

## New Phytologist Supporting Information Figs S1-S3 and Methods S1

Article title: The variation of productivity and its allocation along a tropical elevation gradient: a whole carbon budget perspective

Authors: Yadvinder Malhi, Cécile A. J. Girardin, Gregory R. Goldsmith, Christopher E. Doughty, Norma Salinas, Daniel B. Metcalfe, Walter Huaraca Huasco, Javier E. Silva-Espejo, Jhon del Aguilla-Pasquell, Filio Farfán Amézquita, Luiz E. O. C. Aragão, Rossella Guerrieri, Françoise Yoko Ishida, Nur H. A. Bahar, William Farfan-Rios, Oliver L. Phillips, Patrick Meir and Miles Silman

Article acceptance date: 12 July 2016

The following Supporting Information is available for this article:

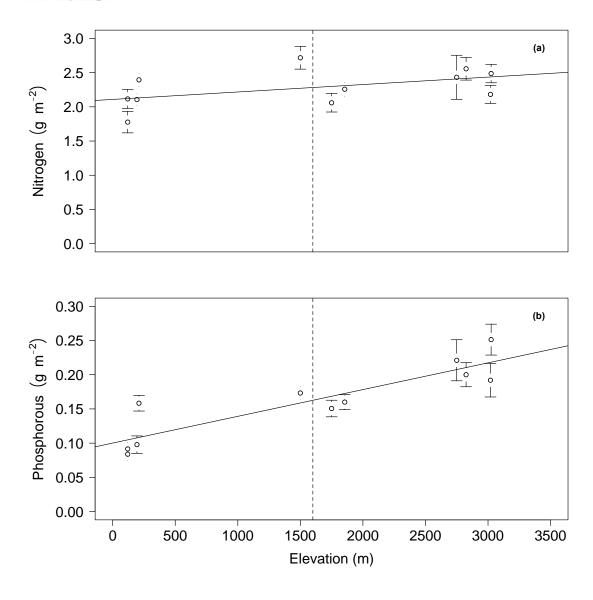
**Fig. S1** Relationship between foliar nutrient concentrations (nitrogen and phosphorus) and elevation.

Fig. S2 Relationship between primary productivity and temperature.

Fig. S3 Relationship between primary productivity and elevation without influential plot.

Methods S1 Detailed explanation of the measurements and data analysis procedures.





**Fig. S1** Variation in foliar (a) nitrogen and (b) phosphorus concentration along a 3300 m tropical montane elevation transect in Peru. The vertical dashed line indicates the submontane–cloud forest ecotone. Error bars indicate ± SE.



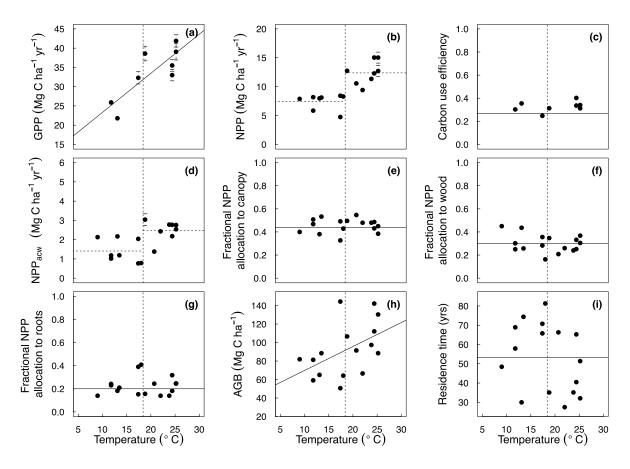
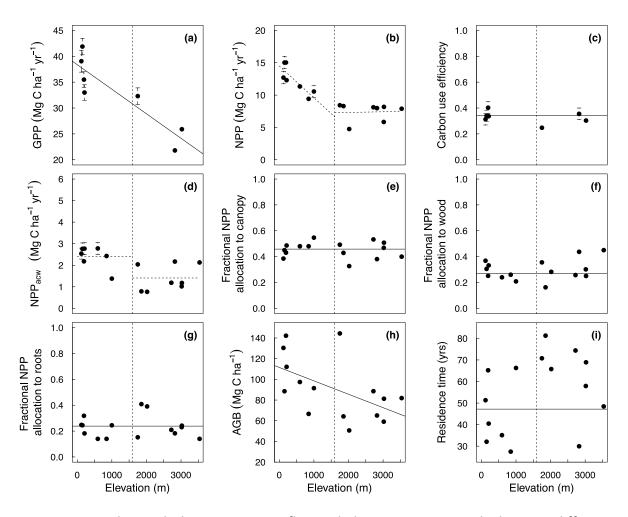


Fig. S2 As Fig. 3, but with all variables plotted against temperature instead of elevation.

Variation in carbon cycle characteristics along the 3300 m tropical montane elevation transect, including (a) gross primary productivity (GPP), (b) net primary productivity (NPP), (c) carbon use efficiency, the fraction NPP/GPP, (d) aboveground course woody NPP (NPP<sub>acw</sub>), (e) fractional NPP allocation to canopy components, (f) fractional NPP allocation to woody components, (g) fractional NPP allocation to roots, (h) aboveground live biomass (AGB) and (i) woody residence time. The best model fit (according to AIC) is shown when significant, either a single horizontal line or slope, or two lines split at 1600 m (cloud base). The vertical dashed line indicates the temperature at the submontane forest–cloud forest ecotone. Error bars ± SE.





**Fig. S3** As Fig. 3, but with the sometimes influential plot SPD-02 removed. The main difference compared to Fig. 3 is in the plot of NPP.



**Methods S1** This section gives a more detailed explanation of the measurements and data analysis procedures.

Our approach in the 1 ha GEM plots is to quantify the major components of the autotrophic carbon cycle at multiple sites. Herein, 'autotrophic' implies focusing on the plant processes of photosynthesis, productivity, autotrophic respiration and allocation, rather than heterotrophic processes such as decay and soil organic matter respiration. We adopt the field protocol of the GEM network (http://gem.tropicalforests.ox.ac.uk). These methods are described in detail in an online manual on the GEM website and are summarised here only briefly. Methods and descriptions of individual pairs of plots along our gradients have recently been published, but there has been no systematic, cross-site comparison of annual averages along the entirety of our lowland dataset.

Here we summarise the protocols and the primary differences among sites based on the site-specific papers (Girardin *et al.*, 2014; Araujo-Murakami *et al.*, 2014; da Costa *et al.*, 2014; Doughty *et al.*, 2014; Metcalfe *et al.*, 2010; Rocha *et al.*, 2014; Huaraca Huasco *et al.*, 2014; del Aguila-Pasquel *et al.*, 2014; Malhi *et al.*, 2014).

#### Site characteristics and disturbance history

All sites included in this analysis show little evidence of anthropogenic disturbance of the forest community structure, hosting mixed-age tree communities with little net increment in biomass. SPD-02 was lightly logged at some point several decades ago, as evidenced by the presence of a large tree stump within the plot. The montane sites are likely impacted by occasional landslips and more rare landslides (Clark *et al.*, 2016)

# Above ground net primary productivity

Measured aboveground net primary productivity (NPP<sub>AG</sub>) components included:

Above ground coarse wood net primary productivity ( $NPP_{ACW \ge 10}$ ): All trees  $\ge 10$  cm DBH were censused to determine growth rate of existing, surviving trees and rate of recruitment of new



trees. Stem biomass was calculated using an allometric equation for tropical moist forests, employing diameter, height, and wood density data (Chave *et al.*, 2005). To convert biomass values into carbon, we assumed that dry stem biomass is 47.3% carbon (Martin & Thomas, 2011). Where tree height data were not available, height was estimated from an allometric equation appropriate for each region (Feldpausch *et al.*, 2011).

Above ground coarse wood net primary productivity ( $NPP_{ACW \le 10}$ ): All trees < 10 cm DBH (details on minimum size provided in Table S1) were censused in subplots within each site to estimate the contribution of smaller stems to  $NPP_{ACW}$ .

Branch turnover net primary productivity (NPP<sub>branch turnover</sub>): The turnover of branches, where trees shed branches and grow new ones, can generate a significant component of woody NPP that is not accounted for by the static tree allometries used above. Branches >2 cm diameter (excluding those fallen from dead trees) were surveyed along fixed transects; small branches were cut to include only the transect-crossing component, removed and weighed. Larger branches had their dimensions taken (diameter at two or more points) and all were assigned a wood density value according to their decomposition class. Details of decomposition status and surface area formulas are available in the RAINFOR-GEM network field manual (www.gem.tropicalforests.ox.ac.uk).

Litterfall net primary productivity ( $NPP_{litterfall}$ ): Annual values of  $NPP_{litterfall}$  were estimated from dead organic material <2 cm diameter collected in 0.25 m<sup>2</sup> (50 × 50 cm) litter traps placed at 1 m above the ground at the centre of each of 25 subplots established within each plot. Litter is separated into its components, oven dried at 80°C to constant mass and weighed. Leaf carbon content was estimated to be 49.2% (Tambopata and montane plots) and 53.2% (Allpahuayo) carbon based on direct measurements on sampled leaves.

Leaf Area Index (LAI): Canopy images recorded with a digital camera and hemispherical lens near the centre of each of the 25 subplots, at a standard height of 1 m, and during overcast



conditions LAI estimated using 'true LAI' output from the CANEYE program (INRA, Avignon, France) which accounts for clumping of foliage Leaves were separated into sunlit and shaded fractions using the following equation: Fsunlit =  $(1 - \exp(-K \times LAI))/K$  where K is the light extinction coefficient, and Fsunlit is the sunlit leaf fraction (Doughty & Goulden, 2008). The model assumptions are randomly distributed leaves, and  $K = 0.5/\cos(Z)$ , where Z is the solar zenith angle, which was set to  $30^\circ$ .

Loss to leaf herbivory (NPP<sub>herbivory</sub>): Loss to leaf herbivory is the fraction of NPP<sub>canopy</sub> lost to herbivory before litterfall. At Tambopata, Wayqecha, Esperanza and San Pedro, leaves collected in the 25 litterfall traps in each plot were scanned before being dried and the leaf area calculated using image analysis software (ImageJ, NIH, USA). The fractional herbivory (H) for each leaf was calculated as:  $H = (A_{nh} - A_h)/A_{nh}$ , where  $A_h$  is the area of each individual leaf including the damage incurred by herbivory and  $A_{nh}$  is the leaf area before herbivory. The mean values of H were calculated across all leaves collected both per litterfall trap and per plot. Data on leaf herbivory are further explored by Metcalfe *et al.* (2014). The mean herbivory fraction observed at Wayqecha was assigned to all montane plots, that observed at SPD-02 assigned to the sub-montane plots (Pantiacolla), and that observed at Tambopata also assigned to Allpahuayo.

## Belowground net primary productivity

Below-ground net primary productivity (*NPP*<sub>BG</sub>) components consisted of fine and coarse roots *NPP*:

Coarse root net primary productivity ( $NPP_{coarse\ root}$ ): Due to potential damage to the trees, this is not measured directly. Instead,  $NPP_{coarse\ root}$  is estimated as  $0.21\pm0.03$  of aboveground woody productivity, based on the published values of coarse root biomass to above ground biomass (Jackson *et al.*, 1996; Cairns *et al.*, 1997). Details and the range of the root : shoot ratio are available in the RAINFOR-GEM network field manual (www.gem.tropicalforests.ox.ac.uk).



Fine root net primary productivity (NPP<sub>fine root</sub>): Sixteen ingrowth cores (mesh cages 12 cm diameter, installed to 30 cm depth) were installed in each plot. Cores were extracted and roots were manually removed from the soil samples in four 10 min time steps and the pattern of cumulative extraction over time was used to predict root extraction beyond 40 min. Root-free soil was then reinserted into the ingrowth core. Collected roots were thoroughly rinsed, oven dried at 80°C to constant mass, and weighed. An additional correction factor was applied for fine roots not collected within 40 min. A further correction was applied for unmeasured roots below 30 cm depth according to fine root biomass profiles extrapolated to the observed soil depth (or to 1 m in the case of the deep soiled plots in the lowlands).

#### Autotrophic and heterotrophic respiration

Total Soil  $CO_2$  efflux ( $R_{soil}$ ): Total soil  $CO_2$  efflux was measured using a closed dynamic chamber method, employed at the centre of each of the 25 subplots every 20 min, with an infrared gas analyser and soil respiration chamber (EGM-4 IRGA and SRC-1 chamber, PP Systems, Hitchin, UK) sealed to a permanent collar in the soil. Soil surface temperature (T260 probe, Testo Ltd, Hampshire, UK) and moisture (Hydrosense probe, Campbell Scientific Ltd., Loughborough, UK) were recorded at each point after efflux measurement.

Soil  $CO_2$  efflux partitioned into autotrophic ( $R_{rhizosphere}$ ) and heterotrophic ( $R_{soilhet}$ ) components: The autotrophic and heterotrophic components of soil respiration were quantified using a partitioning experiment similar to that described in Metcalfe et al. (2007). The partitioning experiment allows estimation of the relative contributions of surface organic litter, rhizosphere and soil organic matter to total soil  $CO_2$  efflux. At four points at each corner of the plot, plastic tubes of 12 cm diameter were placed; three tubes with short collars (10 cm depth) allowing both heterotrophic and rhizosphere respiration, three tubes with longer collars (40 cm depth) with no windows to exclude both roots and mycorrhizae.

Canopy respiration ( $R_{leaves}$ ): Leaf gas exchange measurements of  $R_{dark}$  were performed for at least 20 trees using infrared gas analysers. To obtain the leaves, one branch each from sunlit



and shaded portions of canopy trees were randomly selected and immediately recut under water to restore hydraulic connectivity for subsequent gas exchange measurement. The leaves were fully darkened for 30 min before measuring  $R_{\rm dark}$ . To scale to whole-canopy respiration, mean dark respiration for sunlit and shade-lit leaves were multiplied by the respective estimated fractions of total LAI.

The mean  $R_{\text{leaves}}$  measured for sun leaves and shade leaves was applied to the sun and shade fractions, respectively. The estimation of sun and shade fractions is described in the section on LAI above.

The wet season respiration mean was applied to all months with > 100 mm rain; for the dry season months, measured dry season respiration was linearly scaled by the soil moisture saturation to allow for more continuous variation of leaf respiration. To account for daytime light inhibition of leaf dark respiration, we apply an inhibition factor: 67% of daytime leaf dark respiration, 33% of total leaf dark respiration (Malhi *et al.*, 2009). These were calculated by applying the Atkin *et al.* (2000) equations for light inhibition of leaf respiration to a plot in Tapajós forest in Brazil (Malhi *et al.*, 2009; Lloyd *et al.*, 2010). In recognition of the substantial uncertainty in this estimate, we assigned a 30% error to the multiplying factor.

Above ground live wood respiration ( $R_{stems}$ ): Bole respiration was measured using a closed dynamic chamber method, from 25 trees distributed evenly throughout each plot at 1.3 m height with an IRGA (EGM-4) and soil respiration chamber (SRC-1) connected to a permanent collar, sealed to the tree bole surface. To estimate plot-level stem respiration, tree respiration per unit bole area was multiplied by bole surface area (SA in  $m^2$ ) for each tree, estimated with the following equation (Chambers *et al.*, 2004):  $\log_{10}(SA) = -0.105 - 0.686 \log_{(DBH)} + 2.208 \log_{(DBH)2} - 0.627 \log_{(DBH)3}$ , where DBH is bole diameter in cm at 1.3 m height. Finally, for all 25 trees together, we regressed mean annual bole respiration against total annual growth. In recognition of the substantial uncertainty in this estimate, we assigned a 30% error to the multiplying factor.



Coarse root respiration ( $R_{coarse\ roots}$ ): A substantial amount of wood respiration may occur in or near the root core immediately below the bole, but this has rarely been measured and is not included in our soil respiration partitioning methodology. In addition, even small coarse roots are too slow-growing to be present in 3-monthly ingrowth cores. We therefore estimate this term separately. This component of respiration was not measured directly but estimated by multiplying above-ground live wood respiration by 0.21 (same ratio used in these studies to estimate coarse root biomass and growth – see above). To our knowledge, there are no available data on below-ground course root surface area for tropical forests, so a mass-based approach was used. In recognition of the substantial uncertainty in this estimate, we assigned a 50% error ( $\pm$  0.10) to the multiplying factor. Malhi *et al.* (2009) did not account for this term, but it seems appropriate to include it for a more complete description of the below-ground carbon budget.

#### **Summations**

NPP is then calculated as:

$$NPP = NPP_{ACW \ge 10 \text{ cm}} + NPP_{ACW < 10 \text{ cm}} + NPP_{litterfall} + NPP_{branch turnover} + NPP_{herbivory} + NPP_{fineroot} + NPP_{coarse root}$$
Eqn S1

Total autotrophic respiration is estimated as

$$R_a = R_{leaf} + R_{stem} + R_{rhizosphere} + R_{coarse root}$$
 Eqn S2

In plant-level autotrophic steady state conditions (and on annual timescales or longer where there is little net nonstructural carbohydrate storage), gross primary productivity (GPP), the carbon taken up via photosynthesis, should be approximately equal to plant carbon expenditure (PCE), the amount of carbon used for NPP and autotrophic plant respiration ( $R_a$ ). Note that the autotrophic steady state condition does not require the total plot carbon cycle to be in equilibrium, the plot can still be gaining or losing biomass or soil carbon stocks, as long as there



is no substantial accumulation or loss of non-structural carbohydrates. Hence, we estimated GPP as

$$GPP = NPP + R_a$$
 Eqn S3

We calculated the carbon use efficiency (CUE) as the proportion of total GPP invested in NPP rather than  $R_a$ :

$$CUE = NPP / GPP = NPP / (NPP + R_a)$$
 Eqn S4

The aboveground NPP estimation neglects several small NPP terms, such as NPP lost as volatile organic emissions, non-measured litter trapped in the canopy, or dropped from ground flora below the litter traps. At a site in central Amazonia volatile emissions were found to be a minor component of the carbon budget (0.13±0.06 Mg C ha<sup>-1</sup> yr<sup>-1</sup>; Malhi *et al.*, 2009), and a close comparison with flux tower data and near-closure of the carbon budget in three well-studied Brazilian Amazonian sites, suggests that the other neglected NPP terms are relatively minor (Malhi *et al.*, 2009). For belowground NPP, the allocation to root exudates and to mycorrhizae is neglected. In effect, we treat root exudation and transfer to mycorrhizae as rhizosphere autotrophic respiration rather than as NPP, which could potentially impact our CUE numbers. Given that these exudates are labile and rapidly respired by mycorrhizae and soil microfauna in the rhizosphere, in terms of carbon cycling this exudate NPP term is very similar to fine root autotrophic respiration.

#### Statistics and error analysis

A key consideration was assignment and propagation of uncertainty in our measurements. There were two primary types of uncertainty. First, there was uncertainty associated with the spatial heterogeneity of the study plot and the limited number of samples. Examples include the variability among litter traps, or among fine root ingrowth cores. Second, there was uncertainty associated with either unknown error in measurement, or error in scaling



measurements to the plot level. Examples of unknown biases included the possibility of soil-derived CO<sub>2</sub> in the transpiration stream affecting the stem CO<sub>2</sub> efflux measurements, and uncertainties in scaling include the allometry of scaling of bole stem CO<sub>2</sub> efflux to whole tree stem respiration, or leaf dark respiration to whole canopy dark respiration. Here we assumed that most NPP terms were measured fairly precisely and sampled without large biases; hence the NPP component measurements were dominated by sampling uncertainty, which could be reliably estimated, assuming a normal distribution. On the other hand, some of the main autotrophic respiration terms were dominated by systematic uncertainty. This systematic uncertainty can be very hard to reliably quantify; here, in each case we made an explicit and conservative estimate of the systematic uncertainty of key variables. Key sources of systematic uncertainty applied are detailed above and in the site-specific papers.

All estimated fluxes reported in this study are in Mg C ha<sup>-1</sup> yr<sup>-1</sup> (or month<sup>-1</sup> for seasonal fluxes) and all reported errors are  $\pm$  1 SE; error propagation was carried out for all combination quantities using standard rules of quadrature (Hughes & Hase, 2010), assuming that uncertainties were independent and normally distributed. One Mg C ha<sup>-1</sup> yr<sup>-1</sup> is equal to 100 g C m<sup>-2</sup> yr<sup>-1</sup>, or 0.264  $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>.



## References

- Araujo-Murakami A, Doughty CE, Metcalfe DB, Silva-Espejo JE, Arroyo L, Heredia JP, Flores M, Sibler R, Mendizabal LM, Pardo-Toledo E et al. 2014. The productivity, allocation and cycling of carbon in forests at the dry margin of the Amazon forest in Bolivia. Plant Ecology & Diversity 7: 1–15.
- **Atkin OK, Evans JR, Ball MC, Lambers H, Pons TL**. **2000**. Leaf respiration of snow gum in the light and dark. Interactions between temperature and irradiance. *Plant Physiology* **122**: 915–923.
- **Cairns MA, Brown S, Helmer EH, Baumgardner GA**. **1997**. Root biomass allocation in the world's upland forests. *Oecologia* **111**: 1–11.
- Chambers JQ, Tribuzy ES, Toledo LC, Crispim BF, Higuchi N, Santos JD, Araújo AC, Kruijt B, Nobre AD, Trumbore SE. 2004. Respiration from a tropical forest ecosystem: partitioning of sources and low carbon use efficiency. *Ecological Applications* 14: 72–88.
- Chave J, Andalo C, Brown S, Cairns MA, Chambers JQ, Eamus D, Fölster H, Fromard F, Higuchi N, Kira T *et al.* 2005. Tree allometry and improved estimation of carbon stocks and balance in tropical forests. *Oecologia* 145: 87–99.
- Clark KE, West AJ, Hilton RG, Asner GP, Quesada CA, Silman MR, Saatchi SS, Farfan-Rios W, Martin RE, Horwath AB *et al.* 2016. Storm-triggered landslides in the Peruvian Andes and implications for topography, carbon cycles, and biodiversity. *Earth Surface Dynamics*, 4, 47-70.
- da Costa ACL, Metcalfe DB, Doughty CE, de Oliveira AAR, Neto GFC, da Costa MC, Silva Junior J de A, Aragão LEOC, Almeida S, Galbraith DR *et al.* 2014. Ecosystem respiration and net primary productivity after 8–10 years of experimental through-fall reduction in an eastern Amazon forest. *Plant Ecology & Diversity* 7: 1–18.
- del Aguila-Pasquel J, Doughty CE, Metcalfe DB, Silva-Espejo JE, Girardin CAJ, Gutierrez JAC, Navarro-Aguilar GE, Quesada CA, Hidalgo CG, Huaymacari JMR *et al.* 2014. The seasonal cycle of productivity, metabolism and carbon dynamics in a wet aseasonal forest in northwest Amazonia (Iquitos, Peru). *Plant Ecology & Diversity* 7: 1–13.
- **Doughty CE, Goulden ML. 2008.** Seasonal patterns of tropical forest leaf area index and CO<sub>2</sub>



- exchange. Journal of Geophysical Research 113: G00B06.
- Doughty CE, Metcalfe DB, da Costa MC, de Oliveira AAR, Neto GFC, Silva JA, Aragão LEOC, Almeida SS, Quesada CA, Girardin CAJ *et al.* 2014. The production, allocation and cycling of carbon in a forest on fertile terra preta soil in eastern Amazonia compared with a forest on adjacent infertile soil. *Plant Ecology & Diversity* 7: 1–13.
- Feldpausch TR, Banin L, Phillips OL, Baker TR, Lewis SL, Quesada CA, Affum-Baffoe K, Arets EJMM, Berry NJ, Bird M *et al.* 2011. Height-diameter allometry of tropical forest trees. *Biogeosciences* 8: 1081–1106.
- Girardin CAJ, Espejo JES, Doughty CE, Huasco WH, Metcalfe DB, Durand-Baca L, Marthews TR, Aragão LEOC, Farfán-Rios W, García-Cabrera K et al. 2014. Productivity and carbon allocation in a tropical montane cloud forest in the Peruvian Andes. *Plant Ecology & Diversity* 7: 107–123.
- Huaraca Huasco W, Girardin CAJ, Doughty CE, Metcalfe DB, Baca LD, Silva-Espejo JE, Cabrera DG, Aragão LEOC, Davila AR, Marthews TR *et al.* 2014. Seasonal production, allocation and cycling of carbon in two mid-elevation tropical montane forest plots in the Peruvian Andes. *Plant Ecology & Diversity* 7: 1–18.
- **Hughes I, Hase T 2010.** *Measurements and their uncertainties.* Oxford, UK: Oxford University Press.
- **Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED 1996.** A global analysis of root distributions for terrestrial biomes. *Oecologia* **108**: 389–411.
- Lloyd J, Patino S, Paiva RQ, Nardoto GB, Quesada CA, Santos AJB, Baker TR, Brand WA, Hilke I, Gielmann H et al. 2010. Optimisation of photosynthetic carbon gain and within-canopy gradients of associated foliar traits for Amazon forest trees. *Biogeosciences* 7: 1833–1859.
- Malhi Y, Amézquita FF, Doughty CE, Silva-Espejo JE, Girardin CAJ, Metcalfe DB, Aragão LEOC, Huaraca-Quispe LP, Alzamora-Taype I, Eguiluz-Mora L et al 2014. The productivity, metabolism and carbon cycle of two lowland tropical forest plots in south-western Amazonia, Peru. Plant Ecology & Diversity 7: 1–21.
- Malhi Y, Aragão LEOC, Metcalfe DB, Paiva R, Quesada CA, Almeida S, Anderson L, Brando P, Chambers JQ, da Costa ACL *et al.* 2009. Comprehensive assessment of carbon productivity,



- allocation and storage in three Amazonian forests. Global Change Biology 15: 1255–1274.
- **Martin AR, Thomas SC 2011.** A reassessment of carbon content in tropical trees. *PLoS ONE* **6**: e23533.
- Metcalfe DB, Asner GP, Martin RE, Silva-Espejo JE, Huaraca Huasco W, Farfán Amézquita FF, Carranza-Jimenez L, Galiano Cabrera DF, Durand Baca L, Sinca F et al. 2014. Herbivory makes major contributions to ecosystem carbon and nutrient cycling in tropical forests. *Ecology Letters* 17: 324-332.
- Metcalfe DB, Meir P, Aragao LEOC, Lobo-do-Vale R, Galbraith D, Fisher RA, Chaves MM,

  Maroco JP, da Costa ACL, de Almeida SS *et al.* 2010. Shifts in plant respiration and carbon use efficiency at a large-scale drought experiment in the eastern Amazon. *New Phytologist* 187: 608–621.
- Metcalfe DB, Williams M, Aragao LEOC, da Costa ACL, de Almeida SS, Braga AP, Gonçalves PHL, de Athaydes J, Junior S, Malhi Y *et al.* 2007. A method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy. *New Phytologist* 174: 697–703.
- Rocha W, Metcalfe DB, Doughty CE, Brando P, Silvério D, Halladay K, Nepstad DC, Balch JK, Malhi Y. 2014. Ecosystem productivity and carbon cycling in intact and annually burnt forest at the dry southern limit of the Amazon rainforest (Mato Grosso, Brazil). *Plant Ecology & Diversity* 7: 1–16.