 ± 10% of total respiration on the control and

TFE plot, respectively (Table 1; Fig. 5a). Furthermore, we find limited seasonal change in these values when averaging across trees of all size classes (Fig. 5a; Table 1). When trees were divided into size classes there were, however, strong shifts in the percentage division of *R*_m and *R*_g. On the control plot in the small trees 80 ± 10% of the respiration was *R*_m, and this declined to 60 ± 22% and 43 ± 27% in the medium and large trees, respectively (Fig. 5b; Table 1). By contrast, on the TFE the small trees had a lower percentage *R*_m, 62 ± 14%, and this increased in the medium and large trees to 75 ± 20% and 78 ± 21%, respectively (Fig. 5b; Table 1). This suggests that *R*_m increases substantially in larger trees as a consequence of drought.

Scaling CO_{2,stem}

On the control plot in both the wet and dry season data, there was a stronger correlation between log-transformed CO_{2,stem} on an area basis (*r*² = 0.20–0.28) and dbh, than on a volume basis and 1/dbh (*r*² = 0.08; Fig. 6a,b,e,f). On the TFE plot, the relationships with dbh and 1/dbh were generally weaker than on the control plot (*r*² = 0.10–0.18; Fig. 6). However, on both the

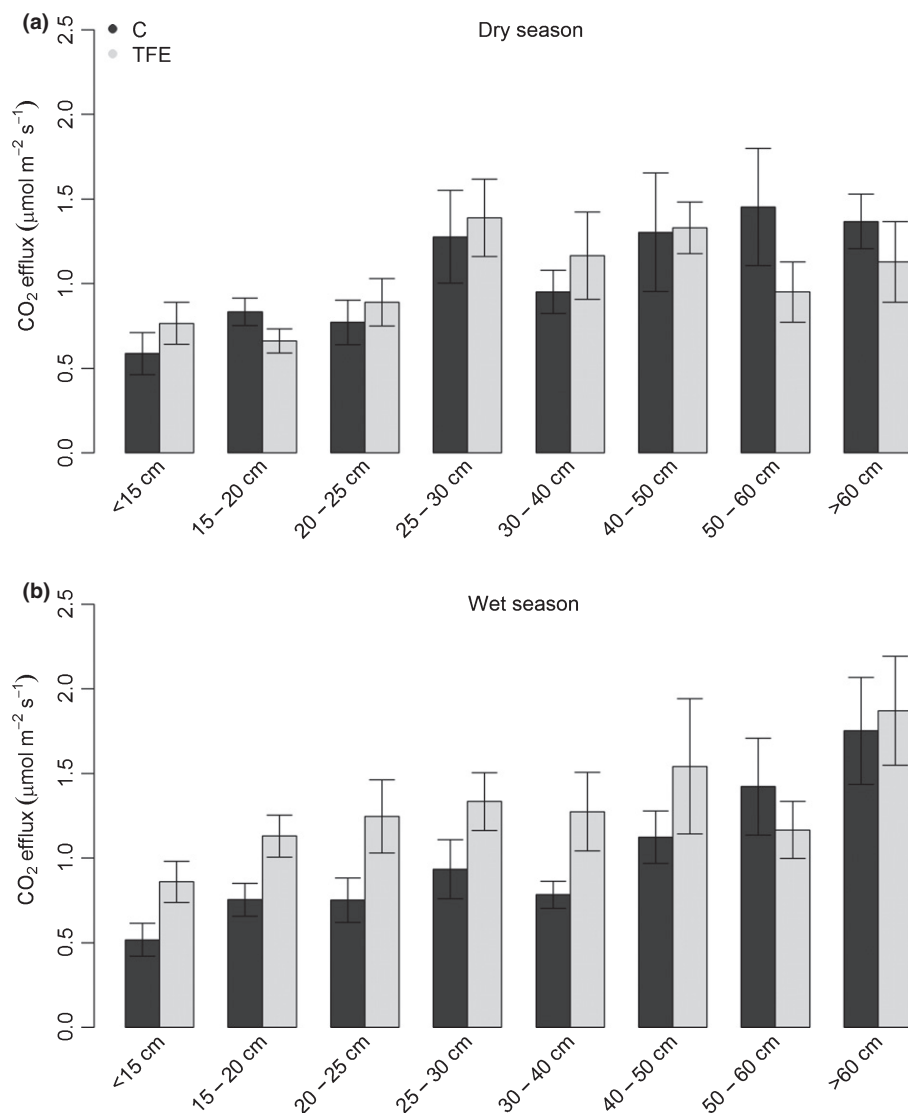


Fig. 2 Mean stem CO₂ efflux (μmol m⁻² s⁻¹) in (a) mid dry season (October 2016) and (b) mid wet season (April 2017) on the control (C, black) and through-fall exclusion (TFE) plot (grey) for trees divided into diameter at breast height (1.3 m; dbh) size classes of < 15, 15–20, 20–25, 25–30, 30–40, 40–50, 50–60 and > 60 cm. Error bars show ± SE.

control and the TFE such low r^2 values created substantial uncertainty concerning whether area or volume is a better scalar; for example, the slope values for CO₂_{stem} by area against dbh and CO₂_{stem} by volume against 1/dbh in the dry season indicated a range of the percentage of the data which scaled with area from 50 ± 10% to 70 ± 10% on the control plot and of 39 ± 10% to 62 ± 10% on the TFE.

When we scaled up the CO₂_{stem} values to S_CO₂_{stem} for each plot, the various estimates for the stand-scale flux of the control ranged by 4.7 Mg C ha⁻¹ yr⁻¹ and those of the TFE plot by 5.1 Mg C ha⁻¹ yr⁻¹ (Table 2). Furthermore, the percentage reduction in the S_CO₂_{stem} on the TFE relative to the control ranged from 0.7–22.9%, depending on the method of scaling (Table 2). The highest estimates of S_CO₂_{stem} came from using surface area as the scalar; however, these values were similar to the scaling outcome using the method of assuming volume as the scalar for wood < 10 cm diameter and area as the scalar for bole diameters > 10 cm. The area and the area–volume scaling methods both produced very small percentage differences between the

control and the TFE S_CO₂_{stem}. By contrast, scaling by sapwood volume alone produced substantially larger differences between the plots (in both absolute and relative terms), which were well-conserved across the range of percentage sapwood volume used (34–80%). Scaling by sapwood volume produced far lower S_CO₂_{stem} values, which were also highly sensitive to the percentage value of sapwood volume used (Table 2).

Discussion

Using the world's longest-running drought experiment in tropical forest and measurements of CO₂ efflux from 215 stems in the wet and dry seasons, we demonstrated that the efflux of CO₂ from stems (CO₂_{stem}) increased by 27% on drought-treated TFE (through-fall exclusion) trees relative to control trees in the wet season. The increases in CO₂_{stem} were caused by large increases, of up to 40%, in the efflux rate of CO₂ released from trees < 40 cm diameter at breast height (dbh) in the wet season, increases which were absent in the dry season. Furthermore, we

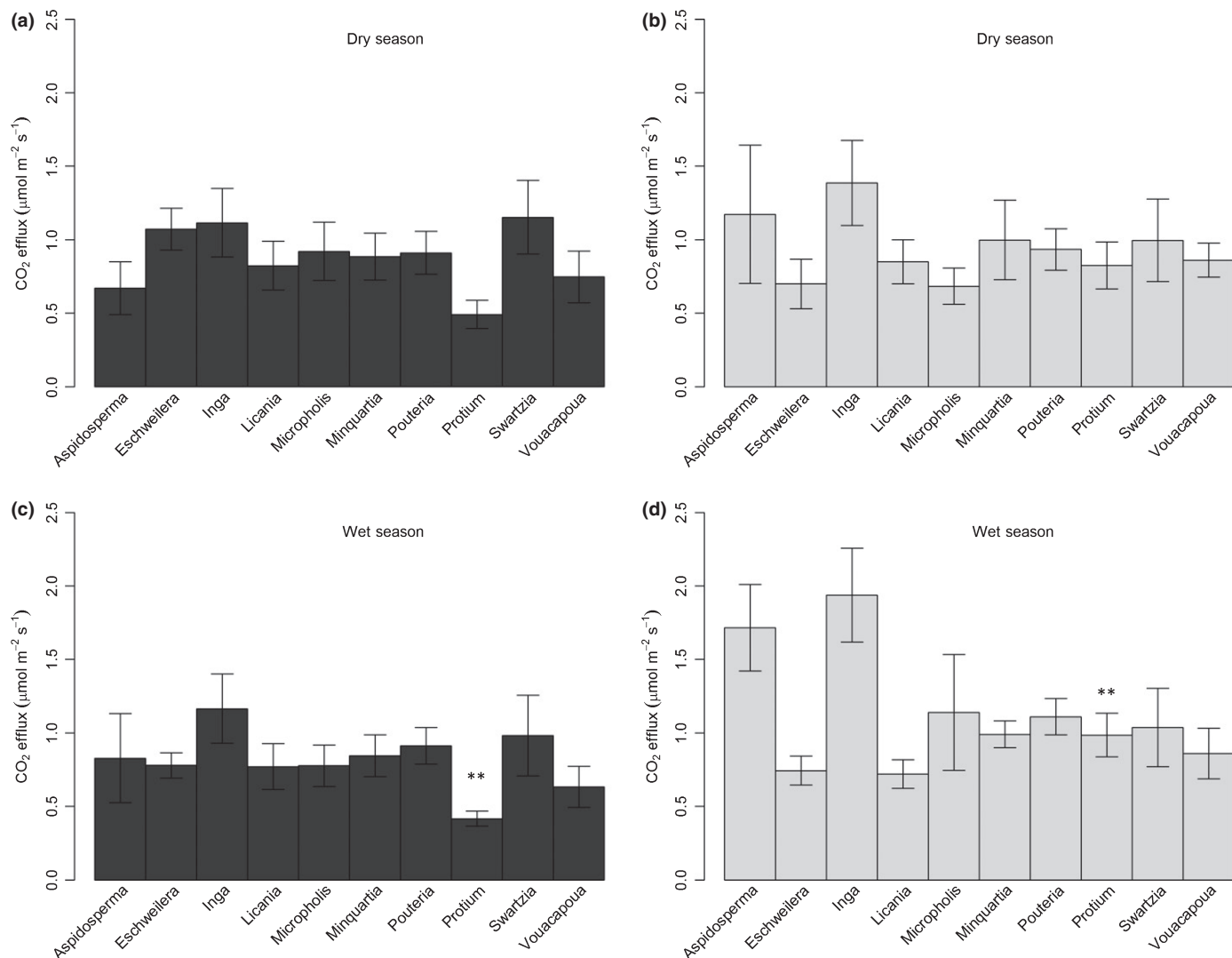


Fig. 3 Mean stem CO₂ efflux (μmol m⁻² s⁻¹) in (a, b) mid dry season (October 2016) and (c, d) mid wet season (April 2017) on (a, c) the control (C, black) and (b, d) through-fall exclusion (TFE) plot (grey) for trees divided into genus groups, with greater than two individuals per group (see Supporting Information Table S1). Error bars show ± SE. Matching symbols indicate that columns are different at $P < 0.05$.

found that there was a substantial increase in the percentage of total respiration that is associated with respiration resulting from maintenance (R_m) on the TFE relative to the control, driven by reduced efflux associated with respiration resulting from growth (R_g) and increased efflux associated with R_m in the medium and large trees. Finally we show that the stand-scale CO_{2,stem} ($S_{CO_{2,stem}}$) estimates, as well as the differences in $S_{CO_{2,stem}}$ between plots are highly sensitive to the scaling method used, with absolute values varying by > 300% within plots and the percentage change between the plots varying by up to 22%.

Following 15 yr of rainfall exclusion, wet season CO_{2,stem} rates on the TFE plot were 27% higher (Figs 1, 3). This result contrasts with findings in temperate forests, where CO_{2,stem} declined, but with short-term water stress (Saveyn *et al.*, 2007; Rodríguez-Calcerrada *et al.*, 2014). However, our result is consistent with several reports elsewhere of drought-related increases in respiration (Miranda *et al.*, 2005; Varone & Gratani, 2015) and corroborates previous results from this site

which showed substantial increases in leaf dark respiration on the TFE plot following extended periods of reduced soil moisture availability (Metcalf *et al.*, 2010; Rowland *et al.*, 2015b), and evidence of coupled increases in root respiration (Metcalf *et al.*, 2007; Meir *et al.*, 2008). Given that the elevated CO_{2,stem} occurs only in the wet season, we speculate that this could be caused by increased growth rates in the small and medium trees found to occur on the TFE (Rowland *et al.*, 2015a) or potentially because the xylem tissue is undergoing hydraulic recovery (Brodersen & McElrone, 2013), following high hydraulic stress which is likely to occur during periods of extreme vapour pressure deficit (VPD) and low rainfall during the dry season on the TFE (Rowland *et al.*, 2015a). This hypothesis is supported further by the significant increase in percentage of R_m on the TFE relative to the control during the wet season (Fig. 1a; Table 1), suggesting that the cost of maintaining existing tissues may be substantially higher on the TFE plot, especially in the largest trees.

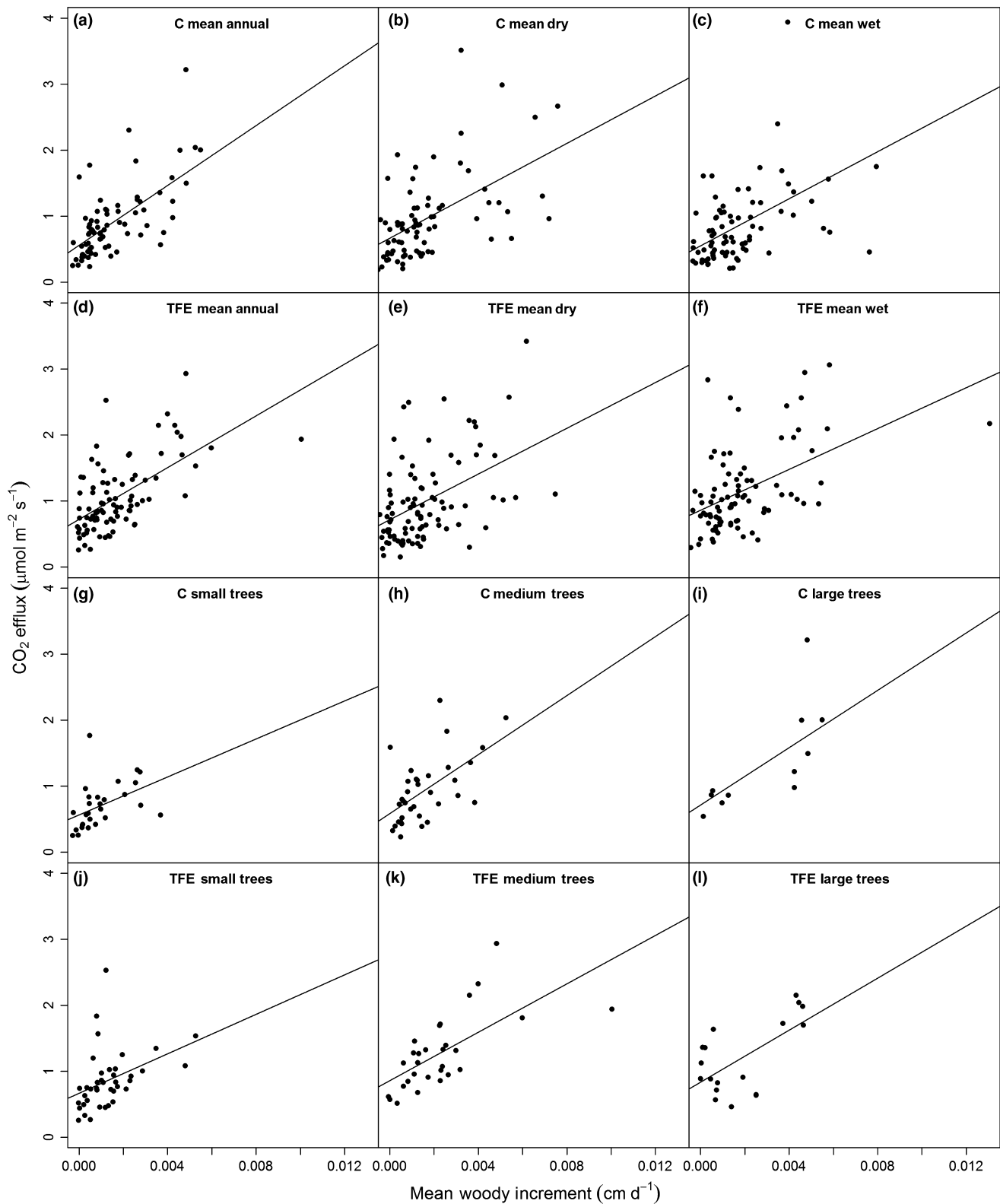


Fig. 4 Relationships between mean stem CO_2 efflux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and the 2010–2015 mean annual woody increment (cm d^{-1}) for the control (C) and through-fall exclusion (TFE) plots for (a, d) mean annual stem CO_2 efflux, (b, e) dry season CO_2 efflux, (c, f) wet season stem CO_2 efflux, and mean annual CO_2 efflux split into (g, j) small (10–20 cm), (h, k) medium (20–40 cm) and (i, l) large trees (> 40 cm). Linear fit lines indicate significant ($P < 0.05$) linear relationships. Correlation coefficients, P -values and intercepts are shown in Table 1.

Table 1 Intercept (Int.), correlation coefficient (r^2) and P -values (P), mean total stem CO_2 efflux (CO_{2_stem} ; standard error given as $\text{CO}_{2_stem_se}$) and the percentage (%) of total CO_2 efflux associated with R_m and R_g for the panels in Fig. 5 (pan.) representing CO_{2_stem} values on the control (C) and through-fall exclusion (TFE) averaged annually, for the wet and dry seasons, and average annual values separated by tree size (small, 10–20 cm diameter at breast height (dbh); medium, 20–40 cm dbh; large > 40 cm dbh)

Panel	Variable	r^2	P	Int.	CO_{2_stem}	$\text{CO}_{2_stem_se}$	% R_m	% R_g
(a)	C annual	0.61	0.00	0.55	0.95	0.07	58	42
(b)	C dry	0.44	0.00	0.68	1.02	0.08	66	34
(c)	C wet	0.44	0.00	0.53	0.89	0.08	60	40
(d)	TFE annual	0.37	0.00	0.72	1.07	0.06	67	33
(e)	TFE dry	0.17	0.00	0.75	0.99	0.07	76	24
(f)	TFE wet	0.25	0.00	0.85	1.15	0.07	74	26
(g)	C small	0.19	0.01	0.56	0.70	0.06	80	20
(h)	C medium	0.41	0.00	0.58	0.98	0.11	60	40
(i)	C large	0.73	0.00	0.68	1.59	0.31	43	57
(j)	TFE small	0.14	0.01	0.67	1.07	0.10	62	38
(k)	TFE medium	0.46	0.00	0.80	1.07	0.10	75	25
(l)	TFE large	0.36	0.01	0.83	1.07	0.16	78	22

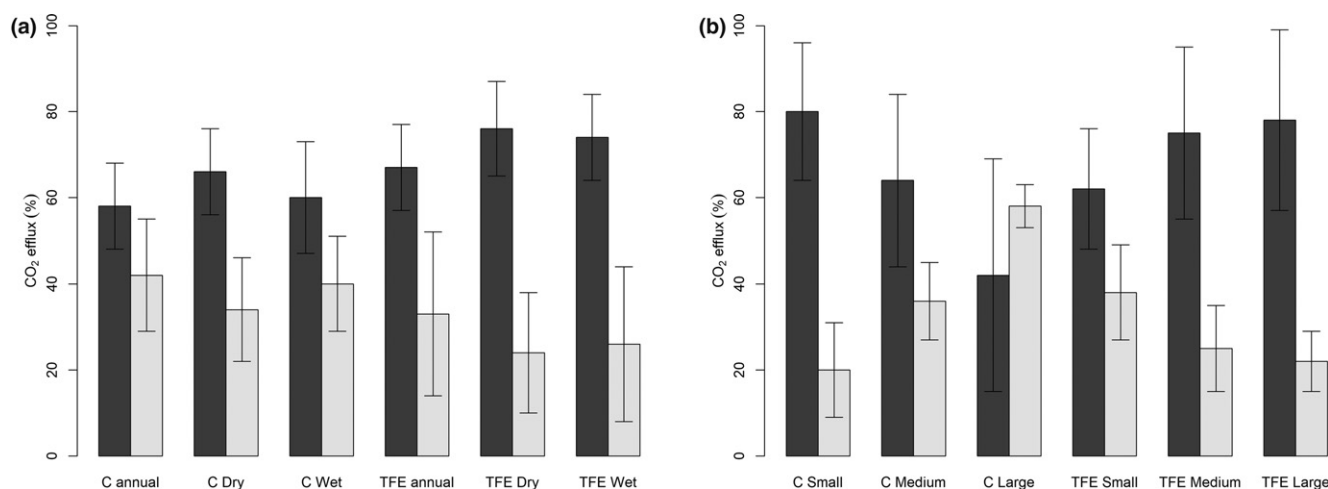


Fig. 5 Estimated percentage of maintenance respiration (black) and growth respiration (grey) for the control plot (C) and through-fall exclusion (TFE) plot, divided by (a) plot and season and (b) by tree size, averaging respiration across seasons. Error bars show \pm SE.

Previously, maintenance respiration was estimated to comprise *c.* 80% of total respiration in mature trees in closed tropical rainforests (Ryan *et al.*, 1994; Meir & Grace, 2002). Our analysis indicates that the division of CO_{2_stem} associated with R_g and R_m varies substantially by tree size class and with drought in tropical forest. On the control plot there was a strong trend toward decreases in percentage R_m with increasing tree size and increasing percentage R_g (Fig. 5b). This strong percentage decline in R_m with tree size was absent from the TFE plot trees, where percentage R_g declined with tree size (Table 1; Fig. 5b). Instead, on the TFE we observed a substantial increase in R_m in the largest trees relative to the control plot (Fig. 5b). As the largest trees are mostly likely to suffer damage, particular hydraulic damage, following drought stress (Bennett *et al.*, 2015; McDowell & Allen, 2015; Rowland *et al.*, 2015a), these results may suggest that these trees are unable to invest as much carbohydrate resource into R_g . This may be driven by elevated maintenance costs associated with repairing drought-damaged cells, removing reactive oxygen species, elevated phloem transport regulation or repair and/or replacement of hydraulically damaged xylem tissue. However, we

note that the errors on our estimates of maintenance respiration are large for certain tree size classes (Table 1), due to smaller proportions of variance in CO_{2_stem} being explained by growth in some size classes than others. This may suggest that other unmeasured interaction variables are necessary to quantify the proportions of growth and maintenance respiration with greater accuracy.

In our analysis, we find no clear evidence of whether scaling by surface area or sapwood volume is more appropriate (Fig. 6). However we note that having used a relationship to estimate sapwood volume, we have estimates of sapwood volume, rather than a direct measurement and CO_{2_stem} may be more prone to error when calculated on a sapwood volume basis, than when calculated on a surface area to CO_{2_stem} . Consequently we tested a variety of scaling methods to estimate our fluxes at the plot level. Competitive release of smaller trees on the TFE plot following a 40% loss of biomass from the mortality of the largest trees (da Costa *et al.*, 2010; Rowland *et al.*, 2015a) enhanced the growth and recruitment of the smallest size-class trees, which also have the largest surface area to volume ratio. This shift in size

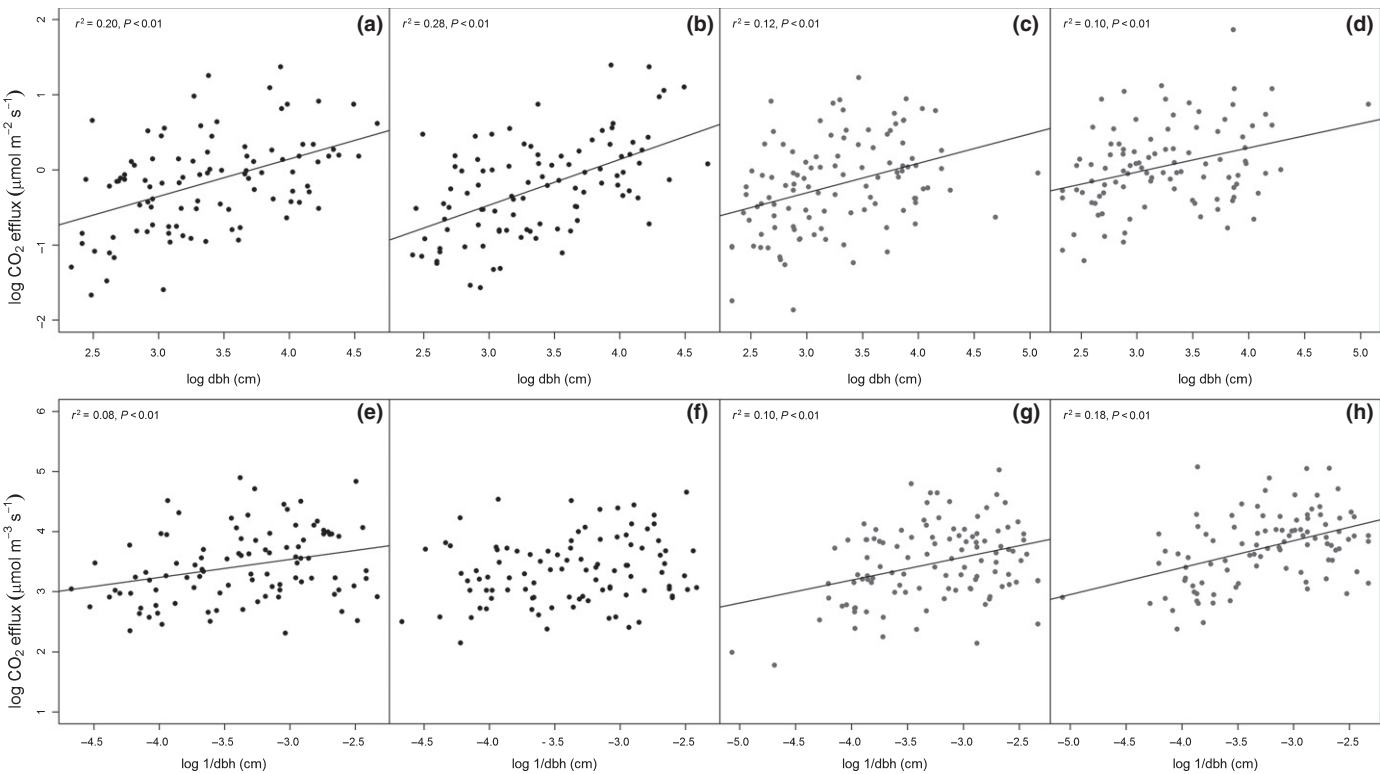


Fig. 6 Relationships between log stem CO₂ efflux by area (μmol m⁻² s⁻¹) and log diameter at breast height (dbh, cm) for the control (black) and through-fall exclusion (TFE) (grey) plot in (a, c) dry and (b, d) wet. Relationships between log stem CO₂ efflux by volume (μmol m⁻³ s⁻¹) and log₁/diameter are also shown for the control and TFE plot in (e, g) dry and (f, h) wet season. Linear regression fits, *r*² and *P*-values are shown for significant (*P* < 0.05) relationships.

Table 2 StemCO₂ efflux (CO₂_{stem}) values scaled to plot level (Mg C ha⁻¹ yr⁻¹) for the control and the through-fall exclusion (TFE) plots, calculated according to: surface area scaling; volume scaling assuming 34%, 50% and 80% of the volume is sapwood (SW); scaling assuming CO₂_{stem} scales with volume for tree boles < 10 cm and with area for all woody sections > 10 cm diameter at breast height (dbh)

	Control	TFE	Change (%)
Surface area	7.07 ± 0.72	6.94 ± 0.63	1.8
Volume, 80% SW	5.53 ± 0.56	4.26 ± 0.39	22.9
Volume, 50% SW	3.46 ± 0.35	2.67 ± 0.24	22.8
Volume, 34% SW	2.40 ± 0.24	1.86 ± 0.17	22.5
Volume bole > 10 cm, area < 10 cm	6.81 ± 0.69	6.76 ± 0.61	0.7

Error term shows the ± SE propagated from the error on the measured CO₂ efflux values only. The final column demonstrates the percentage change of the TFE relative to the control. The frequency distribution of trees across size categories for each plot can be seen in Supporting Information Table S2.

distribution caused the TFE plot to have S_CO₂_{stem} that was almost equal to the S_CO₂_{stem} for the control plot when surface area, or mostly surface area-based scaling was used, but substantially lower S_CO₂_{stem} when volume was used as the scalar. Scaling by area is the most common form of scaling of CO₂_{stem} to the canopy (Chambers *et al.*, 2004; Malhi *et al.*, 2013). Given the radial live-cell distribution in woody tissue it is unlikely, particularly in large diameter woody sections, that

CO₂_{stem} scales directly with area, because CO₂ production occurs in the living sapwood and phloem tissue (Fig. 5; Meir & Grace, 2002; Cavaleri *et al.*, 2006; Levy & Jarvis, 1998). Scaling by sapwood volume does, however, introduce very large uncertainties into S_CO₂_{stem} estimates (Table 2), because the proportion of tree volume that is sapwood remains uncertain, as does the fraction of sapwood cells that are metabolically active. How sapwood volume scales with diameter within trees and between species in tropical forests is very sparsely studied (Meir *et al.*, 2017), with no current estimates on how to calculate the sapwood volume of a tree (including the canopy), or its variation among species. In addition, the allometric scaling equations used for calculating tree volume and surface area (Methods S1) are also likely to introduce large errors into S_CO₂_{stem} estimates, the magnitudes of which are hard to estimate. Biomass studies have shown this may be particularly true for the largest trees (Calders *et al.*, 2015), and this may suggest that greater unknown error exists in the S_CO₂_{stem} value for the control plot, where there are more large trees.

Throughout this study we present all absolute measured values as CO₂_{stem} while acknowledging that there are likely to be many other processes occurring within the stem, which may result in raw chamber-based measurements of CO₂ efflux from the stem, leading to over- or underestimates of the actually woody stem respiration underlying the measurement chamber (McCree, 1970; Levy *et al.*, 1999; McGuire *et al.*, 2007; Berveiller & Damesin, 2008; Saveyn *et al.*, 2008; Teskey *et al.*, 2008; Aubrey & Teskey,

2009; Angert *et al.*, 2012; Trumbore *et al.*, 2013; Hilman & Angert, 2016). However, we do note that we found limited diurnal changes in CO_{2_stem} (Fig. S1), suggesting, as found elsewhere (Ubierna *et al.*, 2009; Stahl *et al.*, 2011), that the upward transport of 'excess' CO_2 from the soil or roots or the upward transport of CO_2 from the point of measurement may be limited in this forest, or compensated for by other processes. Measurements of woody tissue respiration using techniques for measuring oxygen absorption were not feasible at our remote study site, nor on the number of trees presented here. However, given the number of trees sampled, the limited evidence of diurnal variation in CO_{2_stem} , and the good replication of tree genera and tree sizes between the plots, we believe that our study does give as accurate a representation as is currently possible of the changes in stem CO_2 efflux and the proportions of associated R_m and R_g which occur as a result of long-term drought.

Our results suggest that under prolonged periods of drought stress, increasing CO_{2_stem} , particularly from small and medium trees, is likely to augment carbon losses from vegetation to atmosphere, which are already likely from drought-induced mortality. At large scales this response will either further weaken or potentially reverse the tropical forest carbon sink. However, we demonstrate that scaling CO_{2_stem} values to the stand-scale is currently subject to very high levels of uncertainty, limiting predictions of both the absolute values of stand-scale CO_{2_stem} and their proportional variation. This will be especially relevant when ecosystems are subject to climatic stresses, such as drought, which are likely to alter ecosystem size structure and related growth, and related physiological-response regimens.

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Author contributions

The research was designed by L.R., P.M., M.M., A.C.L.d.C., R.S.O., L.V.F. and S.S.V.; data collection, interpretation and analysis was carried out by L.R., A.C.L.d.C., A.A.R.O., P.L.B., P.B.C., A.L.G., A.I.S., I.C., J.L.G., J.A.S.J., M.M. and P.M.; and the manuscript was written by L.R. with contributions from all other authors.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Diurnal variation of stem CO₂ efflux from trees on the control and TFE plots.

Fig. S2 Relationships between sapwood depth and tree diameter and basal area.

Table S1 List of the tree diameter and species of all trees sampled in this study

Table S2 Distribution of trees across size classes for all trees >10 cm diameter at 1.3 m above ground on the control and TFE plots

Methods S1 Additional methods relating to the measurement and scaling of stem CO₂ efflux data.

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