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8 **Stand dynamics modulate water cycling and mortality risk in droughted tropical forest**

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30   Running title: Tropical forest drought & water cycling

31   Primary Research Article

32   Abstract:

33   Transpiration from the Amazon rainforest generates an essential water source at a global and

34   local scale. However, changes in rainforest function with climate change can disrupt this

35   process, causing significant reductions in precipitation across Amazonia, and potentially at a

36   global scale. We report the only study of forest transpiration following a long-term (>10 year)

37   experimental drought treatment in Amazonian forest. After 15 years of receiving half the

38   normal rainfall, drought-related tree mortality caused total forest transpiration to decrease by

39   30%. However, the surviving droughted trees maintained or increased transpiration because

40   of reduced competition for water and increased light availability, which is consistent with

41   increased growth rates. Consequently, the amount of water supplied as rainfall reaching the

42   soil and directly recycled as transpiration increased to 100%. This value was 25% greater

43   than for adjacent non-droughted forest. If these drought conditions were accompanied by a

44   modest increase in temperature (e.g. 1.5°C), water demand would exceed supply, making the

45   forest more prone to increased tree mortality.

46

47   Introduction

48   In South America, 25-35% of precipitation is estimated to be recycled via repeated

49   precipitation-evaporation processes as air masses travel west over Amazonian rainforest

50   (Eltahir and Bras, 1994; Zemp et al., 2014). Up to 70% of the water resources of the

51   extensive Rio de La Plata basin are dependent on evapotranspiration from Amazonia (van der

52   Ent et al., 2010). Changes in land cover properties in the Amazon basin can disrupt this

53   recycling process, potentially causing significant reductions in precipitation both in

54   Amazonia and regionally to the La Plata basin (Spracklen et al., 2012), with large economic

55   consequences (Marengo et al., 2016). However, how tropical forest transpiration will respond

56   to future drought and temperature change remains uncertain. Despite the climatological

57   importance of large gross fluxes of transpiration from the world's tropical rainforests

58   (Lawrence and Vandecar, 2015; Spracklen et al., 2012), predictions of how water recycling

59   from tropical rainforest may change with climate, in particular climate extremes, are poorly

60 constrained by data for this biome (Kume et al., 2011; Restrepo-Coupe et al., 2013). The

61 frequency and intensity of sub-regional extremes in precipitation and temperature are

62 predicted to increase this century, leading to increased drought at seasonal, interannual and

63 decadal timescales (Duffy et al., 2015; Fu et al., 2013). How water use by forests will change

64 remains unclear. Tropical rainforests generally transpire 30-70% of incoming rainfall

65 (Kumagai, 2016), but at their climatic margins, where annual rainfall is 1200-1500 mm/yr

66 (Zelazowski et al., 2011), this value rises to above 90% placing a cap on regional moisture

67 supply, deep soil recharge and river runoff (Kume et al., 2011; van der Ent et al., 2010).

68 Processes ranging in scale from plant tissue to ecosystem can control how the proportion of

69 rainfall that is recycled changes in response to drought. For individual trees, long-term

70 responses may include physiological changes in water use efficiency, turgor regulation and

71 the sensitivity of xylem hydraulics to cavitation, structural acclimation in new root growth

72 (Eller et al., 2016; Oliveira et al., 2005), or changes in leaf to sapwood or root area ratios

73 (Wolfe et al., 2016). These responses can help regulate gross water demand by the canopy,

74 but ultimately it will be the demographic regulation of stand density via competition for water

75 that will determine whole-system water use and stability (Meir et al. 2015a).

76 Measurements of sapflux (J_s) are a powerful method to understand the annual and seasonal

77 shifts in forest water use, including the relationship of transpiration to environmental

78 variables (Eller et al., 2015; Fisher et al., 2007; Poyatos et al., 2013) and the physiological

79 plasticity associated with stomatal regulation in trees (Martinez-Vilalta et al., 2014). There

80 are however relatively few reports of continuous sapflux (J_s) measurements in tropical

81 rainforest (Fisher et al., 2007; Granier et al., 1996), none of which have been conducted

82 following long-term drought (>5 years). Studies during long-term drought are essential to

83 establish whether tropical trees can adjust their water use to drier soils over timescales

84 approaching those of possible changes in climate. By imposing a reduction in soil water

85 availability, large scale through-fall exclusion (TFE) provides a unique way to examine the

86 processes underlying long-term responses to increased deficit in soil water potential, and to

87 examine how water use and stand-scale water cycling are altered.

88 Here we quantify the effects of a prolonged experimental soil drought on water use as a

89 proportion of available rainfall by an old-growth tropical rainforest in eastern Amazonia. We

90 use the world's only long-running tropical forest TFE experiment, at the Caxiuanã National

91 Forest Reserve, Pará State, Brazil (da Costa et al., 2010; Meir et al., 2015b; Rowland et al.,

92   2015b), to compare how transpiration and through-fall recycling (the percentage of canopy

93   through-fall transpired by the forest) are altered between a normal forest and a drought-

94   treated forest, with the latter having experienced a 50% TFE treatment since 2002. We

95   previously reported (Rowland et al., 2015a) the loss of about 40% biomass after 14 years

96   since the TFE started. Because measurements of stand scale transpiration were also available

97   for the years 2002-2003, i.e., at the start of the experiment, but before the large waves of

98   mortality occurred, we are also able to determine how total water use and its partitioning

99   changed in response to changes in stand density and structure.

100

101   Materials and Methods

102   Site

103   The site is a long-term through-fall exclusion (TFE) experiment located at the Caxiuanã

104   National Forest Reserve in the eastern Amazon (1°43'S. 51°27W). The site has a mean

105   rainfall of 2000-2500 mm yr^{-1}, a pronounced dry season between June and November

106   (rainfall <100 mm $month^{-1}$) and is situated on *terra firme* forest, with yellow oxisol soils

107   (Ruivo and Cuhha, 2003).

108   The TFE experiment consists of two 1 ha plots located on old-growth tropical forest. The

109   treatment plot (TFE) has been covered with plastic panels and guttering 1-2 m in height since

110   2002. This structure excludes 50% of the incoming canopy through-fall. A control plot, on

111   which no rainfall exclusion has taken place, is located <50 m from the TFE. For further

112   details on the experimental design and results see: da Costa et al., 2010, Meir et al. 2015 and

113   Rowland et al., 2015. Following 14 years of continuous drought the plot has experienced a

114   40% loss in biomass (equivalent to 100 Mg C ha^{-1}), this loss generated a substantial reduction

115   in basal and thus sapwood area, a reduction in leaf area index (LAI) and an increase in light

116   interception in the lower canopy (see Rowland et al., 2015a).

117

118   Meteorological and soil moisture data

119   All meteorological variables were obtained from a weather station situated at the top of a 40

120   m tower located in the control forest. During the period of 2014-2016 air temperature,

121   relative humidity, solar radiation and rainfall were monitored half hourly using HC2S3

122 (Campbell Scientific, Logan, USA), CM3 sensors (Kipp and Zonen, Delft, The Netherlands),

123 and a tipping bucket rain gauge (TE525MM, Campbell Scientific, Logan, USA) respectively.

124 Vapour pressure deficit (VPD) was calculated from temperature and relative humidity. Soil

125 access pits are located in the control and TFE plots. In each soil access pit volumetric soil

126 water content sensors (CS616, Campbell Scientific, Logan, USA) have been placed at depths

127 of 0, 0.5, 1, 2.5 and 4 m, to monitor soil moisture every hour (*cf.* Fisher et al., 2007, for full

128 methodology). Here we use the data collected during 2014-2016, the period during which

129 sapflux (J_s) data were collected. Hourly relative extractable water (REW) aggregated across

130 the first two meters was calculated using the soil moisture data and following the

131 methodology in Meir et al. 2015. Daily values were calculated using a 30 day running mean

132 so that the seasonal trend of REW was captured, rather than daily or hourly spikes in soil

133 water concentrations.

134

135 J_s Data

136 J_s was measured using the heat balance method (Cermak et al., 1973; Cermak et al., 2004;

137 Kucera et al., 1977) and previously used at the site (Fisher et al., 2007). EMS51 sensors

138 (Environmental monitoring systems; http://www.emsbrno.cz), were used on all trees. The

139 installation process and functioning of these sensors are described in the supplementary

140 information. Between November 2014 and December 2016 the EMS51 sensors were

141 installed on 16 trees in the control plot and 13 trees in the TFE plot. The start date of

142 sampling varied among trees (see Table S1). Trees for which sensors were installed in 2016

143 (seven on the control and three on the TFE) were excluded from the upscaling analysis (see

144 below) on the basis that they had an insufficient data time series. To ensure we could up-scale

145 with confidence, sensors were strategically placed across trees with a range of diameters at

146 breast height (DBH) values (15-56 cm) and on common species in the control and TFE plots

147 known to be both sensitive and resistant to drought stress (see Table S1).

148 Values of J_s obtained from the EMS51 sensors were always offset from zero as a constant

149 part of the heat loss from the heated electrodes is conducted into the xylem tissue. To remove

150 this effect the data were baselined, as performed in other standard sap flux processing

151 protocols (e.g. Poyatos et al., 2013). To baseline the data, the minimum value of the J_s for

152 each night was subtracted from all values for the subsequent day, provided evaporative

153 demand was low (preventing night-time J_s, VPD < 0.15 kPa). If night-time VPD > 0.15 kPa,

154   a minimum value was linearly interpolated from the baseline values from surrounding days

155   using the *approx* function in R (R Core team 2014).

156

157   Gapfilling J_s Data

158   Gaps in the data varied from 0% to 63% (average of 8%) and were generally caused by

159   power failure or broken sensors. Gaps in the hourly baselined J_s data since sensor installation

160   were gap-filled using an autoregressive (AR1) style model, accounting for the autocorrelation

161   in the data. Firstly, the *boxcox* function in R was used to determine the lambda value to

162   power transform the J_s data of each tree (lambda range 0.46-0.84). Secondly, a linear

163   regression was performed between the power-transformed J_s, the three independent variables

164   VPD, radiation, REW and six vectors of the power-transformed J_s preceding the dependent

165   variable by one to six hours. We correlated each J_s data point with the six hourly data points

166   preceding it, as this was the number required to remove the autocorrelation effect across all

167   trees (determined using ACF plots). Data from all but one of the trees were gap filled with a

168   model which had an $r^2 > 0.90$; the mean model fit was $r^2 = 0.93 \pm 0.07$ (s.e.m.), demonstrating a

169   very good fit between modelled and measured J_s.

170

171   Statistical Analysis

172   All statistical analyses of J_s data were conducted within R 3.0.2 (R Core Team[35]) and all

173   errors are shown as standard deviation. To compare diurnal responses between plots and

174   seasons an average diurnal J_s pattern was calculated for the control and TFE plots, during

175   peak wet and dry season. Peak wet and dry season were determined as the two months with

176   the highest (October and November) and lowest (March and April) monthly average VPD.

177   Multiple linear regressions between mean daytime transpiration rate per tree, per plot

178   (calculated as the average J_s from all trees per plot) and environmental conditions were fitted

179   to estimate the most important environmental controls on daily J_s. Initially VPD or

180   temperature with radiation, and REW were included in the model and sequentially non-

181   significant variables were excluded in stepwise linear regressions determined by Akaike's

182   information criterion. For the TFE the use of a single model across both wet and dry season

183   was compared to the model fit of using separate wet and dry season models (considering wet

184   season as Feb-Jul and dry season as Aug-Jan). Two models were most effective on the TFE

185   (see Results) and the same seasonal modelling approach was followed with the control plot.

186   The *relaimpo* package in R (Grömping, 2006) was used to calculate the proportion of the

187   explained variance which was accounted for by each variable retained in each of the final

188   models.

189   Seasonal relationships of VPD to J_s were created by fitting a sigmoidal function using the

190   SSllogis function in R through average hourly J_s data for the trees on the control and TFE,

191   binned by VPD classes. Separate relationships were created for peak wet and dry season and

192   the data were normalised using the maximum average J_s, across plots and seasons, to make

193   the relationships comparable between plots and seasons.

194

195   Scaling J_s to calculate plot-scale transpiration and its temperature sensitivity

196   Scaling J_s from the measured trees for the measured periods to plot level at the yearly time

197   scale involved the following steps in order to properly propagate the sources of uncertainty

198   deriving from tree-to-tree variability in J_s as well as uncertainties in the scaling of J_s with

199   tree DBH.

200   To obtain a scaling relationship between tree J_s and DBH, we regressed J_s data from April-

201   May 2015 (i.e., peak of wet season and when tree DBH were measured) against DBH. This

202   allowed us to additionally included data obtained by Fisher et al. (2007; also collected at peak

203   wet season); all data were obtained using the same measurement method (Environmental

204   monitoring systems; http://www.emsbrno.cz). There was a linear relationship between DBH

205   and mean daytime J_s, with an r^2 of 0.39 and p<0.01 (Fig. S1). This scaling relationship was

206   assumed on the control and TFE plot based on similarity of J_s values across the two plots

207   during the wet season (see Results section), and it was applied to the DBH of all trees on both

208   plots measured in 2015 (see Rowland et al. 2015a, for further details). To account for the

209   uncertainty in the parameters of this relationship, 1000 parameter estimates were randomly

210   generated from the model using the covariance matrix for the intercept and slope. These

211   parameters were used to create 1000 estimates of average daytime April and May J_s for all

212   trees >10 cm DBH on both plots. The average daytime J_s values for each tree, for each of the

213   1000 parameter combinations, were then summed to give 1000 plot-scale estimates of

214   transpiration for April and May of the measurement years, accounting for the error on our

215   DBH to J_s relationship. Following this, a second procedure was employed using similar

216   principles to propagate uncertainty from 1,000 estimates of the measured April-May data to

217 the whole year and across the two plots. We employed the best-fit multilinear model per plot,

218 which described how mean daily J_s varies with climate variables (see above). Because of the

219 strong autocorrelation between VPD, RH and air temperature, only the best regressor among

220 these three was finally employed in the upscaling procedure (see supplementary information

221 for further details). Besides the two estimates for the Control and TFE plots, a third estimate

222 of plot-scale transpiration was generated by applying the estimated J_s from the multiple

223 regression models of Control to the standing biomass of TFE. This estimate gives downscaled

224 values of transpiration on Control with the effect of the loss in basal area on the TFE imposed

225 on Control, and the changes in transpiration rates with environmental variables remaining

226 equal to those on Control.

227 To estimate the effects of increasing temperatures on plot scale transpiration, the 1000 model

228 coefficients from above were re-run with temperature, relative humidity and VPD altered

229 according to a 1.5, 2, 3, 4, and 5˚C increase s in mean air temperature. We emphasise that the

230 purpose of these temperature rise scenarios is not for future prediction, but to estimate the

231 effects of long-term drought on the sensitivity of the forest to other changes in climate. The

232 scaling procedure was then repeated as above. The transpiration rates at each temperature

233 level were then compared to the canopy through-fall received by each plot assuming a canopy

234 storage term of 21.5% on the control plot, as measured at the site in 2008 (Oliveira et al.,

235 2008; and within the ranges of canopy storage terms measured across other Amazonian

236 forests (Czikowsky and Fitzjarrald, 2009)). On the TFE we scaled down this estimate of

237 canopy storage to 18.1% (Oliveira et al., 2008), in proportion with the leaf area index

238 measured in TFE relative to Control (See Rowland et al., 2015a), assuming that canopy

239 interception decreases proportionally with leaf area. The analysis was also repeated using a

240 canopy storage term of 12% (Czikowsky and Fitzjarrald, 2009) to account for uncertainty in

241 throughfall resulting from differences in LAI across plots (see sensitivity to canopy

242 interception term section). Also we would expect it to provide a lower limit to the sensitivity

243 in TFE because of fewer interception surfaces in TFE (i.e., lower LAI and biomass; Rowland

244 et al., 2015a). However due to an inability to accurately estimate LAI on a per tree basis,

245 which may have changed over time due to the treatment effect and due to the likely increase

246 in atmospheric coupling on the drought relative to the control plot due to increasing mortality

247 over time, we were not able to accurately scale sapflux according to leaf area to estimate

248 differences in leaf level conductance between the plots (e.g. Eller et al., 2015).

249

250 Results

251 During the study period (November 2014-December 2016) there were strong seasonal
252 changes in relative extractable water (REW), precipitation and moderate seasonal changes in
253 vapour pressure deficit (VPD) at our study site (Fig. 1). An El Niño event took place across
254 Amazonia in 2015-16, but had limited distinctive influence on climate drivers at our site,
255 which is demonstrated by the El Niño year not creating substantial climate anomalies relative
256 to previous years (Fig. S2). Therefore considering 2015 to represent standard climatological
257 conditions, we find average transpiration is 1389 ± 279 (s.d.) mm yr^{-1} on the control forest
258 plot. On the TFE forest plot a transpiration rate of 964 ± 245 (s.d.) mm yr^{-1} in response to the
259 50% experimental reduction in throughfall was observed; this represents a 30% decline in
260 transpiration relative to the control. Transpiration therefore comprised 75% (s.d. range = 60-
261 90%) of canopy through-fall on the control, compared to 101% (s.d. range = 75-127%) on the
262 TFE (Fig. 2). These estimates of through-fall recycling at Caxiuanã are similar to the mean
263 values previously quantified at the start of the TFE treatment for the years 2002-3 using
264 updated estimates for canopy interception for the plots (59-71% and 78-103%, control and
265 TFE, respectively, Fig. 2). These estimates are robust to assumptions made regarding the
266 magnitude of canopy rainfall interception as a proportion of total rainfall and to differences in
267 canopy storage caused by different values of leaf area index across plots (see Supplementary
268 Table 2).

269 Relative to the control, we observed changes in the transpiration rates of trees on the TFE
270 (Fig. 3). However, there was only a 5% difference between 2015 transpiration on the TFE
271 and the transpiration expected if estimates from the control were downscaled to reflect the
272 40% reduction in biomass and related basal area which occurred between 2002 and 2015
273 (Fig. 2). This small reduction by low dry season transpiration was countered with higher wet
274 season transpiration on the TFE (Fig.3). Increased seasonality in TFE transpiration meant that
275 daytime J_s was modelled more effectively using a separate multiple regression model for dry
276 (Aug-Jan) and wet (Feb-Jul) season on the TFE (r^2 dry = 0.60, r^2 wet = 0.69, r^2 whole year =
277 0.61, all p values <0.01). Dry season variation in transpiration on TFE was explained mostly
278 by REW (44%) and radiation (47%). During the wet season, radiation explained 60% of the
279 variance, VPD 33% and REW 7%. On the control plot air temperature (32%) and radiation
280 (67%) controlled dry season transpiration (r^2=0.81) and radiation (65%) and VPD (35%)
281 were the most important for controlling wet season fluxes (r^2=0.72).

282   The reduced dry season transpiration flux on the TFE (Fig. 3) was caused by substantially
283   lower peak daytime (11am-4pm) fluxes in the dry season (Fig 4b) compared to the wet
284   season. In contrast, the control plot maintained higher J_s throughout the day in the dry season
285   relative to the wet (Fig. 4a), suggesting low REW constrained J_s during periods of high
286   atmospheric demand on the TFE. The REW constraint resulted in an altered relationship
287   between J_s and VPD in the dry season on the TFE, contrasting with the wet season
288   relationship, which was similar to that observed on the control (Fig. 5). However, this
289   increased seasonality had a limited effect on plot-scale reductions in transpiration relative to
290   the effect of the loss of biomass and related basal area and active sapwood area (Fig. 2 & 3).

291   Using the multivariate linear models which specified how J_s varied with environmental
292   conditions on the control and TFE plots (see Methods), we explored how transpiration would
293   vary on both plots if an increase in mean temperature of 1.5-5 °C and the resultant increases
294   in VPD were imposed, assuming all else remained equal. The increase in absolute
295   transpiration with a 5 °C increase in temperature was greater on the control than the TFE, but
296   was proportionally similar (20%, Fig. 6a). However, the TFE would risk exceeding the
297   imposed canopy through-fall supply even at the lowest temperature rise tested (1.5°C, Fig.
298   6a). In contrast, even with a 5°C rise in temperature, the control forest only reaches a
299   through-fall recycling rate of 91% for transpiration, still below that of the TFE within the
300   current climate. In addition, both control and TFE recycle >100% of the water they receive
301   between July-December (dry season) under current climate (Fig. 6b), with this value
302   increasing substantially with a 5°C rise i n temperature (Fig. 6c). Under the current climate,
303   between July and October the TFE forest transpires more than 6 times the precipitation it
304   receives and this rises to almost 8 times with 5°C rise in temperature, creating a
305   substantially greater imbalance between transpiration and precipitation (Fig 6b-c).

306   Discussion

307   Until now the long-term responses of water use in a tropical forest exposed to soil drought
308   stress have not been studied. With new sapflow data spanning a two-year period we are able
309   to demonstrate that the 40% loss of forest biomass observed on the TFE (Rowland et al
310   2015a) resulted in a 30% reduction in total forest transpiration. We are also able to
311   demonstrate for the first time that the surviving trees are able to maintain or increase their
312   transpiration rate on a per-tree basis, causing 100% of the available rainfall received by the
313   droughted forest to be used for transpiration. Furthermore we demonstrate that if such

314 drought conditions were combined with a mild temperature rise, further tree mortality would

315 be inevitable, as forest water demand would substantially exceed supply over an annual and

316 multi-annual timescale.

317 Our estimates of transpiration rates and through-fall recycling rates (Fig. 2, Table S2) are

318 consistent with previous measurements and modelling at this old-growth rainforest site

319 (Carswell et al., 2002; Fisher et al., 2007). They suggest a remarkably constant water flux

320 partitioning over the 15 years of the experiment, despite a substantial shift in forest structure

321 because of high mortality in the TFE-treated plot. The increase in the recycling rate to 100%

322 on the TFE suggests that a high sensitivity by the trees to atmospheric demand for water is

323 maintained even following long-term drought. Our data suggest that drought-induced

324 mortality of the tallest trees changed stand water use patterns, facilitating greater growth

325 competition in the lower canopy, thereby maintaining very high levels of through-fall

326 recycling on the TFE. This is consistent with the observation (Rowland et al. 2015a) that

327 small- and medium-sized trees increased their growth rates after mortality of the taller trees,

328 by responding plastically to increased light availability in the lower canopy. This hypothesis

329 is also consistent with current hydraulic theory, which suggests that trees will continue to

330 compete for, and use up, a limited water supply, provided the advantages accrued from the

331 related carbon gain exceeds the cost of hydraulic damage (Sperry and Love, 2015; Wolf,

332 2016). Plastic reductions in water use as REW declines from wet to dry season on the TFE

333 are likely to only partially alleviate the water stress (Fig. 3 & 4), which would be substantial

334 during climate extremes, and would impose increased tree mortality risk. The intense

335 regrowth by small-to-medium diameter trees (Rowland et al., 2015a) is therefore likely to be

336 the primary driver maintaining through-fall recycling at the high levels seen in 2002-03.

337 Following the mortality of the largest trees, competitive release of small-to-medium diameter

338 trees considerably elevated wet season stem growth on the TFE (Rowland et al., 2015a). As

339 transpiration accompanies photosynthesis and responds to increased radiation availability, it

340 is possible that the TFE trees have acclimated, with elevated water use in the wet season to

341 maximise growth, and restricted growth in the dry season (Figs. 2-3), thus explaining the

342 increased seasonality in transpiration observed on the TFE (Fig. 1). Our sample size prevents

343 us from examining whether sap flux from small-to-medium diameter trees increased relative

344 to large trees. Comparison of sap flux values and canopy through-fall in 2002/03 with those

345 in 2015 provides indirect confirmation of similar levels of competition for water following

346 mortality-related release on TFE. Yearly stand-scale sap flow values on the TFE were

347   estimated as 953 and 805 mm in 2002/03 *vs* 945 mm in 2015 (Table S2). Therefore, despite a

348   40% biomass reduction, water use remained similar over time on a per unit ground area, but

349   increased on a tree-level basis on the TFE, due to having fewer trees per unit ground area.

350   However, we note that our LAI measurements estimate only about a 12-20% reduction in leaf

351   area on the TFE relative to Control (see Rowland et al 2015a), significantly lower than our

352   estimate of a 30% reduction in transpiration. Measurements of LAI in complex multi-layered

353   canopies are notoriously challenging (Breda, 2003) and these difficulties may explain the

354   discrepancy between the two estimates.

355   A shift from radiation and air temperature controlling dry season transpiration on the control

356   plot, to REW and radiation controlling it on the TFE suggests that trees on the TFE adjusted

357   to limit water use during the dry season when REW was low. The strong controlling

358   influence of REW on dry season transpiration on the TFE, but not the control plot suggests

359   low REW restricts dry season transpiration and is most likely linked to significant hydraulic

360   stress as water demand approaches or exceeds supply on seasonal time-scales (Fig. 6).

361   Relative to Control, the TFE forest maintains higher through-fall recycling rates also in the

362   wet season (January to June) when precipitation levels are substantially elevated (Fig 6b-c),

363   resulting in a reduced capacity to recover from dry season water stress. Given predicted

364   changes in VPD, and thus leaf water potential, combined with lower soil water potentials,

365   under some future climate scenarios, there is potential that trees could rapidly be pushed

366   beyond their species-specific hydraulic safety margins (the difference between normally-

367   occurring minimum xylem pressures, and those causing damage to xylem tissues and

368   restricting water transport), potentially causing xylem embolism (Sperry and Love, 2015;

369   Sperry et al., 2016) and/or leaf loss, with the ultimate risk of increased drought-induced

370   mortality. Furthermore, as total annual tropical forest water use approaches total soil water

371   supply, the likelihood of hydraulic damage occurring in the xylem becomes greater. This is

372   particularly the case for large canopy-top trees, which are exposed to greater variability and

373   extremes in VPD, high air temperatures, and larger xylem tensions (Bennett et al., 2015;

374   McDowell and Allen, 2015), which together have been hypothesised to lead to a series of

375   processes causing drought-induced mortality (Anderegg et al., 2016; McDowell and Allen,

376   2015; Mecuccini et al., 2015; Rowland et al., 2015a; Sperry et al., 2016; Wolfe et al., 2016).

377   In future climate scenarios, areas of tropical forest experiencing drought stress are also likely

378   to experience increases in temperature well beyond the moderate levels of 1.5-2 °C

379   (Christensen et al., 2013; Duffy et al., 2015; Fu et al., 2013; Sanderson et al., 2016). Using a

380 novel modelling approach, we demonstrate here that a forest exposed to long term drought is
381 far more likely to have transpiration demand exceed supply than a non-droughted forest (Fig.
382 6). This is driven mostly by transpiration rates exceeding precipitation supply in the dry
383 season by up to eight times in a droughted forest simultaneously experiencing temperature-
384 driven rises in VPD, as would be expected during natural drought. This puts a very large
385 strain on soil water supply, which the non-droughted forest can easily buffer, due to the
386 higher overall wet season recharge of soil water from higher precipitation. Without this re-
387 charge we demonstrate that even a very moderate rise in temperature necessitates tree
388 mortality in order to balance transpiration demand and soil water supply. Although a 50%
389 decline in canopy through-fall on a 10 year time-scale is unlikely within current climate
390 projections, reductions of up to 50% are predicted across parts Amazonia, in a range of recent
391 climate scenario analyses (Christensen et al., 2013, Duffy et al., 2015). This result thus has
392 strong implications for future climate change and carbon cycle feedback predictions, as it
393 suggests that tropical trees will maintain substantial transpiration fluxes even in the face of
394 drought and rising VPD, and that the forest appears to maintain a similar water balance
395 through the process of tree mortality.

396 The overall picture emerging from these results is that compensation processes acting at
397 tissue, tree and stand level have maintained the high levels of through-fall recycling on the
398 TFE-treated forest over more than a decade. While high mortality tended to reduce levels of
399 competition for water, the mortality-related growth release for small-to-medium sized trees
400 tended to increase it. Additional processes, such as acclimation in leaf:sapwood and leaf:root
401 ratios could also have affected competition for water. Estimated through-fall recycling rates
402 are already at approximately 100% on the TFE after 15 years of reduced soil moisture
403 availability, suggesting that further demands for water can only be facilitated by additional
404 tree mortality. As recycling rates are already >100% in the dry season, even in un-droughted
405 forest, it suggests that rainforest trees must rely on soil (and likely internal) water storage to
406 carry them through to the next wet season, potentially limiting their capacity to maintain
407 carbon uptake, whilst simultaneously also elevating their mortality risk. If the effects of our
408 50% rainfall reduction, or indeed a similar reduction in basal area imposed by widespread
409 logging, were to occur at a large scale, even the minimum increase in atmospheric
410 temperature which is now deemed unavoidable in the coming century would imply severely
411 reduced deep soil water recharge and runoff, and increased tree mortality risk. The potential

implications for regional economies, water supply and climate-carbon cycle feedbacks are substantial.

Figure Captions

Figure 1: Meteorological data for the Caxiuanã site during the sapflux measurement period. In panel (a), precipitation (mm day^{-1}) is shown as grey bars alongside average daily relative extractable water (REW) integrated across three meters soil depth for the control plot continuous black line) and TFE plot (dashed grey line). Panel (b) shows average daily air temperature (°C, grey line) and average daily VPD (kPa, black line).

Figure 2: How transpiration per year (red arrows), canopy through-fall per year (blue arrows) and annual through-fall recycling rate (% circular black arrows) change on the control (a, c) and TFE (indicated by panel structure b, d) plots from 2002-3 (a, b) to 2015 (c, d). The diagram depicts the change in above ground biomass and the shift in forest structure which occurred during the full experimental period because of tree mortality on the TFE.

Figure 3: Daily transpiration (mm day^{-1}) from December 2014 - July 2016 for the control plot (black line), the TFE (dashed black line), and the estimated transpiration flux from the control plot if its values were downscaled to reflect only the effect of basal area loss on the TFE plot

441 (dashed grey line). Grey shaded area shows the standard error on the estimates calculated

442 using a bootstrapping technique (see Methods).

443

444 Figure 4: Average diurnal J_s patterns normalised using seasonal maxima per tree during peak

445 wet (March and April, solid black line) and peak dry season (October and November, solid

446 grey line) for trees on control (a.), and TFE (b.). The black dashed line shows the peak wet

447 minus the peak dry season response for each panel and the grey shaded area shows the

448 standard error.

449

450 Figure 5: Optimised sigmoidal relationships between J_s and VPD for trees on the control (C,

451 a. & c.) and TFE (b. & d.) plot in peak dry and peak wet season. J_s is binned by VPD and

452 normalised by max hourly J_s per year to make relationships comparable across plots and

453 season.

454

455 Figure 6: The effect of increasing temperature on annual transpiration fluxes for control (C)

456 and TFE (a.), under current temperature climate (T, year 2015 used) and under the climate of

457 this year + 1.5, 2, 3, 4, and 5˚C, accounting for temperature-driven changes in relative

458 humidity and vapour pressure deficit. Dashed lines (a.) indicate the rainfall reaching the

459 forest floor on control (black) and TFE (grey). Rainfall reaching the forest floor is estimated

460 from rainfall minus a canopy interception estimate of 21.5% (see Methods). Panel b. and c.

461 show the % of seasonal through-fall recycled as transpiration during the four quarters of the

462 year, under the current climate (b.) and with a 5 °C increase in temperature (c.). Solid lines in

463 b. and c. indicate 100%, where transpiration exceeds the rainfall reaching the soil. Error bars

464 show the standard deviation across the 100 estimates made of each scenario (see Methods).

465

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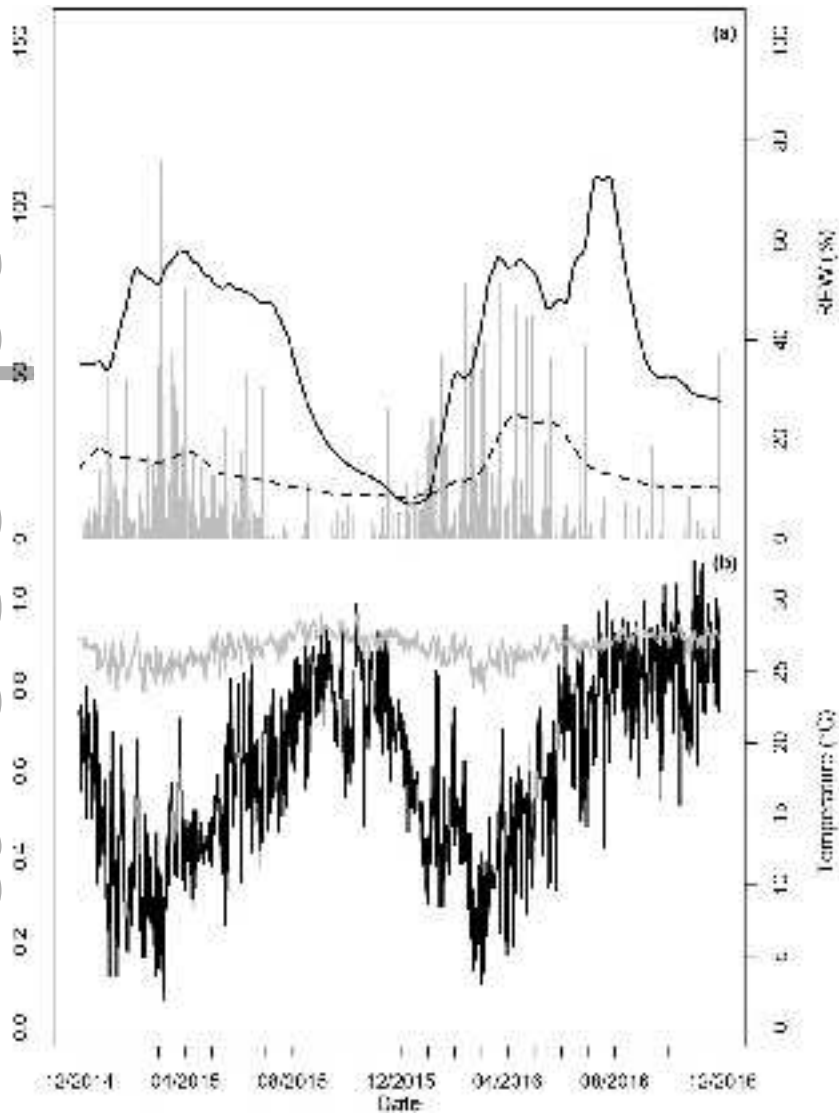504   Academy of Sciences of the United States of America 112, 13172-13177.

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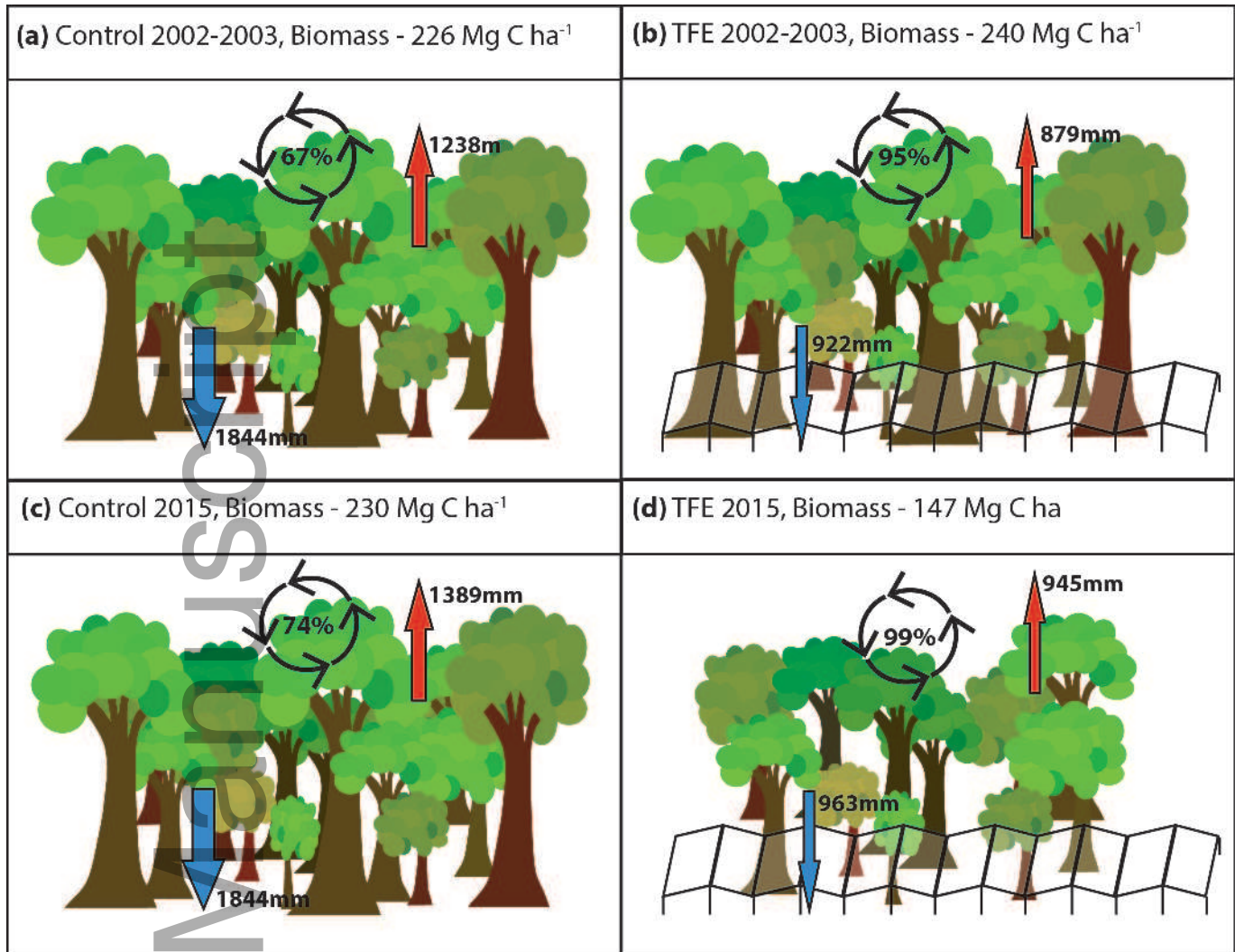570   tropical forests is triggered by hydraulics not carbon starvation. Nature 528, 119-122.

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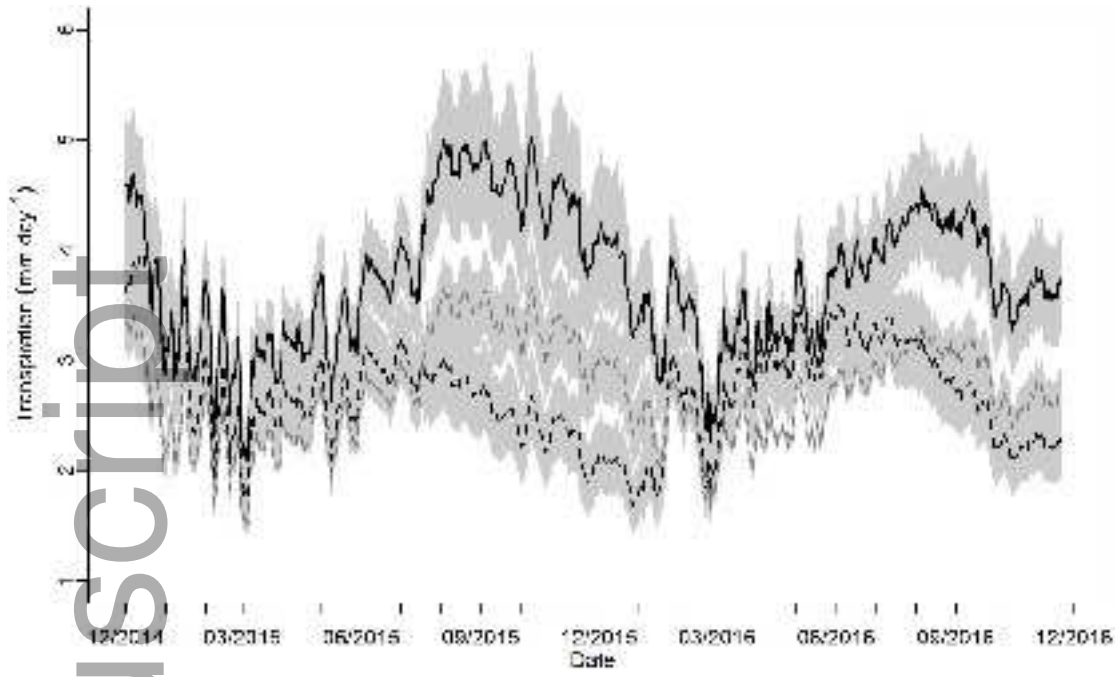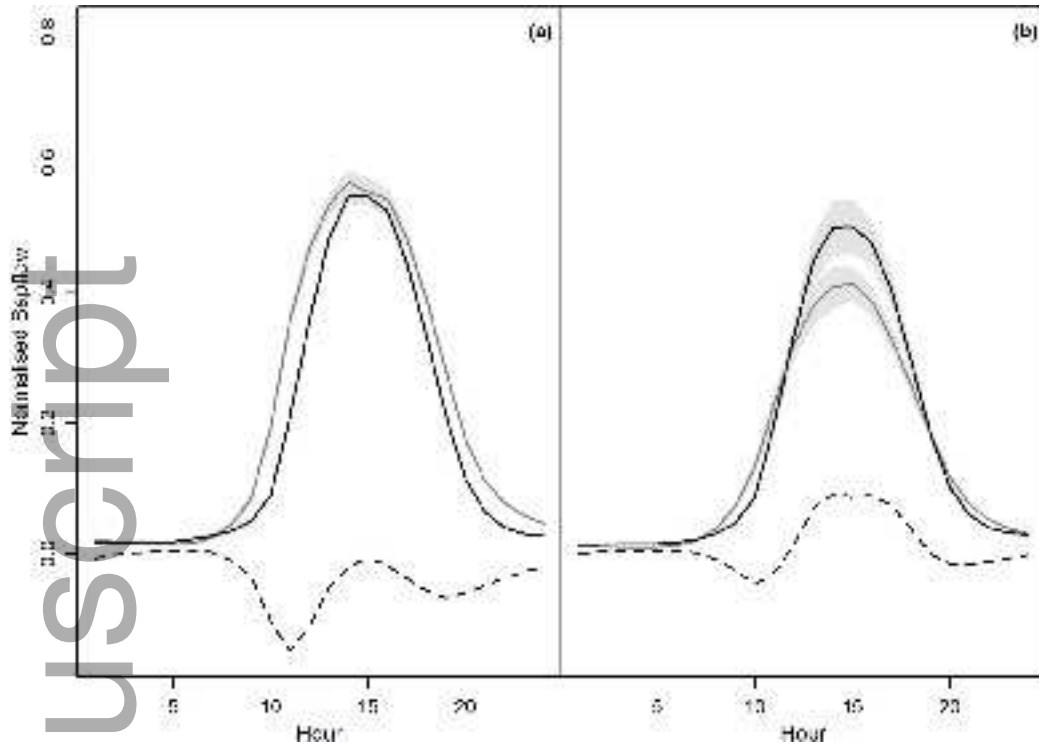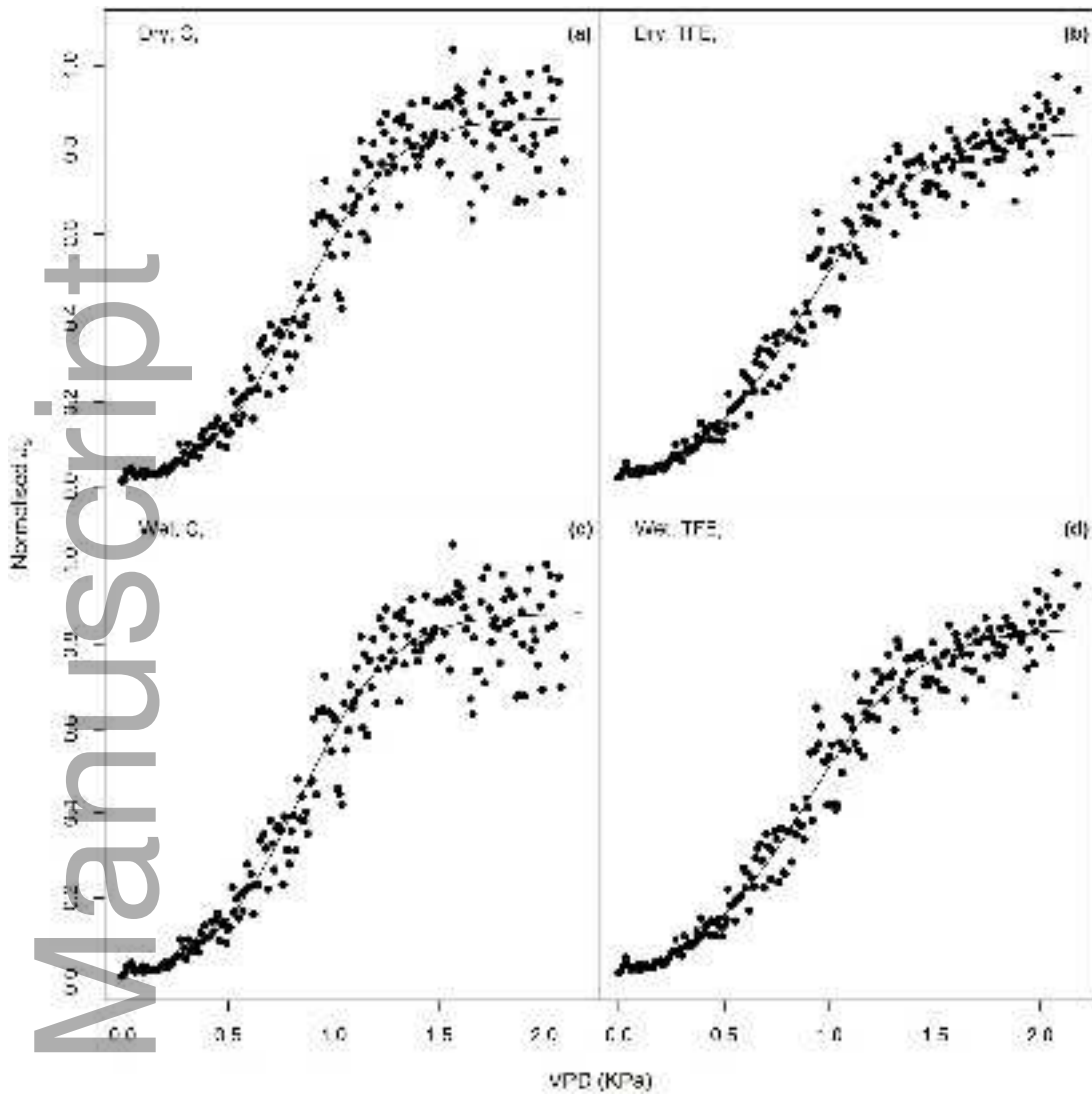
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(a) Control 2002-2003, Biomass - 226 Mg C ha^{-1}

67% 1238m 1844mm

(b) TFE 2002-2003, Biomass - 240 Mg C ha^{-1}

95% 879mm 922mm

(c) Control 2015, Biomass - 230 Mg C ha^{-1}

74% 1389mm 1844mm

(d) TFE 2015, Biomass - 147 Mg C ha

99% 945mm 963mm

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