After more than a decade of soil moisture deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf respiration

Citation for published version:
Rowland, L, Lobo-do-vale, RL, Christoffersen, BO, Melem, EA, Kruijt, B, Vasconcelos, SS, Domingues, T, Binks, OJ, Oliveira, AAR, Metcalfe, D, Da Costa, ACL, Mencuccini, M & Meir, P 2015, 'After more than a decade of soil moisture deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf respiration', Global Change Biology, pp. n/a-n/a. https://doi.org/10.1111/gcb.13035

Digital Object Identifier (DOI):
10.1111/gcb.13035

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Global Change Biology

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
After more than a decade of soil moisture deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf respiration.

Running Head: Tropical forest drought and leaf physiology

Rowland, L. 1*, Lobo-do-Vale, R. L. 2, Christoffersen, B. O. 3,1, Melém, E. A. 4, Kruijt, B. 5, Vasconcelos, S.S. 4, Domingues, T. 6, Binks, O. J. 1, Oliveira., A. A. R. 7, Metcalfe, D. 8, da Costa., A. C. L. 7, Mencuccini, M. 9,1, and Meir, P. 1,10.

1 School of GeoSciences, University of Edinburgh, Edinburgh, UK.
2 Forest Research Centre, School of Agriculture, University of Lisbon, Lisbon, Portugal
3 Earth and Environmental Sciences, Los Alamos National Laboratory, Los Alamos, USA
4 EMBRAPA Amazônia Oriental, Belém, Brasil
5 Alterra, Wageningen UR, Wageningen, Netherlands
6 Departamento de Biologia, FFCLRP - Universidade de São Paulo, Ribeirão Preto, Brasil
7 Centro de Geosciências, Universidade Federal do Pará, Belém, Brasil
8 Department of Physical Geography and Ecosystem Science, Lund University, Lund, Sweden
9 ICREA at CREAF, 08193 Cerdanyola del Vallés, Spain
10 Research School of Biology, Australian National University, Canberra, Australia

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/gcb.13035
This article is protected by copyright. All rights reserved.
Keywords: drought, tropical rainforest, photosynthetic capacity, leaf dark respiration, through-fall exclusion

Article type: Primary research article

Abstract

Determining climate change feedbacks from tropical rainforests requires an understanding of how carbon gain through photosynthesis and loss through respiration will be altered. One of the key changes that tropical rainforests may experience under future climate change scenarios is reduced soil moisture availability. In this study we examine if and how both leaf photosynthesis and leaf dark respiration acclimate following more than 12 years of experimental soil moisture deficit, via a through-fall exclusion experiment (TFE) in an eastern Amazonian rainforest. We find that experimentally drought-stressed trees and taxa maintain the same maximum leaf photosynthetic capacity as trees in corresponding control forest, independent of their susceptibility to drought-induced mortality. We hypothesise that photosynthetic capacity is maintained across all treatments and taxa to take advantage of short-lived periods of high moisture availability, when stomatal conductance ($g_s$) and photosynthesis can increase rapidly, potentially compensating for reduced assimilate supply at other times. Average leaf dark respiration ($R_d$) was elevated in the TFE-treated forest trees relative to the control by 28.2±2.8% (mean ± one standard error). This mean $R_d$ value was dominated by a 48.5±3.6% increase in the $R_d$ of drought-sensitive taxa, and likely reflects the need for additional metabolic support required for stress-related repair, and hydraulic or osmotic maintenance processes. Following soil moisture deficit that is maintained for several
years, our data suggest that changes in respiration drive greater shifts in the canopy carbon balance, than changes in photosynthetic capacity.

Introduction

Tropical rainforests are the world's most productive and biodiverse terrestrial ecosystem (Beer et al., 2010), harbouring a substantial store of carbon, particularly in Amazonia because of its area (Grace et al., 2014). How long tropical rainforests will remain a store of carbon remains uncertain, and may depend upon the intensity and/or frequency of future drought events (Gatti et al., 2014, Phillips et al., 2009). Small shifts in photosynthetic and autotrophic respiratory process responses to seasonal reductions in moisture have previously been shown to shift the carbon balance of tropical rainforests (Bonal et al., 2008, Meir et al., 2008, Rowland et al., 2014); however how photosynthesis and respiration interact and acclimate to multi-year drought in tropical rainforest remains poorly understood.

Evidence from studies of drought-induced changes in photosynthesis and leaf respiration suggest that the effects on photosynthesis are greater than on autotrophic respiration (Catoni & Gratani, 2014, Chastain et al., 2014, Ciais et al., 2005, Doughty et al., 2015, Flexas et al., 2006, Meir et al., 2008). Drought-induced reductions in leaf-level photosynthesis have been found to occur through either reductions in stomatal conductance ($g_s$) and/or mesophyll conductance (Catoni & Gratani, 2014, Chastain et al., 2014, Flexas et al., 2006, Miranda et al., 2005, Stahl et al., 2013), or reductions in biochemical efficiency (Flexas & Medrano, 2002, Galmes et al., 2013, Lawlor & Cornic, 2002, Reddy et al., 2004). During short term or less severe drought events, down-regulation of metabolic processes as a result of low $g_s$ is thought to be the main limitation on photosynthesis (Flexas et al., 2006, Flexas & Medrano, 2002).
Photosynthetic capacity represents the maximum rate at which leaves can photosynthesise in optimum conditions. Provided optimum conditions are realised reductions in photosynthetic capacity will restrict total photosynthesis; in turn increased photosynthetic capacity will only increase photosynthesis in the absence of other limitations such as stomatal conductance or light. If a canopy photosynthesis is optimised to maximise light use efficiency (Hirose et al., 1988, Kull & Kruijt, 1999), photosynthetic capacity is likely to be maintained, or potentially increased during these less severe drought events in order to maximise photosynthetic output at a lower $g_s$. However, as the severity and length of drought increases, accumulation of reactive oxygen species (ROS) can occur if $g_s$ drops below a threshold value; this will result in more permanent reductions in photosynthesis through biochemical changes causing cellular damage (Flexas et al., 2006). Cellular damage occurring during severe drought stress can reduce leaf metabolic function by: decreasing ribulose1, 5-biphosphate (RuBP) regeneration capacity, ATP synthesis or ATP content in the leaf, and Rubisco activity; or, by increasing leaf photochemical reaction rates (chlorophyll fluorescence) or photoinhibition (Flexas et al., 2006, Flexas & Medrano, 2002, Galmes et al., 2013, Reddy et al., 2004). Consequently, under severe drought-stressed conditions photosynthetic capacity (the maximum rates of carboxylation of Rubisco ($V_{\text{cmax}}$) and/or electron transport ($J_{\text{max}}$)) may decline, driving changes in total leaf scale photosynthesis.

The processes involved in leaf respiration remain less well understood than for photosynthesis (Atkin et al., 2015, Atkin & Macherel, 2009, Atkin et al., 2013); particularly in tropical rainforests and in relation to drought (Meir et al., 2008, Meir & Woodward, 2010, Rowland et al., 2014). Short-term, acute water stress is generally found to inhibit $R_d$ because of either reduced substrate supply from photosynthesis with respiratory substrates being diverted to form compounds for osmotic regulation, or through direct reductions in mitochondrial respiratory capacity (Atkin & Macherel, 2009, Ayub et al., 2011, Catoni &
Gratani, 2014, Chastain et al., 2014, O'Brien et al., 2015). However it is also possible that \( R_d \) can increase during periods of drought if substrate demand for processes such as hydraulic maintenance and repair (Brodersen & McElrone, 2013), and phloem transport regulation (Mencuccini & Höltä, 2010) increases, or if drought conditions lead to a greater need to oxidise ROS or other redox equivalents, elevating photorespiration (Atkin & Macherel, 2009). Evidence of increasing \( R_d \) during drought is limited but has been reported for both crop plants and forests (Atkin & Macherel, 2009, Miranda et al., 2005, Varone & Gratani, 2015).

The responses to long-term drought stress are likely to differ from those of short-term drought because certain processes, which may be sustainable over short time periods, are unlikely to facilitate long-term drought survival. Over longer time periods structural or physiological acclimation may occur to facilitate adaptation to drought conditions at a whole tree scale. Acclimation to drought within trees has been reported to occur across various time-scales from seasonal to multi-annual. In response to seasonal drought trees have been reported to increase their leaf-scale water-use efficiency (WUE: unit photosynthesis per unit water loss; Bonal et al., 2007), reduce the rate at which \( R_d \) increases with temperature to minimise leaf carbon loss (Crous et al., 2011) as well as to increase leaf mass per area (Sperlich et al., 2015). Over multi-year drought conditions the key acclimation processes may differ as whole tree-scale structural changes become possible and perhaps necessary to maintain a positive carbon balance. These structural changes may include altered root growth patterns (Metcalf et al., 2007), changes in canopy leaf area (Brando et al., 2008, Metcalfe et al., 2010a) or changes in carbon allocation and stem growth (Brando et al., 2008, da Costa et al., 2010, Metcalfe et al., 2010b). Such structural changes are likely to compensate for, or facilitate further, any changes in physiology. Increased leaf-level respiration was found after five years of experimental drought, on a mix of shaded and sunlit leaves of a tropical...
rainforest (Metcalfe et al., 2010b). Maintaining elevated respiration would require enhanced substrate use, thus also requiring acclimation responses in either allocation to growth or from carbon storage. However whether elevated $R_d$ is related to acclimation responses in photosynthetic capacity or WUE, or even if elevated $R_d$ can be maintained during long-term soil moisture deficit (e.g. for more than a decade), has yet to be determined.

In tropical rainforest, where species diversity is high and drought-sensitivity has been shown to vary with taxonomic identity (da Costa et al., 2010, Nepstad et al., 2007), there is likely to be a range of photosynthetic and respiratory acclimation responses to drought in any forest stand (Stahl et al., 2013). Understanding these processes and their possible acclimation in the context of long-term drought in tropical rainforests is key to constraining the variability which currently exists in model predictions of such processes in future climate change scenarios (Galbraith et al., 2010, Huntingford et al., 2013, Powell et al., 2013, Rowland et al., 2015), or in more conceptual models (Meir et al., 2015a).

We present wet and dry season measurements of photosynthetic capacity ($V_{cmax}$ and $J_{max}$), $R_d$, $g_s$, photosynthesis in saturating light and ambient atmospheric CO$_2$ concentration ($A_{sat}$), WUE and leaf nutrient content for tropical rainforest which has experienced 13 years of experimental soil moisture deficit imposed by a through-fall exclusion (TFE) treatment and corresponding, adjacent control forest. We also compare variables for tree taxa that have been previously determined as sensitive or resistant to drought in terms of their mortality response to experimental soil moisture deficit (da Costa et al., 2010). The results are compared with earlier measurements of photosynthesis and respiration made during the first three years of the experiment. Using these data we test the following hypotheses: (1) $V_{cmax}$, $J_{max}$, and $R_d$, change following prolonged soil moisture deficit; (2) Drought sensitivity (specified as the drought-induced mortality risk of specific tree genera) constrains the physiological response ($V_{cmax}$, $J_{max}$, $R_d$, $A_{sat}$ and WUE) of a tree to seasonal or experimental drought; and (3) the
relationships between $R_d$, photosynthetic capacity and nutrient content change following prolonged soil moisture deficit.

Materials and methods

Site

This study was performed at a long-term TFE experiment in tropical rainforest, located in the Caxiuanã National Forest Reserve in the eastern Amazon ($1°43'S$, $51°27'W$), on terra firme forest, with yellow oxisol soils (Ruivo & Cunha, 2003). The site is 15 m above sea level, has a mean annual rainfall between 2000 and 2500 mm and a pronounced dry season between June and November when monthly rainfall is <100 mm.

The experiment comprises two 1 ha plots, a treatment (TFE) and a control. In the TFE plot plastic panels and gutters are supported at a height of 1-2 m and exclude 50 % of the incident rainfall (da Costa et al., 2010). A corresponding control plot is sited <50 m from the TFE, where there has been no manipulation of incident through-fall. As with many large-scale ecosystem manipulations, this experiment was prohibitively large and expensive to permit replication. The potential insights derived from long-term monitoring at large scale were favoured over the use of smaller plots replicated over large geographical areas (Carpenter, 1996, Osmond et al., 2004). In addition, the large horizontal extent of many tree roots in this forest ecosystem made a small-plot design inappropriate. As described in earlier publications from this experiment (da Costa et al., 2010, Fisher et al., 2007, Meir et al., 2009, Meir et al., 2015b), and as also performed in other un-replicated large scale experiments such as at Hubbard Brook, pre-treatment data were obtained before the start of the experiment, enabling comparison over time as well as by treatment (see also Nepstad et al., 2002, Davidson et al.,...
2004). Perimeter trenching to 1-2 m depth was used to avoid lateral in-flow of soil water, and the litter on each panel was transferred manually to the forest floor immediately below it every few days to maintain these biogeochemical inputs to the soil. For more experimental details see (Fisher et al., 2007, Meir et al., 2009, da Costa et al., 2010). The TFE experiment has been maintained continuously since January 2002 until the present (2015), and thus, prior to this study, the trees had experienced a 50% reduction in through-fall for 12-13 years.

**Species selection and measurement protocol**

*Campaigns during 2013-14*

In October-November 2013 (peak dry season) and July 2014 (peak wet season) leaf photosynthesis and leaf dark respiration measurements were performed on 21 canopy-top trees from the control and TFE forest (42 samples in total; Table 1). The selected trees were from six genera, with 3-6 replicates per genus. We tested the criteria used by da Costa et al., (2010; See Table S1) to identify how sensitive these genera were to drought stress. We found strong consistency with the da Costa et al. (2010) results using six additional years of data, from (i.e., from 2009 to 2014). Of the six genera employed, three were classed as sensitive, and three as resistant to drought in terms of their drought-induced mortality response (Table S1). Branches from the canopy-tops of 21 trees from the TFE plot and 20 trees from the control plot were cut between 10 am and 3 pm, over several days in each sampling season. The stems were then immediately immersed in water, and re-cut to reconstitute the water supply to leaves, as also performed elsewhere for the chosen gas exchange measurements (Domingues et al., 2010). After re-cutting underwater, branches were left to stabilise in a canopy opening, in full ambient irradiance for a minimum of 30 minutes before leaf measurements were conducted. Gas exchange measurements were performed using three
LICOR 6400 portable photosynthesis systems (LI-COR, Nebraska, USA), which were regularly cross-calibrated through checking repeated measures on the same leaves to ensure consistency among measurements. Following careful field tests on-site, no significant effect of branch cutting was found on our measurements (See measurement protocol below, and Figure S1).

\( V_{\text{c max}} \) and \( J_{\text{max}} \) were derived by measuring photosynthesis at different concentrations of CO\(_2\) (50-2000 ppm) in the leaf chamber. A-\( C_i \) curves (\( C_i \), the CO\(_2\) concentration of the leaf intercellular spaces) were performed using a saturating photosynthetically active radiation (PAR) intensity of 2000 µmol m\(^{-2}\) s\(^{-1}\), determined from light curves performed separately on each genus. \( V_{\text{c max}} \) and \( J_{\text{max}} \) were calculated from the A-\( C_i \) curves after temperature-correction to 25 °C using the equations from Sharkey et al. (2007). Model fitting was carried out using the optim function in R, which optimises the fit of an A-\( C_i \) curve to the data (R.3.0.2; R Core Team). As we were unable to measure mesophyll conductance we assumed it to be infinite (i.e. there to be no mesophyll conductance limitation) in our fitting procedure, rather than fitting this parameter separately, to avoid introducing unknown biases in our comparisons of control with treatment. Saturating photosynthesis (\( A_{\text{sat}} \)) was measured separately to the A-\( C_i \) curves at a CO\(_2\) concentration of 400 ppm and a PAR intensity of 2000 µmol m\(^{-2}\) s\(^{-1}\). \( A_{\text{sat}} \) was corrected to 25 °C using standard temperature constants from Sharkey et al. (2007). To standardise for variations in \( A_{\text{sat}} \) caused by differences in the internal CO\(_2\) concentration (\( C_i \)) when leaves are exposed to the same external CO\(_2\) concentrations \( A_{\text{sat}} \) was corrected to a common \( C_i \) value; the median \( C_i \) of all \( A_{\text{sat}} \) measurements was used as the common \( C_i \) value. \( A_{\text{sat}} \) was only measured if photosynthesis and \( g_s \) had remained stable within the LI-COR chamber for a minimum period of 10 minutes. Intrinsic leaf water use efficiency (WUE) was calculated as \( A_{\text{sat}} \) divided by the \( g_s \) measured at \( A_{\text{sat}} \). Since the branches were cut, measured WUE may not be identical to WUE of intact branches at the top of the canopy; however the
effects of cutting on photosynthesis and maximum $g_s$ were non-significant (Fig. S1), and sampling among tree taxa was balanced between the control and TFE (Table 1), thereby minimizing potential biases due to branch cutting. Leaf dark respiration ($R_d$) was measured after keeping the leaf in complete darkness inside tinfoil, for 30 minutes. $R_d$ measurements were corrected to 25 °C using Equation 1, where $R_{dt}$ is respiration at the leaf temperature inside the leaf chamber, $T$ is the leaf temperature inside the leaf chamber and the $Q_{10}$ factor 2.2 represents the factor by which respiration increases for a 10 °C increase in leaf temperature (Atkin & Tjoelker, 2003).

\[
R_d = R_{dt} \cdot 2.2^{\frac{T-25}{10}}
\]

Equation 1

**Measurement campaigns during 2001-03**

$A-C_i$ curves were also recorded on attached leaves from trees adjacent to two walk-up observation towers in each plot, in both the wet and dry seasons of 2001 (pre-TFE treatment), 2002 and 2003. Samples were taken from 11 trees of 10 different species in the control, and nine trees of nine different species in the TFE; the total number of samples taken per period are shown in Table S2. As some of the tree canopies were partially shaded on each plot (Table S2), saturating PAR intensity for these curves was set at 1000 µmol m$^{-2}$ s$^{-1}$. To ensure comparability between the 2001-03 and the 2013-14 protocols, $A-C_i$ curves and leaf respiration were re-measured on these same trees in 2013 and 2014 (Table S2). However, only one $A-C_i$ curve and two $R_d$ measurements could be determined in the TFE because of tree mortality and loss of the branches close to the tower since 2003. $V_{\text{cmax}}$ and $J_{\text{max}}$ were calculated from the $A-C_i$ data using the same method as above. During the 2001-2003 campaigns, light response curves were also performed on the same leaves employed for $A-C_i$
curves in both control and TFE plots. Previously published data on $R_d$ at Caxiuanã (Metcalfe et al., 2010) were derived from these light response curves.

**Additional measurement campaigns**

Additional $R_d$ data were obtained in 2007 on sunlit and shaded leaves from cut branches obtained from both the control and TFE plots (Metcalfe et al., 2010a; Table S3). All these values of $R_d$ were corrected to 25°C using Equation 1. The same trees sampled in 2007 by Metcalfe et al. (2010a) were re-sampled where possible in this study, in peak dry season 2013, and peak wet season 2014 (Table S3). In summary, we synthesised leaf measurements of $V_{cmax}, J_{max}$ and $R_d$ from the experiment over the full experimental period, 2001-2013, to test the hypotheses laid out above.

**Nutrient and leaf mass analysis**

All leaves used for gas exchange were collected, scanned to obtain leaf area, dried to constant mass and weighed. Leaf area was measured from the scanned images using the Image J software (http://imagej.nih.gov/ij/), which, together with the dry mass of the leaf was used to calculate leaf mass per area (LMA, g m$^{-2}$), and its reciprocal, specific leaf area (SLA m$^{-2}$ kg$^{-1}$). Following removal of the petiole and the main vein, and grinding of the dry tissue, nitrogen ($N$) and phosphorus ($P$) content of all leaves were measured in the EMBRAPA laboratories in Belem, Para, Brazil. $N$ concentrations of 0.1 g subsamples were determined by dry combustion using a LECO CNS-2000 analyser (LECO Corporation, Michigan) and tested against laboratory standards. $P$ concentrations of 0.1 g subsamples were determined by the molybdate method (Murphy & Riley, 1962) after digestion using sulphuric acid and peroxide.
Statistics

All statistical analyses were done in R (Version 3.02, R Core Team). Mixed effects modelling was performed following standard methods (Zuur et al., 2009), using the lme4 package. Models were formulated to test for a drought treatment effect on $V_{cmax}$, $J_{max}$, $R_d$, $N$, $P$, LMA and SLA where plot, sensitivity status (whether a tree belonged to a taxon which was considered sensitive or resistant to drought-induced mortality based on the data from this experiment; Table S1) and season were considered as fixed effects, and individual tree, nested within genus was considered as a random effect. Models were also formulated to test if a single relationship was maintained between $V_{cmax}$, $J_{max}$, $R_d$ and leaf nutrients plus the combination of SLA (or LMA), in both the control and TFE, independent of drought stress. In these models $N$, $P$, and either LMA or SLA were considered fixed effects and individual tree, nested within genus, was considered as a random effect. All tests were performed on both a leaf area and a leaf mass basis. To find the most appropriate model for a variable, a likelihood ratio test with one degree of freedom was used to compare models with and without the variable of interest. Models were tested both with and without variable interactions. All other tests for significance throughout the paper were done using the Wilcoxon rank-sum test statistic, and significant results were considered as those with a P value <0.05.

Results:

Campaigns during 2013-14

No significant differences were found for $V_{cmax}$ and $J_{max}$ between the control and the TFE plots, in either wet or dry seasons (Fig. 1a & b). The average values for $V_{cmax}$ and $J_{max}$ across both seasons were 29.1±1.8 µmol m$^{-2}$ s$^{-1}$ (one standard error) and 51.1±2.8 µmol m$^{-2}$ s$^{-1}$, respectively in the control plot, and 26.8±1.6 µmol m$^{-2}$ s$^{-1}$ and 45.2±1.9 µmol m$^{-2}$ s$^{-1}$.
The average \( J_{\text{max}} : V_{\text{cmax}} \) ratio was 1.8±0.1 in both the control and the TFE. Leaf respiration \( (R_d) \) was significantly elevated \((P=0.03)\) in the TFE \((0.9±0.1 \ \mu\text{mol CO}_2 \ \text{m}^{-2} \ \text{s}^{-1})\) relative to the control plot \((0.7±0.1 \ \mu\text{mol CO}_2 \ \text{m}^{-2} \ \text{s}^{-1})\) during the dry season, but no significant differences existed in the wet season (Fig. 1c). These results were very similar when expressed on a leaf mass basis (Fig. S2). The significant increase in \( R_d \) in the TFE during the dry season was driven by elevated respiration in the tree taxa vulnerable to drought-induced mortality, rather than by an overall increase across all trees and taxa (Fig. 2; Table S1). \( R_d \) in drought-vulnerable taxa increased 48.5±3.6 % \((P=0.02; \ \text{Fig. 2})\) in the TFE, relative to the average \( R_d \) of the same taxa in control plot; in contrast no significant changes in the respiration rates of the drought-resistant taxa were observed. The relationships of \( R_d \) with \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were stronger in the control plot than in the TFE (Fig. 3), particularly during the dry season when correlation coefficients were >0.5 in the control plot while no significant relationships were found for the TFE (Fig. 3).

Tree vulnerability status seemed to influence seasonal patterns of WUE, \( A_{\text{sat}} \) and \( g_s \). Resistant taxa in the control plot experienced significantly increased WUE in the dry season \((P=0.03; \ \text{Fig. 4a})\) by delivering an elevated \( A_{\text{sat}} \) at a similar \( g_s \) to the wet season. This did not occur in either the vulnerable or resistant trees in the TFE because \( A_{\text{sat}} \) was not elevated and \( g_s \) was reduced in the dry season (Fig. 4b, c). The vulnerable trees in the TFE experienced a significant 34.0±5.1% \((P=0.02)\) reduction in \( A_{\text{sat}} \) from the peak of the wet season to the peak of the dry season, which was related to a drop of 22.9±9.6% in peak \( g_s \) \((P=0.02; \ \text{Fig. 4b, c})\).

The mixed effect model analysis demonstrated that none of the treatment effect (plot), season, vulnerability status or any combination of these variables contributed significantly to predicting \( V_{\text{cmax}} \), \( J_{\text{max}} \), \( N \), and \( P \), on either a mass or area basis, and the same was also true for modelling LMA and SLA (Table 2). As no plot or seasonal difference was detectable in any of these variables, we found that across both plots and both seasons LMA was significant in
the modelling of $V_{cmax}$ on an area basis and $N$ was significant in the modelling of $V_{cmax}$ and $J_{max}$ on a mass basis (Table 2). In contrast the interaction of plot, vulnerability status and season was a highly significant component of the best model of $R_d$, on both a mass and area basis (Table 2). The importance of plot, season and drought sensitivity in explaining the variance in $R_d$ prevented a more general model of $R_d$ based on $N$, $P$, LMA or SLA, or any combination of these variables, from being significant (Table 2).

**Comparison between the 2013-14 results and earlier campaigns**

Over the 13 years of the TFE experiment, the ratio of TFE: control $V_{cmax}$ remained close to 1.0, suggesting leaf photosynthetic capacity was not altered throughout the experiment (Fig. 5a). In contrast, measurements of TFE: control $R_d$ over 13 years of drought were significantly greater than 1.0. This conclusion was robust to tests carried out to determine the potential influence of using leaf samples taken in different seasons from different species using fundamentally different measurement methods (i.e., cut and uncut branches from shaded and sunlit canopy positions). The magnitude of the difference in $R_d$ between the control and TFE plots is likely to have been affected by the composition of tree taxa, where sampling of taxa differed, and canopy height in the samples, and also the seasonal conditions, but despite these sources of potential variation, the overall $R_d$ signal was significant and remained elevated in the TFE plot (Fig. 5b).

**Discussion**

Although the TFE does not simulate the atmospheric effects of drought, it allows us to isolate the impacts of prolonged soil moisture deficit on leaf physiology, a process which is poorly simulated in ecosystem models, yet responsible for substantial uncertainty in modelled
climate change predictions for Amazonia (Powell et al., 2013; Rowland et al., 2015). Following 13 years of soil moisture deficit in the TFE there has been no change in the photosynthetic capacity of the trees, but a 48.5±3.6% increase in the leaf level $R_d$ of drought-vulnerable trees in the TFE relative to the control (Fig. 1 & 5, Table 2). Our measured values of both $V_{cmax}$ and $J_{max}$ lie well within the ranges previously measured in tropical rainforests (Carswell et al., 2000, Coste et al., 2005, Domingues et al., 2005, Meir et al., 2007, van de Weg et al., 2012) and we demonstrate that substantial long-term reductions in soil moisture availability in the TFE (Rowland et al., submitted) have no impact on the values of these photosynthetic parameters. In addition, no significant difference was observed in $A_{sat}$ between the control and TFE plots in either vulnerable or resistant trees (Fig. 4c); however seasonal differences in $A_{sat}$ and $g_s$ at $A_{sat}$ were greater for the drought vulnerable trees in the TFE (Fig. 4b, c). These results suggest that, in the TFE, even the taxa which are vulnerable to drought-induced mortality can achieve the same maximum photosynthetic rates as the control trees. However, as photosynthetic capacity does not equate to realised photosynthesis, it is possible that the TFE trees may still suffer from lower photosynthesis for example caused by lower daily average $g_s$.

Interestingly the taxa which are resistant to drought-induced mortality in the TFE showed no obvious signs of acclimation of photosynthetic capacity, $R_d$ or $g_s$ at $A_{sat}$ and this may suggest that these trees acclimate to the drought conditions in other ways, such as reducing individual canopy leaf area or by increasing root water uptake (Metcalfe et al., 2010a, Metcalfe et al., 2010b, Metcalfe et al., 2007). However maintenance of photosynthetic capacity in the droughted leaves may instead be an acclimation response itself; provided sufficient light and nutrients are available, investment in maintaining $V_{cmax}$ and $J_{max}$, at the expense of other investments within the tree, would compensate for canopy-scale reductions in $g_s$ and $C_i$, allowing maximization of photosynthetic output during wetter periods when $g_s$ is elevated.
This would, in theory, optimise light-use efficiency in the canopy (Hirose et al., 1988, Meir et al., 2002, Niinemets et al., 2015), provided no other long-term drought-related stress was occurring within the leaf. This potential for the droughted trees to maintain the same maximum photosynthetic output is consistent with the recent demonstration at this TFE experiment of no depletion of stored leaf, stem and branch non-structural carbohydrates (NSC), and no reduced investment into carbon-demanding processes such as growth following 13 years of experimental drought (Rowland et al., submitted).

Previously, Metcalfe et al., (2010a) found $R_d$ to be elevated in the TFE but their sample was taken from a subset of mostly shaded leaves, from trees of widely differing species in the control and TFE, without replication by species or mortality-risk. Using a sample design that balances tree taxa between plots across vulnerability status we show here that increased $R_d$ is a long-term acclimation response in the TFE, and is attributable to the higher respiration of certain taxa vulnerable to drought-induced mortality, during the driest parts of the year. Increased $R_d$ in response to low soil moisture has been observed elsewhere in Amazonia (Miranda et al., 2005) and has also been reported in Mediterranean species, as, albeit during shorter term drought conditions, elevations in $R_d$ were associated with low resistance to water stress in three taxa that had higher plant maintenance costs during drought (Varone & Gratani, 2015). It is therefore possible that an enhanced respiration cost during drought is a trait which has not been selected against in the Amazon because of its adaptive advantage. Significant reductions in $g_s$ and $A_{sat}$ in the drought-vulnerable trees suggest they are more hydraulically vulnerable during the dry season (Fig. 4). Consequently, elevated $R_d$ in these taxa may be explained by an increased necessity for hydraulic repair (Brodersen & McElrone, 2013), for processing of ROS (Atkin & Macherel, 2009, Ribas-Carbo et al., 2005) and/or an increase in photorespiration (Atkin & Macherel, 2009), or, counter intuitively here, use of increased substrate supply, which may occur if processes involved in carbon use or
transport are more impaired by drought than photosynthesis (Körner, 2013). We thus hypothesise that the increased carbon use (this study) and high mortality (da Costa et al., 2010; Table S1) in vulnerable trees in the TFE plot (Fig. 2) are caused by a reduced ability to cope with the physiological stress caused by prolonged reductions in soil water content, but that this physiological stress is not severe enough to decrease photosynthetic capacity.

The biochemistry of photosynthesis and $R_d$ are related, such that a short-term down-regulation of photosynthesis has frequently been observed to lead to a down-regulation of $R_d$ (Catoni & Gratani, 2014, Chastain et al., 2014, Flexas et al., 2006). Reductions in the ratio of photosynthesis to $R_d$ during drought have been reported elsewhere in the literature (Atkin & Macherel, 2009, Catoni & Gratani, 2014, Chastain et al., 2014, Miranda et al., 2005); we find this result at the Caxiuana TFE experiment, but unlike the cited studies we find this to be driven only by increased $R_d$ rather than decreased photosynthetic capacity. Our results also demonstrate that the relationship between photosynthetic capacity ($V_{cmax}$ and $J_{max}$) and $R_d$ are much weaker in the TFE than in control forest (Fig. 3), and that the standard relationships expected between $R_d$ and $N$, $P$, LMA or SLA (Atkin et al., 2015) are not found because of a significant treatment effect on $R_d$, but not on $N$, $P$, LMA or SLA (Table 2). This may suggest that $N$ and $P$ do not substantially limit $R_d$, in these plots. These results also suggest that the normally tight coupling of leaf respiratory processes with photosynthetic processes, and their relationships with leaf nutrient content and LMA or SLA are disrupted during long-term drought stress in tropical trees, and new model formulations are needed to simulate this response to drought.

This study examines how photosynthetic capacity and leaf dark respiration ($R_d$) acclimate to long-term (>12 years) soil moisture deficit in a tropical rainforest. Using a sampling design with a balanced selection of tree taxa between control and treatment we demonstrate that, following 12-13 years of soil moisture deficit, photosynthetic capacity is unchanged even in
taxa which are prone to drought-induced mortality. The rate of $R_d$ in the dry season in the experimentally droughted forest was, however, significantly elevated because of a 48.5±3.6% increase in the $R_d$ of taxa which are prone to drought-induced mortality. We conclude that, even after a severe and prolonged reduction in soil moisture availability, trees are able to maintain the same photosynthetic capacity, despite increasing $R_d$. Such results suggest that better models of acclimation in $R_d$ and overall gas exchange may be necessary to accurately simulate the responses of tropical rainforests to drought.

Acknowledgements

This work is a product of UK NERC grant NE/J011002/1 to PM and MM, CNPQ grant 457914/2013-0/MCTI/CNPq/FNDCT/LBA/ESECAFLOR to ACLD, and ARC grant FT110100457 to PM. It was previously supported by NERC NER/A/S/2002/00487, NERC GR3/11706, and EU FP5-Carbonsink and EU FP7-Amazalert no. 282664 to PM, & BK. LR MM and PM would also like to acknowledge support from Stephen Sitch, University of Exeter. We would also like to thank two anonymous reviewers for the contributions to this manuscript.

References

Atkin OK, Bloomfield KJ, Reich PB et al. (2015) Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. New Phytol, 206, 614-636.

Atkin OK, Macherel D (2009) The crucial role of plant mitochondria in orchestrating drought tolerance. Ann Bot, 103, 581-597.

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science, 8, 343-351.

This article is protected by copyright. All rights reserved.
Atkin OK, Turnbull MH, Zaragoza-Castells J, Fyllas NM, Lloyd J, Meir P, Griffin KL (2013) Light inhibition of leaf respiration as soil fertility declines along a post-glacial chronosequence in New Zealand: an analysis using the Kok method. *Plant and Soil*, 367, 163-182.

Ayub G, Smith RA, Tissue DT, Atkin OK (2011) Impacts of drought on leaf respiration in darkness and light in Eucalyptus saligna exposed to industrial-age atmospheric CO(2) and growth temperature. *New Phytol*, 190, 1003-1018.

Beer C, Reichstein M, Tomelleri E et al. (2010) Terrestrial Gross Carbon Dioxide Uptake: Global Distribution and Covariation with Climate. *Science*, 329, 834-838.

Bonal D, Born C, Brechet C, Coste S, Marcon E, Roggy JC, Guehl JM (2007) The successional status of tropical rainforest tree species is associated with differences in leaf carbon isotope discrimination and functional traits. *Annals of Forest Science*, 64, 169-176.

Bonal D, Bosc A, Ponton S et al. (2008) Impact of severe dry season on net ecosystem exchange in the Neotropical rainforest of French Guiana. *Glob Chang Biol*, 14, 1917-1933.

Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P (2008) Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 363, 1839-1848.

Brodersen CR, McElrone AJ (2013) Maintenance of xylem network transport capacity: a review of embolism repair in vascular plants. *Frontiers in Plant Science*, 4.

Carpenter SR (1996) Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology*, 77, 677-680.

This article is protected by copyright. All rights reserved.
Carswell FE, Meir P, Wandelli EV et al. (2000) Photosynthetic capacity in a central Amazonian rain forest. *Tree Physiology*, 20, 179-186.

Catoni R, Gratani L (2014) Variations in leaf respiration and photosynthesis ratio in response to air temperature and water availability among Mediterranean evergreen species. *Journal of Arid Environments*, 102, 82-88.

Chastain DR, Snider JL, Collins GD, Perry CD, Whitaker J, Byrd SA (2014) Water deficit in field-grown Gossypium hirsutum primarily limits net photosynthesis by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis. *J Plant Physiol*, 171, 1576-1585.

Ciais P, Reichstein M, Viovy N et al. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, 437, 529-533.

Coste S, Roggy JC, Imbert P, Born C, Bonal D, Dreyer E (2005) Leaf photosynthetic traits of 14 tropical rain forest species in relation to leaf nitrogen concentration and shade tolerance. *Tree Physiology*, 25, 1127-1137.

Crous KY, Zaragoza-Castells J, Low M et al. (2011) Seasonal acclimation of leaf respiration in Eucalyptus saligna trees: impacts of elevated atmospheric CO2 and summer drought. *Glob Chang Biol*, 17, 1560-1576.

Da Costa ACL, Galbraith D, Almeida S et al. (2010) Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. *New Phytologist*, 187, 579-591.

Davidson EA, Ishida FY, Nepstad DC (2004) Effects of an experimental drought on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Glob Chang Biol*, 10, 718-730.

Domingues TF, Berry JA, Martinelli LA, Ometto JPHB, Ehleringer JR (2005) Parameterization of canopy structure and leaf-level gas exchange for an eastern

This article is protected by copyright. All rights reserved.
Amazonian tropical rain forest (Tapajos National Forest, Para, Brazil). *Earth Interactions*, 9.

Domingues TF, Meir P, Feldpausch TR *et al.* (2010) Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant Cell and Environment*, 33, 959-980.

Doughty CE, Metcalfe DB, Girardin CaJ *et al.* (2015) Drought impact on forest carbon dynamics and fluxes in Amazonia. *Nature*, 519, 78-U140.

Fisher RA, Williams M, Da Costa AL, Malhi Y, Da Costa RF, Almeida S, Meir P (2007) The response of an Eastern Amazonian rain forest to drought stress: results and modelling analyses from a throughfall exclusion experiment. *Glob Chang Biol*, 13, 2361-2378.

Flexas J, Bota J, Galmes J, Medrano H, Ribas-Carbo M (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum*, 127, 343-352.

Flexas J, Medrano H (2002) Drought-inhibition of photosynthesis in C-3 plants: Stomatal and non-stomatal limitations revisited. *Ann Bot*, 89, 183-189.

Galbraith D, Levy PE, Sitch S, Huntingford C, Cox P, Williams M, Meir P (2010) Multiple mechanisms of Amazonian forest biomass losses in three dynamic global vegetation models under climate change. *New Phytologist*, 187, 647-665.

Galmes J, Aranjuelo I, Medrano H, Flexas J (2013) Variation in Rubisco content and activity under variable climatic factors. *Photosynth Res*, 117, 73-90.

Gatti LV, Gloor M, Miller JB *et al.* (2014) Drought sensitivity of Amazonian carbon balance revealed by atmospheric measurements. *Nature*, 506, 76-+.

Grace J, Mitchard E, Gloor E (2014) Perturbations in the carbon budget of the tropics. *Glob Chang Biol*, 20, 3238-3255.
Hirose T, Werger MJA, Pons TL, Vanrheenen JWA (1988) Canopy Structure and Leaf Nitrogen Distribution in a Stand of Lysimachia-Vulgaris L as Influenced by Stand Density. *Oecologia, 77*, 145-150.

Huntingford C, Zelazowski P, Galbraith D *et al.* (2013) Simulated resilience of tropical rainforests to CO2-induced climate change. *Nature Geoscience, 6*, 268-273.

Körner C (2013) Growth Controls Photosynthesis – Mostly. *Nova Acta Leopoldina, 391*, 273–283.

Kull O, Kruijt B (1999) Acclimation of photosynthesis to light: a mechanistic approach. *Functional Ecology, 13*, 24-36.

Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell and Environment, 25*, 275-294.

Meir P, Brando PM, Nepstad D *et al.* (2009) The Effects of Drought on Amazonian Rain Forests. *Amazonia and Global Change, 186*, 429-449.

Meir P, Kruijt B, Broadmeadow M *et al.* (2002) Acclimation of photosynthetic capacity to irradiance in tree canopies in relation to leaf nitrogen concentration and leaf mass per unit area. *Plant Cell and Environment, 25*, 343-357.

Meir P, Levy PE, Grace J, Jarvis PG (2007) Photosynthetic parameters from two contrasting woody vegetation types in West Africa. *Plant Ecology, 192*, 277-287.

Meir P, Mencuccini M, Dewar RC (2015a) Drought-related tree mortality: addressing the gaps in understanding and prediction. *New Phytologist, n/a-n/a.*

Meir P, Metcalfe DB, Costa ACL, Fisher RA (2008) The fate of assimilated carbon during drought: impacts on respiration in Amazon rainforests. *Philosophical Transactions of the Royal Society B-Biological Sciences, 363*, 1849-1855.

This article is protected by copyright. All rights reserved.
Meir P, Wood TE, Galbraith DR, Brando PM, Da Costa ACL, L. R, V. FL (2015b) Threshold responses to soil moisture deficit by trees and soil in tropical rain forests: insights from field experiments. Bioscience.

Meir P, Woodward FI (2010) Amazonian rain forests and drought: response and vulnerability. New Phytologist, 187, 553-557.

Mencuccini M, Hölttä T (2010) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytologist, 185, 189-203.

Metcalfe DB, Lobo-Do-Vale R, Chaves MM et al. (2010a) Impacts of experimentally imposed drought on leaf respiration and morphology in an Amazon rain forest. Functional Ecology, 24, 524-533.

Metcalfe DB, Meir P, Aragao LEOC et al. (2010b) 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, Meir P, Aragao LEOC et al. (2007) Factors controlling spatio-temporal variation in carbon dioxide efflux from surface litter, roots, and soil organic matter at four rain forest sites in the eastern Amazon. Journal of Geophysical Research-Biogeosciences, 112.

Miranda EJ, Vourlitis GL, Priante N et al. (2005) Seasonal variation in the leaf gas exchange of tropical forest trees in the rain forest-savanna transition of the southern Amazon Basin. Journal of Tropical Ecology, 21, 451-460.

Murphy J, Riley JP (1962) A Modified Single Solution Method for Determination of Phosphate in Natural Waters. Analytica Chimica Acta, 26, 31-.

This article is protected by copyright. All rights reserved.
Nepstad DC, Moutinho P, Dias MB et al. (2002) The effects of partial throughfall exclusion on canopy processes, aboveground production, and biogeochemistry of an Amazon forest. *Journal of Geophysical Research-Atmospheres*, **107**.

Nepstad DC, Tohver IM, Ray D, Moutinho P, Cardinot G (2007) Mortality of large trees and lianas following experimental drought in an amazon forest. *Ecology*, **88**, 2259-2269.

Niinemets U, Keenan TF, Hallik L (2015) A worldwide analysis of within-canopy variations in leaf structural, chemical and physiological traits across plant functional types. *New Phytologist*, **205**, 973-993.

O'brien MJ, Burslem DFRP, Caduff A, Tay J, Hector A (2015) Contrasting nonstructural carbohydrate dynamics of tropical tree seedlings under water deficit and variability. *New Phytologist*, **205**, 1083-1094.

Osmond B, Ananyev G, Berry J et al. (2004) Changing the way we think about global change research: scaling up in experimental ecosystem science. *Glob Chang Biol*, **10**, 393-407.

Phillips OL, Aragao LEOC, Lewis SL et al. (2009) Drought Sensitivity of the Amazon Rainforest. *Science*, **323**, 1344-1347.

Powell TL, Galbraith DR, Christoffersen BO et al. (2013) Confronting model predictions of carbon fluxes with measurements of Amazon forests subjected to experimental drought. *New Phytologist*, **200**, 350-364.

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol*, **161**, 1189-1202.

Ribas-Carbo M, Taylor NL, Giles L et al. (2005) Effects of water stress on respiration in soybean leaves. *Plant Physiol*, **139**, 466-473.
Rowland L, da costa ACL, Mencuccini M, et al. (Submitted) Long-term drought in tropical forest: death from hydraulic failure, not carbon starvation. *Nature*

Rowland L, Harper A, Christoffersen BO et al. (2015) Modelling climate change responses in tropical forests: similar productivity estimates across five models, but different mechanisms and responses. *Geoscientific Model Development, 8*, 1097-1110.

Rowland L, Hill TC, Stahl C et al. (2014) Evidence for strong seasonality in the carbon storage and carbon use efficiency of an Amazonian forest. *Glob Chang Biol, 20*, 979-991.

Ruivo M, Cunha E (2003) Mineral and organic components in archaeological black earth and yellow latosol in Caxiuanã, Amazon, Brazil. In: *Ecosystems and sustainable development.* (ed Tiezzi E, Brebbia, C. A., Uso, J. L., (Eds)) pp Page. Southampton, UK, WIT Press.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C(3) leaves. *Plant Cell Environ, 30*, 1035-1040.

Sperlich D, Chang CT, Peñuelas J, Gracia C, Sabaté S (2015) Seasonal variability of foliar photosynthetic and morphological traits and drought impacts in a Mediterranean mixed forest. *Tree Physiology.*

Stahl C, Burban B, Wagner F, Goret JY, Bompy F, Bonal D (2013) Influence of Seasonal Variations in Soil Water Availability on Gas Exchange of Tropical Canopy Trees. *Biotropica, 45*, 155-164.

Van De Weg MJ, Meir P, Grace J, Ramos GD (2012) Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest. *Oecologia, 168*, 23-34.

Varone L, Gratani L (2015) Leaf respiration responsiveness to induced water stress in Mediterranean species. *Environmental and Experimental Botany, 109*, 141-150.
Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith G (2009) Mixed Effects Models and Extensions in Ecology with R. , New York, Springer.

**Figure Captions**

Fig. 1: Average $V_{cmax}$ (a), $J_{max}$ (b) and $R_d$ (c) expressed in $\mu$mol $m^{-2}$ $s^{-1}$ in the control (C; white) and TFE (grey) plots, in peak dry season 2013 (November) and peak wet season 2014 (June). Error bars show the standard error.

Fig. 2: $R_d$ for the resistant and vulnerable tree taxa in the control (white) and TFE (grey) plot in peak dry season of 2013. Columns with a * indicates significant difference with $P<0.05$. Error bars show standard error.

Fig. 3: Relationships between $V_{cmax}$ and $R_d$ (a-d) and $J_{max}$ and $R_d$ (e-h) during the wet and dry season in the control and TFE plot. If the linear line is significant ($P<0.05$), the linear relationship, correlation coefficient ($r^2$) and significance value ($P$) are shown.

Fig. 4: Box plots of water use efficiency (WUE; a), stomatal conductance ($g_s$; b) and light-saturated photosynthesis at 400 ppm of CO$_2$ ($A_{sat}$) during peak dry season 2013 and peak wet season (2014) for vulnerable and resistant tree taxa in the control (white) and TFE (grey) plots. * indicates significant difference with $p<0.05$.

Fig. 5: Ratio of TFE: control plot $V_{cmax}$ (a) and $R_d$ (b) values for various measurement campaigns made at the Caxiuanã TFE experiment from prior to the start of the experiment (2001) to 2013; see Methods for details. The symbols indicate the differences in the sample selection. ‘Uncut tower trees’ are data from leaves on attached branches accessed via a walk-up through-canopy tower. ‘Metcalfe sample’ are from leaves on the same trees sampled by Metcalfe et al. (2010). This study used cut branches with fully sunlit leaves and the results from Fig. 2 are re-plotted here.

This article is protected by copyright. All rights reserved.
Tables

Table 1: Genus, species with genus and sensitivity to drought-induced mortality (see table S1) for the number of trees sampled in the control and TFE plots for the 2013 and 2014 analysis of $R_d$, $V_{cmax}$, $J_{max}$, $A_{sat}$, $g_s$, leaf nutrients and LMA.

| Genus       | Species sampled within Genus | Vulnerability | No° sampled on control plot | No° sampled on TFE plot |
|-------------|------------------------------|---------------|-----------------------------|-------------------------|
| *Eschweilera* | *coriacea, grandiflora, pedicellata* | Vulnerable | 5                           | 6                       |
| *Licania*    | *membranacea, octandra*       | Resistant     | 3                           | 3                       |
| *Manilkara*  | *bidentata*                  | Vulnerable     | 3                           | 3                       |
| *Pouteria*   | *anomala*                    | Vulnerable     | 3                           | 3                       |
| *Protium*    | *tenufolium, paniculatum*     | Resistant     | 3                           | 3                       |
| *Swartzia*   | *racemosa*                   | Resistant     | 3                           | 3                       |

Table 2: Mixed effect model results on a leaf area and a leaf mass basis, to test for: a) the drought treatment effect on $V_{cmax}$, $J_{max}$, $R_d$, nitrogen (N), phosphorus (P), leaf mass per area (LMA), and specific leaf area (SLA), using the treatment effect (control versus TFE plot (Pl)), tree vulnerable or resistant status (V), and season (S) as fixed variables and tree nested within genus as the random component of the model. b) the effect of nutrients and leaf area and mass on $V_{cmax}$, $J_{max}$ and $R_d$, using N, P and either LMA or SLA as fixed variables and tree nested within genus as the random component of the model. The significance of the intercept ($p$) and valid fixed variables are shown as well as the proportion of the model variance accounted for by the random component of the model (R variance, %). * indicates variable interaction.
### a. Models to test treatment effects

| Y variable          | Fixed variables tested | Significant fixed variables | R Variance, % |
|---------------------|------------------------|----------------------------|---------------|
| $V_{cmax}$ area basis | Pl, V, S               | None                       | 64.41         |
| $J_{max}$ area basis  | Pl, V, S               | None                       | 43.53         |
| $R_d$ area basis     | Pl, V, S               | Pl*V*S ($p=0.02$)          | 31.54         |
| N area basis         | Pl, V, S               | None                       | 78.34         |
| P area basis         | Pl, V, S               | None                       | 17.36         |
| LMA                 | Pl, V, S               | None                       | 52.46         |
| $V_{cmax}$ mass basis | Pl, V, S               | None                       | 52.35         |
| $J_{max}$ mass basis  | Pl, V, S               | None                       | 32.61         |
| $R_d$ mass basis     | Pl, V, S               | Pl*V*S ($p=0.02$)          | 43.60         |
| N mass basis         | Pl, V, S               | None                       | 71.21         |
| P mass basis         | Pl, V, S               | None                       | 30.78         |
| SLA                 | Pl, V, S               | None                       | 60.30         |

### b. Models to test Nutrient and Leaf mass/area effects

| Y variable          | Fixed variables tested | Significant fixed variables | R Variance, % |
|---------------------|------------------------|----------------------------|---------------|
| $V_{cmax}$ area basis | N, P, LMA             | LMA ($p<0.01$)             | 68.86         |
| $J_{max}$ area basis  | N, P, LMA             | None                       | 45.01         |
| $R_d$ area basis     | N, P, LMA             | None                       | 4.62          |
| $V_{cmax}$ mass basis | N, P, SLA            | N ($p<0.01$)              | 44.98         |
| $J_{max}$ mass basis  | N, P, SLA            | N ($p<0.01$)              | 14.97         |
| $R_d$ mass basis     | N, P, SLA            | None                       | 27.73         |
