*Plant, Cell and Environment* (2004) **27**, 1239–1256

# Functional diversity of photosynthesis during drought in a model tropical rainforest – the contributions of leaf area, photosynthetic electron transport and stomatal conductance to reduction in net ecosystem carbon exchange

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## ABSTRACT

**The tropical rainforest mesocosm within the Biosphere 2 Laboratory, a model system of some 110 species developed over 12 years under controlled environmental conditions, has been subjected to a series of comparable drought experiments during 2000–2002. In each study, the mesocosm was subjected to a 4–6 week drought, with well-defined rainfall events before and after the treatment. Ecosystem $CO_2$ uptake rate ($A_{eco}$) declined 32% in response to the drought, with changes occurring within days and being reversible within weeks, even though the deeper soil layers did not become significantly drier and leaf-level water status of most large trees was not greatly affected. The reduced $A_{eco}$ during the drought reflected both morphological and physiological responses. It is estimated that the drought-induced 32% reduction of $A_{eco}$ has three principal components: (1) leaf fall increased two-fold whereas leaf expansion growth of some canopy dominants declined to 60%, leading to a 10% decrease in foliage coverage of the canopy. This might be the main reason for the persistent reduction of $A_{eco}$ after rewatering. (2) The maximum photosynthetic electron transport rate at high light intensities in remaining leaves was reduced to 71% for three of the four species measured, even though no chronic photoinhibition occurred. (3) Stomata closed, leading to a reduced ecosystem water conductance to water vapour (33% of pre-drought values), which not only reduced ecosystem carbon uptake rate, but may also have implications for water and energy budgets of tropical ecosystems. Additionally, individual rainforest trees responded differently, expressing different levels of stress and stress avoiding mechanisms. This functional diversity renders the individual response heterogeneous and has fundamental implications to scale leaf level responses to ecosystem dynamics.**

*Key-words*: chlorophyll fluorescence; drought; leaf area; leaf fall; leaf growth; net ecosystem $CO_2$ exchange; photosynthesis; photosynthetic electron transport; tropical rainforest; tropical trees.

*Abbreviations*: $A_{eco}$, ecosystem photosynthetic $CO_2$ uptake rate ($\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$); $c_i$, apparent intercellular $CO_2$ concentration (p.p.m.); ET, ecosystem evapotranspiration (mmol $H_2O$ $m^{-2}$ $s^{-1}$); ETR, photosynthetic electron transport rate ($\mu$mol electrons $m^{-2}$ $s^{-1}$); $F_0$, ground fluorescence of the dark-adapted leaf; $F_m$, maximum fluorescence of the dark-adapted leaf; $F$, fluorescence of the light-adapted leaf; $F_m'$, maximum fluorescence of the light-adapted leaf; $F_v/F_m$, pre-dawn potential quantum yield of photosystem (PS) II ($F_v = F_m - F_0$); $\Delta F/F_m'$, effective quantum yield of PS II ($\Delta F = F_m' - F$) measured at ambient light; NEE, net ecosystem $CO_2$ exchange rate ($\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$); PFD, photon

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flux density ($\lambda = 400$–$700$ nm) ($\mu$mol photons $m^{-2}$ $s^{-1}$); $R_{eco}$, ecosystem respiration ($\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$); RGR, relative growth rate (% $\alpha^{-1}$); VPD, atmospheric vapour pressure deficit (mol $mol^{-1}$); $\Psi_{leaf}$, leaf water potential (MPa); $\Psi_{soil}$, soil water potential (MPa).

## INTRODUCTION

Ultimately all life on earth depends on the photosynthetic light capture and the use of this energy to convert $CO_2$ to carbohydrates. Stress factors such as nutrient limitations, availability of water and extreme temperatures affect the efficiency of photosynthesis by influencing the function, biosynthesis, molecular assembly, and co-ordination of the components of the photosynthetic apparatus (Schulze & Caldwell 1996). These molecular mechanisms occur in the context of plant- and ecosystem-level responses to stress (Fitter & Hay 2001) that, in the case of drought, include changes in stomatal conductance, alteration of leaf growth and leaf abscission and changes in the water, energy and carbon balance. Terrestrial ecosystems are complex assemblies of photosynthetic green material, above- and below-ground respiratory compartments, and chemical and physical resource pools, which may serve as carbon sinks and sources (Melillo *et al*. 1993; Dixon *et al*. 1994) and influence energy flow in the ecosystem. Variations in external parameters and biotic and abiotic stress may significantly alter those pools and the fluxes between them (Randerson *et al*. 2002). Furthermore, stresses may alter the climate at regional to continental scales, by affecting the water and energy balance of the land surface, energizing positive and negative feedback loops (de Rosnay *et al*. 2002; Moorcroft 2003). Although drought responses have been extensively studied at the leaf and plant scales, these studies are not necessarily sufficient to understand the complex and coupled responses that occur at the ecosystem-scale. Modelling studies that link global circulation models to atmospheric transport and physiological models have shown that ignoring stress responses may lead to erroneous conclusions (Sellers *et al*. 1996), emphasizing the need for more research concerning the effects of abiotic influences on plant ecosystem physiology.

The need to scale leaf-level physiology to ecosystem responses and climate feed-backs has been emphasized recently in the context of global climate change research (National Research Council 2004), and remains a challenge of future ecosystem research (Ehleringer & Field 1993; Enquist *et al*. 2003). One approach to such scaling issues may be to expand the scale of controlled environment experiments using complex model systems such as the mesocosms of Biosphere 2 (Osmond *et al*. 2004). Hypothesis driven experimental evaluation of key issues concerning the influence of atmospheric $CO_2$ on growth of calcifying marine organisms (Langdon *et al*. 2000), and the $CO_2$ balance of the carbon cycle in the Biosphere 2 ocean mesocosm (Langdon *et al*. 2003), illustrate the power of this approach. Our goal here is to extend this approach to the tropical forest mesocosm (Lin *et al*. 1998, 1999).

Global climate change will affect global water budgets and may also alter the seasonal drought in tropical ecosystems with prolonged drought periods in parts of the equatorial tropics (Hulme & Viner 1998). In a classic modelling approach a canopy is seen as a single- or multi-layer 'big leaf', mediating the gas-exchange between soil, plant, and atmosphere (e.g. Running & Coughlan 1988; Amthor 1994). Drought influences the natural water balance (Field, Jackson & Mooney 1995) and reduces the canopy conductance to water vapour of the 'big leaf', as demonstrated for several ecosystems (Hollinger *et al*. 1998; Arneth *et al*. 1999; Lai *et al*. 2000; Rambal *et al*. 2003; Novick *et al*. 2004). The goal of the present study is to investigate the mechanisms, which underlie the response of canopy water vapour conductance to drought. Specifically, the drought experiments in Biosphere 2 were designed to test the following hypotheses: (1) the mechanism of reduced canopy conductance during drought is composed of a number of underlying physiological responses, such as direct limitation of photosynthetic capacity, stomatal closure, reduced leaf growth and increased leaf abscission. Each of these effects can be quantified separately. (2) The response of individual plants to changing water relations may be different. This inherent heterogeneity of physiology ('functional diversity') renders the ecosystem behaviour asynchronous and thus scaling from the leaf to the ecosystem non-linear. (3) Simultaneous studies of these complex individual responses and the response of the whole system $CO_2$ and water exchange will help to put this complexity into context.

Tropical trees are known to sensitively react to seasonal drought (Bonal *et al*. 2000a), however, the individual responses may differ greatly between species (Hogan, Smith & Samaniego 1995; Bonal *et al*. 2000b). Rainforest soils are usually highly weathered and deep (Brady & Weil 1996) and, especially during the dry seasons for seasonal forests, can vary greatly in their water content with respect to depth (Meinzer *et al*. 1999). Although the distribution of roots for most growth forms can be heterogeneous in the soil profile (Sternberg *et al*. 1998), larger trees usually root deeply, with some having access to groundwater, whereas the roots of smaller trees tended to be shallower (Dawson 1996). In dry tropical forests, trees that can use deepwater sources can maintain their water-use during drought and do not have a great seasonal variation in their leaf fall (Meinzer *et al*. 1999). Although such variability may be an important aspect of functional diversity, it renders budgeting carbon, water, and energy fluxes of tropical ecosystems more difficult. Recently, two rainfall exclusion experiments have been set-up within the Amazon rainforest, addressing the intermittent effect of limited water supply on ecosystem physiology on various scales (Nepstad *et al*. 2002). Even though these experiments will deliver valuable information about drought effects of individuals within a complex ecosystem, it will be difficult to address the integrated effects of these responses on whole ecosystem exchange and feedbacks of a drought-stressed vegetation on the regional scale atmosphere.

The mechanisms of water stress are still under debate. Primarily it is considered a hydraulic effect with reductions in leaf water potential leading to stomatal closure (Cornic & Fresneau 2002), negatively influencing plant carbon gain and the light reactions of photosynthesis (Schulze & Hall 1982; Martinez-Carrasco, Sanchez-Rodriguez & Perez 2002; Chaves, Maroco & Pereira 2003). However, the importance of stomatal conductance to water vapour in restricting the supply of $CO_2$ to metabolism, which in turn reduces the rate of carbon uptake, is still unclear and metabolic effects may further reduce carbon uptake during drought (Lawlor 2002). Plants sense drought using hydraulic and chemical signals (Magnani & Grace 2000; Chaves *et al*. 2003), which decelerate cell division and expansion (Heckenberger, Roggatz & Schurr 1998). Growth effects are especially well studied for leaves of herbaceous plants because they respond to drought quickly (Durand *et al*. 1995). The dynamics of trees are much less thoroughly investigated, mostly due to technical difficulties of investigating growth for a large plant non-destructively and evidence of drought in tree canopies is usually limited to canopy dieback and reduced production of new leaves and branches (Fernandez, Perry & Flore 1997; Horton, Kolb & Hart 2001). Because growing tissues are characterized by the simultaneous development of structure and function, any environmental stress affecting overall plant growth and photosynthesis will manifest itself in newly emerging plant organs that are good biomonitors for the overall performance of the analysed tree or stand.

The enclosed and controllable tropical rainforest mesocosm of Columbia University's Biosphere 2 Laboratory was used to test the eco-physiological effects of prolonged drought by measuring carbon fluxes for the entire mesocosm. The tropical rainforest mesocosm within Biosphere 2 Laboratory was not intended to represent any particular natural rainforest, however, its plant species composition, leaf area index (LAI: 4–5), canopy height (15 m), and other factors (Leigh 1999; Leigh *et al*. 1999) are similar to natural rainforests.

## MATERIALS AND METHODS

### The rainforest within the Biosphere 2 Laboratory

The rainforest mesocosm within Biosphere 2 Laboratory (32°35′ N, 110°51′ W and 1200 m a.s.l) is a unique experimental model system, which is encased in a glass and metal shell controlled for temperature, humidity, atmospheric gas composition, and precipitation. Water is supplied to the mesocosm via an overhead sprinkler system and is removed by condensation and soil drainage. Atmospheric gas composition, climatic conditions (PFD, temperature and humidity), and energy and trace gas fluxes throughout the canopy were monitored continuously by an array of sensors (for details see Lin *et al*. 1999; Fig. 1a). The mesocosm has a total projected area of 1940 $m^2$ and an atmospheric volume of 26 700 $m^3$. The rainforest was planted with a mixture of some 410 species (110 remaining today) from humid rainforests from the old world and neotropics (Leigh 1999; Leigh *et al*. 1999) and today has a leaf area index (LAI) of 4–5. The mesocosm was regularly watered with an average daily precipitation of 3.6 mm.

### Soil water relations

Soil water content was measured gravimetrically before and at the end of the drought from soil pits at the following incremental depths in the soil: 0–0.05, 0.05–0.10, 0.10–0.20, 0.20–0.30, 0.30–0.40, 0.40–0.60, 0.60–0.80 and 0.80–1.00 m. Bulk density was determined from blocks of soil removed from the side of the pits for incremental depths of 0–0.30, 0.30–0.60 and 0.60–1.00 m. Volumetric water content for each sample was then determined as $(m_{\text{soil water}}/m_{\text{soil}}) \times$ soil bulk density. Soil water potentials for soil samples from depths ranging from 0 to 0.30 m and from 0.30 to 0.60 m were determined from soil moisture characteristics developed using a WP4 Dewpoint Potential Meter (Decagon Devices, Pullman, WA, USA) in the manner suggested by the manufacturer.

### Leaf level measurements

#### *Plant species*

The following eight plant species were chosen and monitored for various growth and physiological parameters before, during, and after the drought: *Averrhoa carambola* L., *Ceiba pentandra* L., *Clitoria racemosa* G. Don., *Hura crepitans* L., *Inga cf sapindoides* Willd., *Pachira aquatica* Aubl., *Phytolacca dioica* L., *Pterocarpus indicus* Willd. (Table 1). Locations of monitored individuals within the rainforest mesocosm are indicated in Fig. 1a. In general, measurements were taken in the outer canopy. Growth and photosynthesis measurements were performed on the same leaves throughout the experiment. Leaves were reached either from the surrounding construction ('space frame') or using rope-assisted climbing techniques.

#### *Plant water relations*

Pre-dawn and midday water potential measurements were performed for leaves of the outer canopy using a PMS 1003 digital pressure-bomb (PMS Instruments, Corvallis, OR, USA) for a representative of each of the following tree species: *C. pentandra*, *Cl. racemosa*, and *H. crepitans*. Unfortunately it was impossible to use bulky gas-exchange instrumentation and to measure leaf-level transpiration within the tall canopy, however, we measured sap flow at the trunk of a 15-m-tall *C. pentandra* tree using the constant heat method (Granier 1987), data were averaged and stored every 30 min. As sapflow represents the integrated transpiration over the leaf-area of this tree, we could calculate transpiration and stomatal conductance to water vapour.

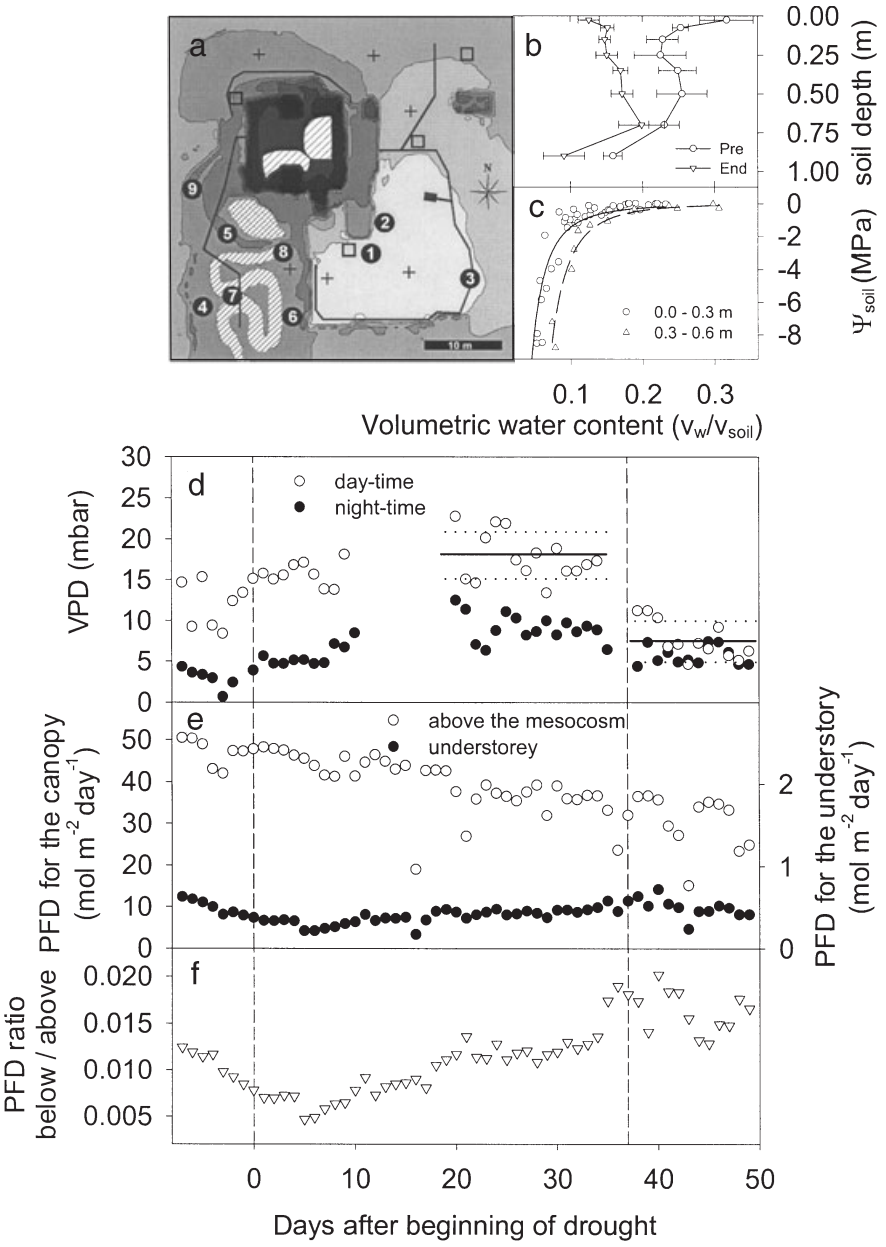


**Figure 1.** The rainforest mesocosm of Columbia University's Biosphere 2 Laboratory during the Sept/October 2002 drought experiment (III in Fig. 2). (a) Map of the tropical biome and the sampling locations. Circles, locations of the monitored plants: 1, *Ceiba pentandra*; 2, *Clitoria racemosa*; 3, *Hura crepitans*; 4, *Phytolacca dioica*;, 5, *Inga* cf. *sapindoides*; 6, 7, *Pterocarpus indicus*; 8, *Pachira aquatica*; 9, *Averrhoa carambola*. Crosses: locations of the soil cores and soil measurements; squares: locations of the temperature, light, and humidity sensors. Grey values encode for ground elevation (1 m steps), hatched areas indicate a pond and the drainage river, which flow was stopped during drought. Length bar indicates 10 m. (b), (c), Water relations for the soil before and at the end of the drought experiment. (b) Soil volumetric water content in relation to soil depth prior to (○) and at the end of (△) the drought (mean values ±SE; $n = 5$ locations). (c) Soil moisture characteristics for the upper 0.60 m of the mesocosm ($n = 5$ locations). The solid line is the fitted curve for soil from depths ranging from 0 to 0.30 m [$\Psi_{soil} = -0.0059(\text{volumetric water content})^{-2.39}$, $r^2 = 0.96$] and the dashed line is for depths ranging from 0.30 to 0.60 m [$\Psi_{soil} = -0.0033(\text{volumetric water content})^{-3.02}$, $r^2 = 0.94$]. (d) Daytime (– ○ –) and nighttime (– ● –) vapour pressure deficit (VPD) measured within the canopy. Horizontal lines indicate average daytime VPD ± SD at the end of the drought and after rewatering. (e) PFD measured outside the rainforest mesocosm (– ○ –) and 1 m above the soil in the understorey (– ● –) ($n = 4$ sensors), data are averages over 24 h. (f) Ratio of PFD in the understorey and above the mesocosm. This ratio describes the fraction of light, which was not absorbed by the structure of the building and the canopy.

### *Leaf fall*

Leaf fall was measured using 21 traps arranged randomly throughout the entire biome. Each trap, which has a frame constructed of PVC pipe covered with 1 mm thick fibreglass screening, had an area of 0.48 $m^2$. Leaves were collected monthly before the drought and biweekly during the autumn 2002 drought experiment. After collection, the leaves were dried at 65 °C in a forced-draught oven for 1 week, separated by species (*Cl. racemosa, C. pentandra, H. crepitans,* and *Pt. indicus*), and weighed. 1 $m^2$ of shed leaf material has an approximate dry weight of 50 g.

### *Leaf growth*

The length and width of growing leaves from 6 to 12 branches (totalling 40–60 leaves) of each tree were measured two to three times per week with a ruler (precision 0.5 mm). Areas and relative growth rates (RGR) of those leaves were calculated according to Walter & Schurr (1999). All investigated leaves were fully exposed in the outer canopy, 1–1.5 m away from the surrounding glass and steel structure. Effects of a mild drought on plant growth are often difficult to assess because of the inter-individual variability of plants, thus a dynamic parameter of canopy leaf growth was used to compare growth during the initial

**Table 1.** Plant species monitored during the drought in September/October 2002, including the height (m) of the measured individual, the number of specimens in the Biosphere 2 mesocosm and the measurements performed on these species.

| | Measurements | | | | | | |
|---|---|---|---|---|---|---|---|
| Plant species | Height | No. | $\Psi_{leaf}$ | Sap flow | Growth | Fluorescence | Pigments |
| *Averrhoa carambola* L. (Oxalidaceae) | 6 | 2 | | | × | | |
| *Ceiba pentandra* L. (Bombacaceae) | 15 | 3 | × | × | × | × | × |
| *Clitoria racemosa* G.Don. (Fabaceae) | 8 | 6 | × | | | | |
| *Hura crepitans* L. (Euphorbiaceae) | 13 | 1 | × | | | | |
| *Inga* cf. *sapindoides* Willd. (Fabaceae) | 4 | 3 | | | | × | × |
| *Pachira aquatica* Aubl. (Bombaceae) | 5 | 10 | | | | × | × |
| *Phytolacca dioica* L. (Phytolaccaceae) | 10 | 7 | | | × | | |
| *Pterocarpus indicus* Willd. (Fabaceae) | 12 | 2 | | | × | × | × |

well-watered state, the drought, and during the recovery period. The time series of a group of young leaves from each tree was followed over the entire course of the experiment. Data from leaves with areas between 0 and 30% of the final leaf area at a given date were pooled for this analysis because RGR was almost constant during this developmental phase (see also Fig. 5a).

### *Pigment analyses*

Leaf samples for pigment analyses were taken immediately after harvest using a cork-borer (diameter: 8.1 mm; $n \approx 20$ for each leaf) and stored in liquid nitrogen until use. Pigments were extracted from six leaf discs according to Matsubara, Gilmore & Osmond (2001) with a slight modification of extraction volume (2 mL 80% acetone followed by 3 mL 100% acetone) and then analysed quantitatively using a modified high-performance liquid chromatography assay (Gilmore & Yamamoto 1991; solvent A: acetonitrile–methanol–Tris·HCl buffer 0.1 M pH 8.0, 72 : 15 : 5.5). The following pigments, associated with the photosynthetic apparatus, were analysed quantitatively: chlorophyll *a*, chlorophyll *b*, $\alpha$-carotene, $\beta$-carotene, violaxanthin, antheraxanthin, neoxanthin, zeaxanthin, lutein and lutein-epoxide.

### *Chlorophyll fluorescence measurements*

Gas-exchange techniques, which require bulky and heavy instruments, could not be applied within the tall canopy of Biosphere 2. Alternatively, the non-invasive quantification of the fluorescence signal of chlorophyll *a* can accurately be quantified with small instruments, which can be carried into the canopy by climbing techniques and were used to measure photosynthetic activity and non-photochemical energy dissipation processes (Schreiber & Bilger 1993; Schreiber, Bilger & Neubauer 1994, Maxwell & Johnson 2000). Chlorophyll *a* fluorescence was measured using the miniaturized pulse-amplitude modulated photosynthesis yield analyser (Mini-PAM) of H. Walz (Effeltrich, Germany) with a leaf clip holder described by Bilger, Schreiber & Bock (1995). Spot measurements of light intensity ($\lambda$ = 380–710 nm) were taken inside the measuring field by the microquantum sensor of the Mini-PAM. Pre-dawn values of optimal quantum yield of PS II ($F_v/F_m$) were performed once per week between 0400 and 0500 h and were calculated as $F_v/F_m = (F_m - F_0)/F_m$, with $F_m$ being the maximum fluorescence of the dark-adapted leaf when a saturating light pulse of 800 ms duration (intensity $\approx$ 4000 $\mu$mol $m^{-2}$ $s^{-1}$) was applied. The effective quantum yield of PS II ($\Delta F/F_m'$) was measured between 0900 and 1200 h and was calculated as $(F_m' - F)/F_m'$, where $F$ is fluorescence yield of the light-adapted sample and $F_m'$ is the maximum light-adapted fluorescence yield when a saturating light pulse (as described above) was superimposed on the prevailing environmental light levels (Genty, Briantais & Baker 1989; Schreiber & Bilger 1993). During these measurements special care was taken not to change the ambient conditions, such as the angle of the leaf or shading. Non-photochemical processes (NPQ) were calculated as $(F_m - F_m')/F_m'$ (Bilger & Björkman 1990). Prior to and just after each measurement, a fluorescence standard was measured, which was used to correct absolute values. The apparent rate of photosynthetic electron transport of PS II (ETR) was obtained as $ETR = \Delta F/F_m' \times PPFD \times 0.5$, where the factor 0.5 assumes equal excitation of both PS II and PS I; no correction was made for reflection as it was not known numerically.

Light within the canopy changed during the morning hours and showed patches of varying intensity. Thus, leaves were exposed to rapid changes in PFD of various duration and intensity, which could not be determined analytically. $\Delta F/F_m'$, ETR and NPQ values dynamically adapt primarily to these changes in light intensity, but may also reflect manifold underlying physiological mechanisms, such as light-stress-induced activation of the xanthophyll cycle (Demmig *et al*. 1987; Bassi & Caffarri 2000) or drought stress (Grieu, Robin & Guckert 1995; Valentini *et al*. 1995). Additional parameters, such as maximum apparent electron transport rate ($ETR_{max}$) and saturating photosynthetically active radiation ($PFD_{sat}$), can be derived from light-response curves. In general, measurements of light-response curves lead to a deeper insight into characteristic parameters of a plant species, which are not related to the momentary ambient light conditions, but rather to the ontogeny of a leaf and to the range of physiological plasticity of a plant (Rascher,

Liebig & Lüttge 2000). In order to obtain light response characteristics, all data of single species measured between 0900 and 1200 h were grouped weekly and plotted over PFD. Light-dependency data plotted in such a way can be mathematically fitted using single exponential functions (Eqns 1 and 2) in order to quantify the characteristic cardinal points of photosynthesis (Rascher *et al*. 2000).

$$f(x) = m + ae^{-bx} \quad (1)$$

$$f(x) = a(1 - e^{-bx}) \quad (2)$$

where $a$, $b$ and $m$ are independent parameters.

From the results of Eqns 1 and 2 the initial slope of $\Delta F/F_m'$ and ETR, maximum electron transport rate ($ETR_{max}$) and saturating light intensity ($PFD_{sat}$; reached at 0.9 $ETR_{max}$), were calculated. The parameters were tested statistically using the Wald test, as follows:

$$W = \frac{a_1 - a_2}{\sqrt{SE^2(a_1) + SE^2(a_2)}} \quad (3)$$

where $a_1$ and $a_2$ are the parameters tested against each other and $W$ has a standard normal distribution for high sample sizes.

### Whole ecosystem gas exchange measurements

The tropical rainforest mesocosm was separated from the rest of the Biosphere facility in 1997, increasing the controllability of this mesocosm greatly. During the day, $CO_2$ concentration was controlled at 410 p.p.m. by a setpoint control system with a mass flow controller and infrared gas analyser, which added $CO_2$ to replace that taken up in photosynthesis and maintained the $CO_2$ concentration at 418 ± 14 p.p.m. (mean of 15 min averages ±SD). At night, when respiration predominated, $CO_2$ was controlled by flowing ambient air ($CO_2 \approx 370$ p.p.m) through the biome using variable speed fans and ducts fitted with sonic anemometers. Even though $CO_2$ concentrations inevitably increased, flow was adjusted so that the $CO_2$ concentration at night did not exceed 700 p.p.m., which are typical levels seen in tropical forest canopies at night. The $CO_2$ concentration in the rainforest, as well as in the in- and outcoming air was measured continuously using LI-6262 $CO_2/H_2O$ gas analysers (LI-COR Inc., Lincoln, NE, USA). In all four drought experiments net ecosystem exchange rate of $CO_2$ (NEE) was calculated every 15 min with positive values representing ecosystem efflux and negative values representing $CO_2$ influx (Lin *et al*. 1998). Daytime ecosystem respiration was estimated using night-time respiration with a temperature correction based on the daytime soil temperature (Lin *et al*. 1999, 2002). Ecosystem photosynthetic uptake rate ($A_{eco}$) was calculated as $A_{eco} = -NEE + R_{eco}$, where ecosystem respiration ($R_{eco}$) was modelled as a function of temperature. Daily means were tested statistically using General Linear Models analyses (SAS Procedures Guide, Release 6.03; SAS Institute Inc., Cary, NC, USA).

The mass balance method, as for $CO_2$, was used to estimate water budget in the mesocosm. Input flows included the water of rains ($W_r$), fogger moisture addition ($W_f$) and water vapour brought into the mesocosm, when the fans were on ($W_{in}$). Output flows were condensation ($W_c$), soil drainage ($W_d$), the moisture existing the mesocosm by the fan ($W_{out}$), as well as the moisture accumulation in the air ($W_v$) and in the soil ($W_s$). When the water budget was balanced, all inputs should equal all outputs, namely

$$W_r + W_f + W_{in} = W_c + W_d + W_{out} + W_v + W_s \quad (4)$$

Evapotranspiration ($ET$) rate was estimated every 15 min only when the rain and fogger inputs were stopped, as:

$$ET = W_c + W_v - (W_{in} - W_{out}) \quad (5)$$

In analogy to leaf level measurements, apparent ecosystem conductance to water vapour ($gH_2O$) was calculated as $gH_2O = ET/VPD$ and intercellular $CO_2$ concentration ($c_i$) according to Eqn 6, with $c_{ext}$ being ambient $CO_2$ concentration.

$$c_i = c_{ext} - 1.6 \cdot \frac{A_{eco}}{gH_2O} \quad (6)$$

## RESULTS

### Whole ecosystem processes

Three drought experiments were conducted in January/February of 2000, April/May of 2002 and September/October of 2002, according to essentially similar protocols. A map of the biome, Fig. 1a shows the location of temperature, light and humidity sensor arrays, soil pits, and the canopy trees that were studied in detail during the study. A summary of the measurements conducted on these trees is given in Table 1, and the results of these will be presented below. Prior to the drought periods the rainforest was heavily wetted for 2 weeks (7.7 mm $d^{-1}$), then droughted for 27–37 d by stopping artificial rainfall, and re-watered for 2 weeks using the pre-drought regime. These drought treatments were calibrated to result in mild stress, still permitting rapid and reversible recovery of the biome and may well represent an extended dry season in the Amazon. Similar dry periods are predicted for El Niño years (Trenberth & Hoar 1997) and may be more likely if deforestation continues (Costa & Foley 2000). Following the success of the first two drought experiments a team of researchers was assembled to make more comprehensive measurements in the study of autumn 2002, which are presented in this communication.

Soil volumetric water content decreased significantly during drought for the top 0.60 m of soil, with a 60% decrease in the top 0.05 m of the soil, an average decrease of 37% at depths from 0.05 to 0.20 m, and an average decrease of 33% at depths of 0.20–0.60 m ($P < 0.05$ in all cases for paired $t$-test; Fig. 1b). The corresponding $\Psi_{soil}$ decreased from –0.12 to –1.19 MPa in the top 0.05 m of the soil, from an average of –0.20 to –0.58 MPa at depths of 0.05–0.20 m, from –0.41 to –0.64 MPa at depths of 0.20–0.30 m, and from an average of –0.31 to –0.71 MPa at depths of 0.30–0.60 m (Fig. 1b & c). Soil volumetric water

content did not decrease significantly below 0.60 m during the drought. On day 28 an isolated soil compartment (approximately 20% of the whole soil surface) was watered with 30 000 L (36 mm) in order to test the effect of a singular, isolated watering event.

Ambient day/night temperatures were maintained at 27/23 °C at a height of 1 m above the soil surface. Temperature stratification in the upper canopy, the principal artefact of enclosure (Arain *et al*. 2000), was reduced by high-capacity fans, which were used to ensure adequate gas mixing in the atmosphere. Nevertheless, canopy daytime temperature in the upper canopy often reached daily maxima of 34–37 °C. High canopy temperature during direct sun exposure can result in temporarily high values for atmospheric vapour pressure deficit (VPD) within the canopy. The flow of dry desert air through the system during the night maintained a significant VPD during the night. Due to the decreased evaporation and the increased sensible heat transfer to the air, VPD increased during the drought. Average VPD at the end of the drought was 17.81 ± 2.85 mbar and dropped to an average level of 7.63 ± 2.31 mbar after rewatering resumed (Fig. 1d).

### *Whole ecosystem gas exchange*

Figure 2 shows the mean daily courses ($n = 5$ d before, within and after the drought) of net ecosystem exchange rate (NEE, using the atmospheric convention) and gross photosynthetic $CO_2$ uptake rate ($A_{eco}$) for the three drought experiments. This approach attenuates the effect of day-to-day differences in cloud cover, exposing the effect of the drought cycle on the diel pattern of $CO_2$ exchange.

NEE during wet conditions was constant during the night with a mean carbon release rate of 7.61 ± 0.15 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ and reached minimum carbon uptake during midday with values between –11.4 and –14.6 $\mu$mol $m^{-2}$ $s^{-1}$ and generally more negative NEE values during the summer months (Fig. 2a, c & e). Eddy covariance measured fluxes at sites in the wet tropics show very similar mean day- and night-time NEE between –18 and –26 and between 5 and 8 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$, respectively (Fan *et al*. 1990; Grace *et al*. 1995; Malhi *et al*. 1998; Löscher *et al*. 2003). During drought mean night-time NEE decreased to 5.27 ± 0.62 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ as minimum values increased being between –7.8 and –9.6 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$. The reduced night-time $CO_2$ release slowly recovered after rewatering was resumed. Daytime NEE fully recovered to pre-drought values 2 weeks after rewatering was resumed (Fig. 2a, c & e), which was similar to the response of natural rainforest to seasonal drought in Rhondonia (Andreae *et al*. 2002). Integrated over 24 h drought-induced changes of NEE were small with compensating changes in ecosystem photosynthesis ($A_{eco}$) and ecosystem respiration ($R_{eco}$) (Table 2) and differences in day length and maximum solar

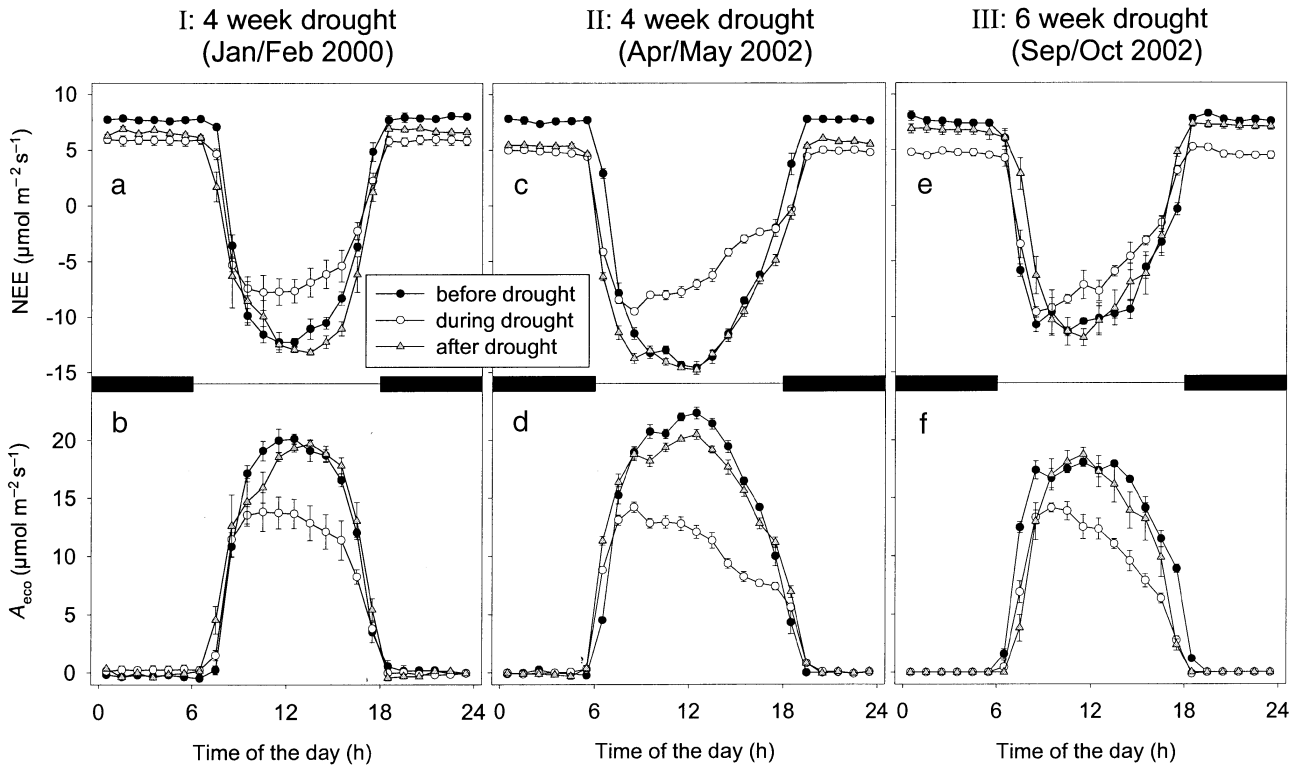


**Figure 2.** Total daily (a), (c), (e) net ecosystem $CO_2$ exchange rate (NEE) and (b), (d), (f) photosynthetic $CO_2$ uptake rate ($A_{eco}$) before the drought (– ● –), 21–25 d within the drought (– ○ –), and 6–11 d after the drought ended (– ▲ –). Data are hourly averages of five subsequent day courses. Data are mean ± SE ($n = 5$). The three drought experiments (I, II and III) were conducted at different times of the year (see headlines) and drought lasted for 27, 28 and 36 d, respectively.

**Table 2.** Daily integrals of NEE and $A_{eco}$

| | Experiment | before drought | during drought | after drought |
|---|---|---|---|---|
| 24 h net ecosystem $CO_2$ uptake NEE (mmol $m^{-2}$ $d^{-1}$) | I | 107.51 *a* | 96.11 *a* | –14.50 |
| | | (12.71) | (30.58) | (25.55) |
| | II | –92.70 *b* | –67.14 *b* | –268.77 |
| | | (16.86) | (6.99) | (7.03) |
| | III | –40.11 *c* | –10.14 *c* | –86.81 *c* |
| | | (8.15) | (17.54) | (55.97) |
| 24 h net respiration $R_{eco}$ (mmol $CO_2$ $m^{-2}$ $d^{-1}$)*d* | I | 670.52 | 518.68 *d* | 558.90 |
| | | (12.42) | (18.14) | (8.43) |
| | II | 662.85 | 427.92 | 481.86 |
| | | (11.79) | (10.80) | (6.95) |
| | III | 575.58 *e* | 388.72 *f* | 428.93 *e, f* |
| | | (18.31) | (29.26) | (99.62) |
| Daily photosynthetic $CO_2$ uptake $A_{eco}$ (mmol $m^{-2}$ $d^{-1}$) | I | 563.01 *g* | 422.57 | 573.40 *g* |
| | | (20.66) | (32.40) | (22.83) |
| | | [100%] *1* | [75%] *2* | [102%] *3* |
| | II | 755.54 *h* | 495.07 | 750.63 *h* |
| | | (7.88) | (15.33) | (12.59) |
| | | [100%] *1* | [66%] *2* | [99%] *3* |
| | III | 615.69 | 398.86 | 515.74 |
| | | (10.35) | (15.63) | (45.88) |
| | | [100%] *1* | [65%] *2* | [84%] |
| | mean | 644.74 | 438.83 | 613.26 |
| | | | [68%] | [95%] |

Values are mean (SE) of integrals of the day courses shown in Fig. 2 ($n = 5$). The periods refer to subsequent days before or just at the beginning of the drought, at the end of the drought, and 6–11 d after the rewatering resumed. The Roman numbers refer to the three drought experiments as shown in Fig. 2. Values are tested statistically, similar letters within rows and similar numbers within columns (referring to the normalized values, see text) indicate non-significance ($\alpha = 0.05$).

radiation accounts, in part for small differences between the experiments.

Gross ecosystem $CO_2$ uptake rate ($A_{eco} = -NEE + R_{eco}$) was maximal in April 2002, when pre-drought $A_{eco}$ reached 22.3 $\mu$mol $m^{-2}$ $s^{-1}$ (Fig. 2d). During winter and autumn, when maximum solar radiations were lower, midday values of $A_{eco}$ reached 20.1 and 18.0 $\mu$mol $m^{-2}$ $s^{-1}$, respectively (Fig. 2b & f). During drought $A_{eco}$ was maximum at about 1000 h, even though light intensity peaked between 1200 and 1300 h, hence, $A_{eco}$ was most strongly reduced during midday and afternoon (Fig. 2b, d & f). These drought-induced reductions of $A_{eco}$ were reversible as soon as watering was resumed (▲ in Fig. 2). In order to compare the drought effect on photosynthesis, $A_{eco}$ values were normalized to pre-drought values and the drought-induced reduction (in percent) is given (Table 2). Normalized reduction of daily $A_{eco}$ during drought was the same in all three experiments and after drought values of $A_{eco}$ fully recovered to pre-drought values in experiment I and II. In experiment III (6 weeks of drought) after-drought $A_{eco}$ was slightly lower than in the two previous experiments, which lasted only 4 weeks each ($P = 0.025$ for I versus III and P = 0.047 for II versus III). Considering the non-significant or only slightly significant differences of the three experimental runs, the three experiments were averaged and thus, $A_{eco}$ was determined to be highly significantly reduced to 68% ($P < 0.0001$) because of the drought and recovered to 95% ($P = 0.152$) after the drought (Table 2). These changes in whole ecosystem photosynthesis ($A_{eco}$) could be the result of changes in light interception by the canopy and changes in the efficiency with which light was used for photosynthesis during drought (see below).

Ecosystem evapotranspiration (ET) changed dramatically because of the drought and was reduced to 50–60%, regardless of the time period of calculation (24-h or daytime) (Table 3). VPD was variable and highest during the drought (Fig. 1d). The whole system conductance to water vapour ($gH_2O$) was greatly reduced during the drought (33% of pre-drought conductance) and recovered after watering was resumed. We cannot give quantitative data for the after-drought regime, as massive water inputs during this time rendered water budgeting inaccurate. As a consequence of the reduced $gH_2O$ and reduced $A_{eco}$, apparent intercellular $CO_2$ concentration ($c_i$), was slightly reduced during the drought period (Table 3).

## Single component processes

### *Leaf water relations*

Responses of pre-dawn and midday leaf water potential ($\Psi_{leaf}$) to drought and recovery after drought, varied greatly among the three investigated tree species. The value of $\Psi_{leaf}$ did not vary between pre-dawn and midday for either *C. pentandra* or *H. crepitans* and neither species exhibited a noticeable response to drought. During drought, $\Psi_{leaf}$

**Table 3.** Daily integrals and daytime mean of ecosystem evapotranspiration (ET), apparent daytime water vapour conductance ($gH_2O$), and apparent intercellular $CO_2$ concentration ($c_i$) during the autumn 2002 drought experiment (III) in Table 2 and Fig. 2)

| | Before drought | | During drought | |
|---|---|---|---|---|
| 24 h ecosystem evapotranspiration (ET) (mol $H_2O$ $m^{-2}$ $d^{-1}$) | 234.2 | | 122.4 | |
| | (8.3) | | (7.1) | |
| | [100%] | | [52%] | |
| Daytime ecosystem evapotranspiration (ET) (mol $H_2O$ $m^{-2}$ 12 $h^{-1}$) | 177.2 | | 102.3 | |
| | (6.3) | | (7.8) | |
| | [100%] | | [58%] | |
| Daytime mean apparent water vapour conductance ($gH_2O$) (mmol $H_2O$ $m^{-2}$ $s^{-1}$) | 410.2 | | 121.8 | |
| | (67.9) | | (14.4) | |
| | [100%] | | [30%] | |
| Apparent intercellular $CO_2$ concentration ($c_i$) (p.p.m.) | 379.0 | *c* | 356.2 | *c* |
| | (5.6) | | (29.7) | |
| | [100%] | | [94%] | |

Values are mean (SE) of integrals of the same five days shown in Fig. 2 and used for NEE calculations shown in Table 2. Same letters within rows indicate non-significant differences ($\alpha = 0.05$).

decreased an average of –0.44 MPa for *C. pentandra*; pre-dawn $\Psi_{leaf}$ recovered –0.24 MPa, whereas midday $\Psi_{leaf}$ did not recover (Fig. 3a). *Clitoria racemosa* displayed the largest decrease in $\Psi_{leaf}$ of the three species during the drought with pre-dawn $\Psi_{leaf}$ decreasing around –1.0 MPa and midday $\Psi_{leaf}$ dropping below –1.2 MPa, with no appreciable recovery in either pre-dawn or midday $\Psi_{leaf}$ after rainfall commenced (Fig. 3b). Leaves of *Cl. racemosa* strongly responded to the singular, isolated watering experiment at day 28, with midday $\Psi_{leaf}$ equalling predawn values 7 d after there was a –0.9 MPa difference between the two measurements (Fig. 3b). Both, pre-dawn and midday $\Psi_{leaf}$ of *H. crepitans* were constant at –0.2 MPa throughout, showing no effect to the drought (Fig. 3c).

## Leaf area and light interception

### Leaf area

Daily PFD was usually similar from day to day because of the mostly sunny days in Southern Arizona. However, because the experiment was performed in autumn, PFD slightly decreased throughout the experiment. Despite this seasonal decrease of overall light intensity, PFD in the understorey (1 m above ground) slightly increased during the drought (Fig. 1e). We calculated the ratio of $PFD_{understorey}$ and $PFD_{above\ canopy}$ in order to quantify the percentage of light, which was not absorbed by the construction or the canopy. This ratio increased during the drought and remained constant after rewatering was resumed (Fig. 1f). As the absorption of the construction was constant, this finding points towards a gradual thinning of the canopy during the course of the drought.

### Leaf fall

Average leaf fall (September 2001 to August 2002) was 0.71 g $m^{-2}$ $d^{-1}$. During the first 2 weeks of drought, leaf fall increased dramatically to 1.59 g $m^{-2}$ $d^{-1}$ or 220% of the mean value for the previous year during the same period (Fig. 4a). As drought continued, daily leaf fall declined to pre-drought values and showed a second peak (1.78 g $m^{-2}$ $d^{-1}$ or 245% of annual mean) after rewatering. In general leaf fall increased during the drought, however, varied among species, with *Cl. racemosa* having the greatest before, during, and after the drought. Other species showed different responses of leaf fall during the drought (Fig. 4b–e).

### Leaf growth

In general relative growth rate (RGR) declines with leaf area. However, during drought the size-dependent distribution of RGR was reversibly affected for all leaf areas (see Fig. 5a as an example). In order to follow RGR during the drought, data from leaves with areas between 0 and 30% of the final leaf area were pooled and RGR for these leaves are shown (Fig. 5c–f). RGR decreased more than 90% for leaves from *A. carambola* (Fig. 5c), *Ph. dioica* (Fig. 5d) and *Pt. indicus* (Fig. 5a & e) during drought and RGR recovered after rewatering. For the three species, the average RGR of young leaves declined beginning in the first days of the imposed drought. For *Pt. indicus* and *Ph. dioica*, an increase in RGR occurred 1 week before the end of the drought, which was most likely a response to the localized watering event on day 28. RGR of *C. pentandra* leaves was unaffected by the drought (Fig. 5f). Average normalized values for all four species decreased 40% over the course of the drought with most of the decrease occurring between day 10 and 20 (Fig. 5b) Despite the overall decrease in RGR, the final size of leaves that emerged at the beginning of the drought was similar to the final size of leaves that reached their final size before the drought. Only *Averrhoa carambola* leaves were significantly smaller ($P < 0.01$, Fig. 5c–f, insets). The number of leaves produced per branch was lower during drought than before the drought.

## Light use efficiency

### Potential quantum yield

Potential quantum yield of photosynthesis ($F_v/F_m$) was measured before sunrise and did not change during the drought (Table 4). Except for *I. sapinoides*, which had $F_v/F_m$ values

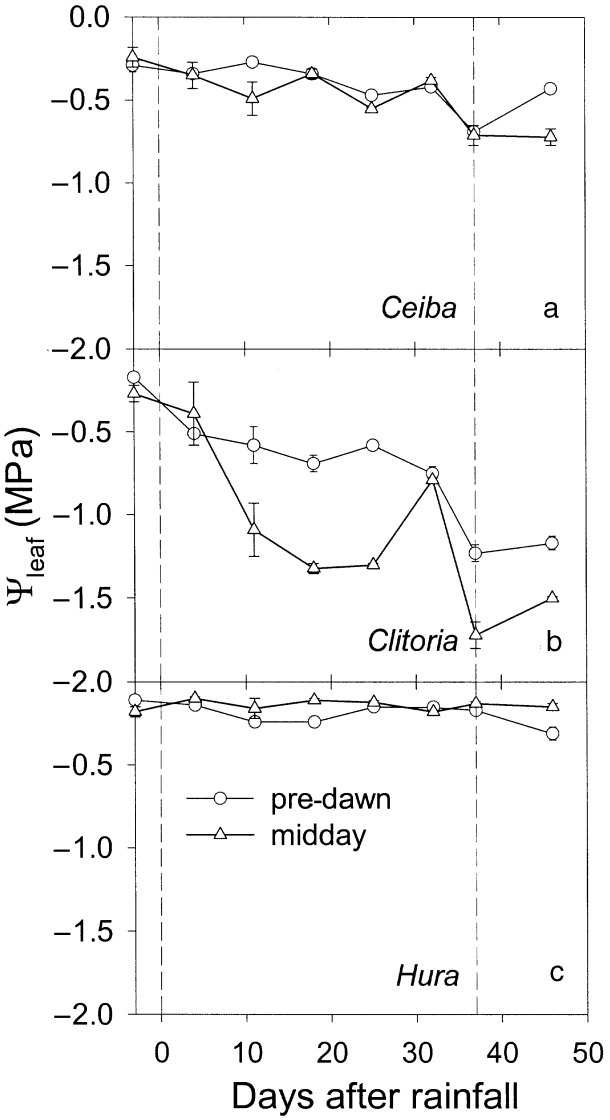


**Figure 3.** Leaf water relations for three large trees during the autumn 2002 drought experiment. Predawn (– ○ –) and midday (– △ –) leaf water potentials ($\Psi_{leaf}$) for (a), *Ceiba pentandra*; (b), *Clitoria racem*osa; and (c) *Hura crepitans* ($n = 4$ leaves for each plant). Dashed lines indicate the beginning and the end of the drought period. Data are means ±SE.

of 0.79, all plants showed maximum values around 0.83. No changes of $F_v/F_m$ related to drought were observed, indicating that light reactions of photosynthesis were in a fully functioning state without any signs of pre-dawn photo-inhibition throughout.

### *Photosynthesis related pigments*

Concentration of pigments associated with the photosynthetic apparatus, namely chlorophyll *a*, chlorophyll *b*, $\alpha$-carotene, $\beta$-carotene, violaxanthin, antheraxanthin, neoxanthin, zeaxanthin, lutein and lutein-epoxide, remained constant during the drought. However, pigment concentrations varied between leaves, no drought-related correlation was detected. Additionally, pigment relations, such as chlorophyll *a*/*b* ratio and the de-epoxidation state of the xanthophylls cycle remained on the same level and did not follow a drought related shift (data not shown).

### *Maximum photosynthetic electron transport at high light intensities*

Light intensity varied greatly within the canopy and thus, in order to extract intrinsic parameters of light driven photosynthesis, $\Delta F/F_m'$ and ETR values were plotted versus PFD. All data points (more than 400 measurements on at least 10 different leaves per species) were grouped (pre-drought, drought and after drought) and then fitted using single exponential functions (material and methods; Fig. 6). The maximum values of ETR at saturating PFD ($ETR_{max}$) of such light dependency curves describe the intrinsic capacity of the photosynthetic apparatus and were tested statistically for drought effects. $ETR_{max}$, was significantly reduced during drought for *Pt. indicus* ($P < 0.001$), *P. aquatica* ($P < 0.001$), and *I. sapinoides* ($P < 0.01$) (Fig. 6, Table 4), with minimum $ETR_{max}$ values occurring 4 weeks in the drought. One week after the rewatering $ETR_{max}$ of *Pt. indicus* and *I. sapinoides* recovered to pre-drought values, while $ETR_{max}$ of *P. aquatica* remained low. $ETR_{max}$ values of *C. pentandra* did not change significantly, however, showed the same trend with a slightly reduced $ETR_{max}$ during drought and a recovery to pre-drought values (Table 4). Fitted exponential functions, which were used to quantify the reduction of $ETR_{max}$, can be considered robust against variations within the single measurements, as all data points are used to extract $ETR_{max}$. ETR measured using chlorophyll fluorescence can be influenced by stomata related reduction of internal carbon availability, but we think this is largely due to down-regulation of the photosynthetic capacity itself. Other cardinal points of light response curves, such as initial slope (i.e. slope for PFD < 200 $\mu$mol $m^{-2}$ $s^{-1}$) or maximum NPQ values, did not change in a significant manner, which was related to the imposed drought.

If we average this reduction of $ETR_{max}$ over the four species, $ETR_{max}$ was reduced to 71% of pre-drought values and recovered to 88% 1 week after rewatering resumed. Single species showed the same trend, however, individual response and magnitude of the effect was highly variable between species. Although we would like to have complementary gas-exchange measurements, it was practically impossible to use bulky instrumentation within the canopy.

### *Transpiration, estimated from the sap-flow*

Sap flow for one emergent tree of *C. pentandra* was reduced during the drought ($25.0 \pm 1.4$ g $m^{-2}$ $s^{-1}$) and recovered significantly after watering was resumed ($27.9 \pm 1.7$ g $m^{-2}$ $s^{-1}$; $P < 0.001$, $n = 5$ d), even though $\Psi_{leaf}$ of this *Ceiba* tree was only slightly affected and leaf area was not reduced by the drought (Figs 4b & 5f). However, mean daytime VPD was higher during the drought (17.82 versus 7.63 mbar). Assuming that sap-flow represents the integrated transpiration over the unchanged leaf-area, we can calculate that tran-

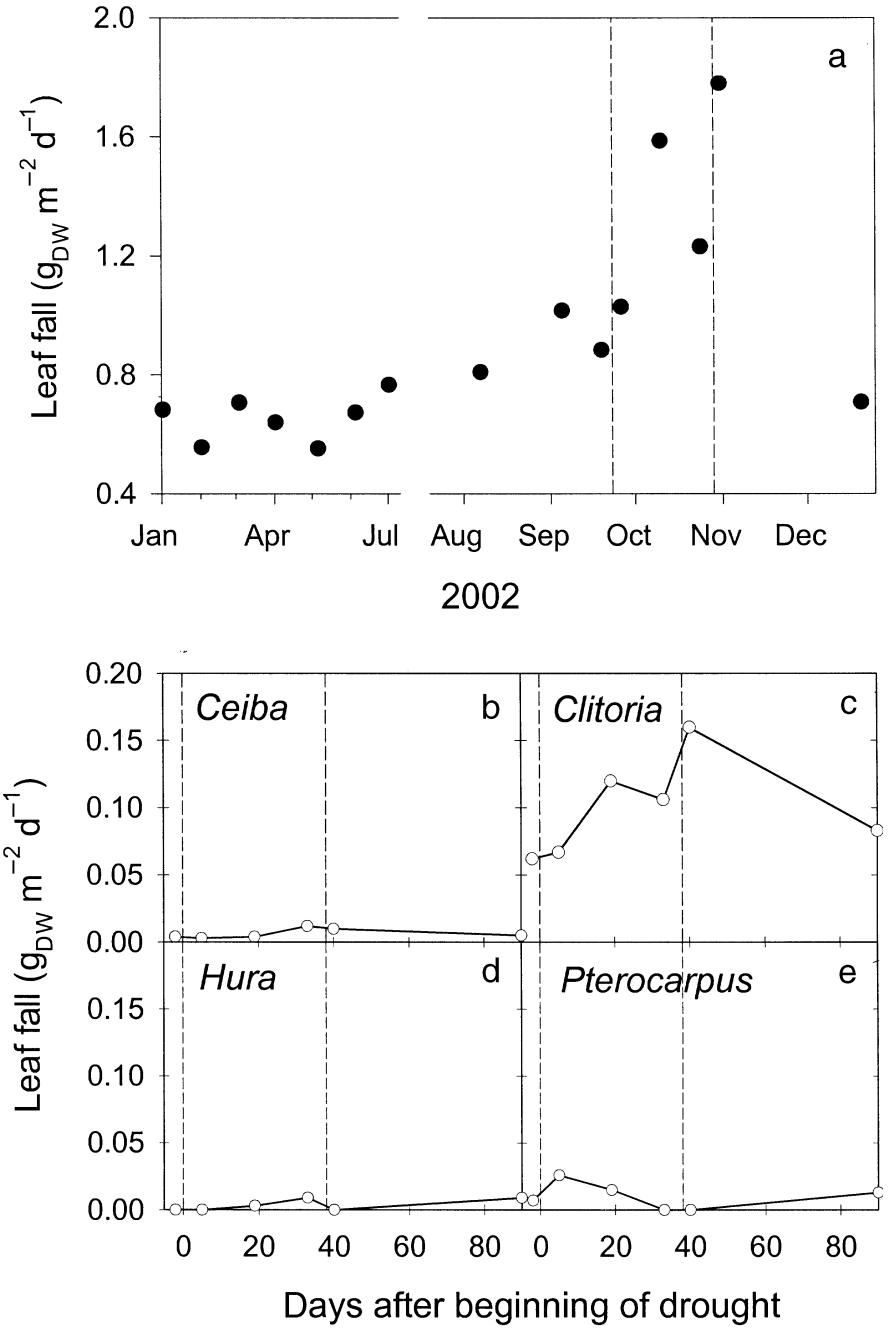


**Figure 4.** Leaf fall from the rainforest mesocosm during 2002 (a) and specific leaf fall from selected species during the drought (b)–(e) ($n = 21$ traps). The vertical lines indicate the beginning and the end of the drought experiment. (a) Overall leaf fall within the mesocosm throughout the year 2002. (b)–(d) Leaf fall separated according to selected species, (b), *Ceiba pentandra*; (c),: *Clitoria racemosa*; (d), *Hura crepitans*; (e), *Pterocarpus indicus*.

spiration was reduced to 90% of that of the well-watered conditions. Allowing for the increased VPD, conductance to water vapour ($gH_2O$) during drought was 38% that after rewatering, which agrees well with the whole system response.

## DISCUSSION

Tropical evergreen rainforests play an important role in global carbon and water cycles, having about 35% of the global net primary production (Löscher *et al*. 2003), however, their capacity as carbon sinks is still under debate. In order to predict the long-term behaviour of tropical rainforests in a changing environment we have to understand the mechanistic and regulatory properties, which determine whole ecosystem dynamics and govern water- and carbon-budget in response to local climate and feed-backs expected to these aspects on the regional-scale climate (Field *et al*. 1995; Cox *et al*. 2000).

Tropical evergreen rainforest may be subjected to increasingly severe drought episodes, caused by El Niño Southern Oscillation (Trenberth & Hoar 1997) or by reduced rainfall, which may be a result of increased deforestation (see models by Nobre, Sellers & Shukla 1991; Lean *et al*. 1996; and Costa & Foley 2000). The effects of drought

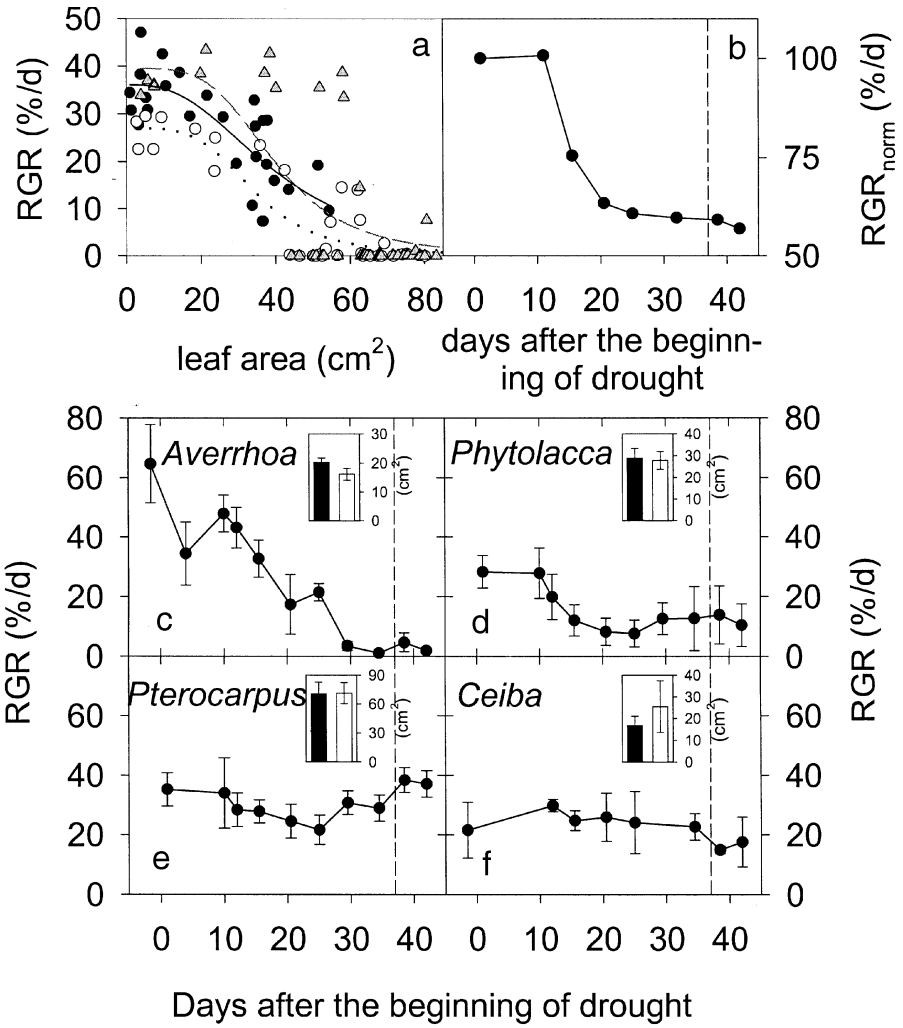


**Figure 5.** Effect of drought on leaf growth. (a) Relation of relative growth rate (RGR) on leaf area, exemplified on *Pterocarpus indicus*. Symbols refer to pre-drought (●, solid line), 2 weeks within drought (○, dotted line), and after drought conditions (▲, dashed line) and were fitted by a three parameter logistic function:

$$y = \frac{a}{1+\left(\frac{x}{x_0}\right)^b}.$$ (c)–(f) Relative growth rates (RGR) of young leaves and final leaf areas (inserts). Depicted are mean values and standard deviations from all leaves smaller than 30% of final leaf size. Insets show final leaf size from control leaves (full-grown before start of drought) and from leaves that reached the full-grown state at the end of the drought (leaves were smaller than 30% of final leaf size at the onset of drought) (c), *Averrhoa carambola*; (d), *Phytolacca dioica*; (e), *Pterocarpus indicus*; (f), *Ceiba pentandra*. (b) Average value of all four species (control value before drought = 100%).

**Table 4.** Potential quantum yield ($F_v/F_m$) and maximum photosynthetic electron transport rate ($ETR_{max}$) of four species before, 4 weeks within and after the drought

| | | Before drought | During drought | After drought |
|---|---|---|---|---|
| *Pterocarpus indicus* | $F_v/F_m$ | 0.837 | 0.830 | 0.837 |
| | | (0.003) | (0.002) | (0.002) |
| | $ETR_{max}$ | 220.24 *a* | 117.00[1] | 231.55 *a* |
| | | (28.16) | (5.45) | (38.55) |
| *Ceiba pentandra* | $F_v/F_m$ | 0.829 | 0.831 | 0.825 |
| | | (0.001) | (0.002) | (0.002) |
| | $ETR_{max}$ | 148.10 *b* | 133.53 *b* | 136.68 *b* |
| | | (28.10) | (4.20) | (6.55) |
| *Pachira aquatica* | $F_v/F_m$ | 0.833 | 0.838 | 0.835 |
| | | (0.002) | (0.002) | (0.001) |
| | $ETR_{max}$ | 205.13[2] | 122.90 | 111.57 |
| | | (13.24) | (4.93) | (3.89) |
| *Inga* cf. *sapindoides* | $F_v/F_m$ | 0.796 | 0.783 | 0.784 |
| | | (0.003) | (0.003) | (0.003) |
| | $ETR_{max}$ | 115.33 *c* | 92.26 | 114.99 *c* |
| | | (12.37) | (4.90) | (6.50) |

$F_v/F_m$ of photosynthesis was measured before sunrise ($n = 20$ leaves for *Pterocarpus*, *Pachira* and *Inga* and 40 leaves for *Ceiba*). Maximum electron transport rate ($ETR_{max}$) was obtained by fitting morning measurements (0900–1200 h) as indicated in Fig. 6 (after drought values were fitted in the same way and the results are given here). Values are mean (SE) and were tested statistically. $F_v/F_m$ values did not change significantly because of the drought ($\alpha = 0.01$). Similar letters within rows indicate non-significance between $ETR_{max}$ values ($\alpha = 0.05$) numbers within rows indicate highly significant difference of the $ERT_{max}$ values ($\alpha = 0.001$).

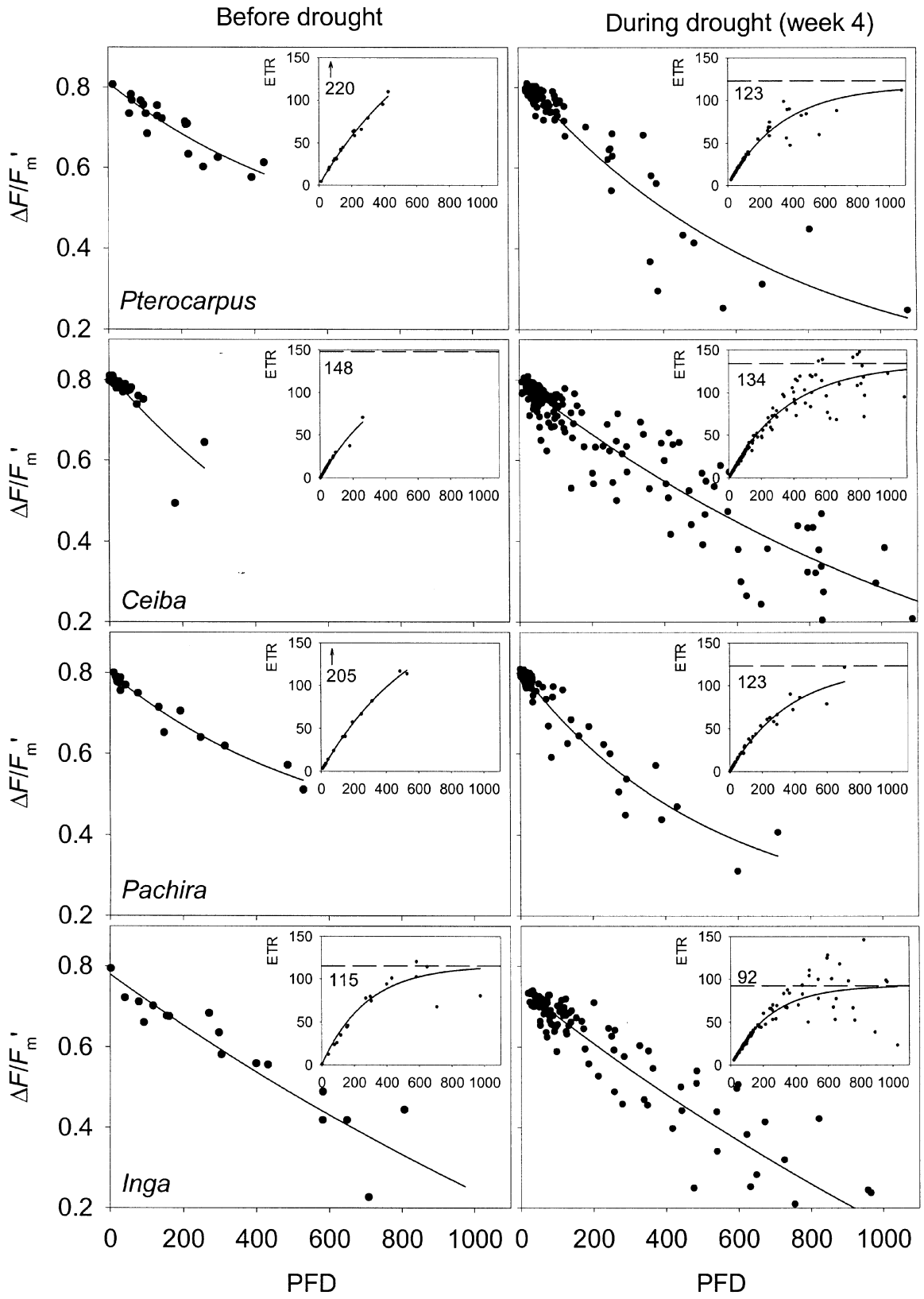


**Figure 6.** Effective quantum yield ($\Delta F/F_m'$) and electron transport rate (ETR, inserts) of *Pterocarpus indicus*, *Ceiba pentandra*, *Pichura aquatica*, and *Inga* cf. *sapinoides* measured at several days before the drought (left panels) and 2–4 weeks within the drought (right panels). Data were obtained between 0900 and 1200 h from different leaves under steady state conditions, i.e. constant light intensities. Data points were fitted using the single exponential equations giving in the material section (lines) and maximum electron transport rate ($ETR_{max}$) were determined [numbers for $ETR_{max}$ ($\mu$mol electrons $m^{-2}$ $s^{-1}$) are given in the inserts].

on tropical rainforests are potentially large. To our knowledge there is only one publication, which addresses the mechanistic effects of an imposed drought on a rainforest ecosystem (Nepstad *et al*. 2002). During this rainfall exclusion experiment in an east-central Amazon forest about 40% of the daily throughfall was excluded by plastic panels in the understorey. A second similar field site is just being set-up in Caxiuana, north-eastern Brazil, in the frame of the Large Scale Biosphere Atmosphere Project in Amazonia (LBA-ECO). The area extent of these experiments, however, is too small to measure or address the possible acceleration of drought events due to plant-ecosystem feedbacks (Cox *et al*. 2000).

The aim of this study was to translate the mechanistic findings, which were obtained on the level of leaves or plants, into their contribution to ecosystem carbon budget. Experimentally the tropical rainforest within the Biosphere 2 Center provided an ideal model system, as the easy canopy access enabled leaf-level measurements in tight correlation with whole ecosystem carbon budgeting (Lin *et al*. 1999, 2002). Steady-state NEE values measured within Biosphere's tropical mesocosm were comparable to those reported for field sites in the wet tropics. We are aware of possible artefacts of an enclosed ecosystem and we do not try to make simple one to one translations from this experimental model system to the field. However, the understanding of the dynamics within Biosphere 2 may serve the understanding of the mechanisms in the diverse tropics and could reveal possible regional scale feed-backs.

Ecosystem photosynthetic $CO_2$ uptake rate ($A_{eco}$) of Biosphere 2 Center's tropical rainforest mesocosm decreased by 32% in average during the three imposed drought treatments, even though the deep-rooted trees had access to an adequate water supply throughout. This is similar to natural conditions within the Amazon Basin, where big trees utilize water from deep resources during drought (Nepstad *et al*. 1994; Meinzer *et al*. 1999). Over 24 h, the reduced $A_{eco}$ was associated with an equally reduced ecosystem respiration, which might be due to the reduced $\Psi_{soil}$ in the top 0.60 m of soil, reducing root and microflora respiration.

Mechanistically, this dramatic drought-induced reduction of photosynthetic $CO_2$ uptake rate could be ascribed to three main underlying reasons: (1) the canopy became thinner; (2) photosynthetic $CO_2$ fixation capacity of the remaining leaves was reduced; and (3) stomata closed.

Leaf area, declined during drought due to increased leaf fall, especially during the first 2 weeks, and reduced rates of leaf growth. Assuming that the four species on which growth measurements were performed, were a representative sample of the trees constituting the canopy, leaf area loss was defined by the following considerations: the average pre-drought leaf fall of 0.71 g $m^{-2}$ $d^{-1}$, which was slightly less than the lowest values reported for tropical evergreen lowland forests (Rai & Proctor 1986). Under steady state, this corresponded to an average daily decrease in leaf area of 27.5 $m^2$ $d^{-1}$ and thus, under steady-state conditions, average leaf growth was also 27.5 $m^2$ $d^{-1}$. During the first 2 weeks of drought, leaf fall increased to 220% while growth decreased to 93% of the average. Thus, average leaf fall was 60.6 $m^2$ $d^{-1}$ and leaf growth was 25.6 $m^2$ $d^{-1}$, and within the first 14 d, the total leaf area of the biome was hence reduced by $14 \times (60.5 - 25.6) = 490$ $m^2$. The estimated whole leaf area of the canopy was between 7760 and 9700 $m^2$ (1940 $m^2$ of projected area of the canopy) for an LAI of 4 or 5, respectively. After 14 d of drought, this area was reduced to an area between 7270 and 9210 $m^2$ or to 93.7 and 94.9%, respectively. During the next 4 weeks, leaf fall reached average values again, while leaf growth was decreased to 60%, leading to a further loss of $28 \times (27.5 - 16.5) = 308$ $m^2$. Thus, the total leaf area at the end of the drought was between 6960 and 8900 $m^2$ or between 89.7 and 91.8% of pre-drought area.

Induced leaf fall is a widely used strategy to reduce leaf area during drought (Greitner, Pell & Winner 1994; Clifton-Brown *et al*. 2002; San Jose *et al*. 2003) even though no effect on leaf water potential may be visible (Wendler & Millard 1996). Total leaf growth within the canopy of around 30 $m^2$ $d^{-1}$ corresponds to an average turnover rate for the leaves of 0.3–0.5% $d^{-1}$ or an average lifetime of the leaves between 200 and 300 d, which is similar to values reported from natural tropical rainforests (Kitajima, Mulkey & Wright 1997). Carbon assimilation of young leaves from the outer canopy of tropical trees is roughly twice than that of old leaves of the given species (Kitajima *et al*. 1997). Hence, the reduction in growth measured in this study may effectively shift the age spectrum of leaves in the outer canopy towards older leaves, which may additionally decrease the photosynthetic capacity of the outer canopy (exemplified for *C. pentandra* by Zotz & Winter 1994). Most studies impose more drastic treatments, for which growth reductions can be deduced from biomass or final leaf area. The effect of a mild drought stress on plant growth is often difficult to assess. In this study decreases in RGR could be detected long before wilting or senescence occurred. The slight recovery of growth rates upon rewatering in the individual leaves cannot be explained by increase in turgor alone, because this mechanism acts on a much shorter time-scale of minutes (Shackel, Matthews & Morrison 1987; Serpe & Matthews 1992). It was not expected that leaf RGR would reach initial values upon rewatering, because the RGR of individual leaves does not increase greatly in a few days. Total recovery of canopy leaf area will involve new emerging branches and leaves from the terminal buds, which may require months. We also want to highlight that individual species responded very differently. While some species, such as *Cl. racemosa*, showed clearly reduced midday $\Psi_{leaf}$ and greatly increased leaf fall, other species (e.g. *C. pentandra*) showed no drought-induced changes in $\Psi_{leaf}$, leaf abscission, or RGR.

The value of $\Psi_{leaf}$ of some species was affected (e.g. *Clitoria*) whereas $\Psi_{leaf}$ of other species (e.g. *Hura* and *Ceiba*) remained at pre-drought levels throughout, even though $gH_2O$ of the *Ceiba* tree decreased to 40% during drought. Unfortunately we cannot give exact numerical leaf-level data for conductance to water vapour, as it was practically

impossible to use gas-exchange instrumentation in the tall canopy. The mechanisms of water stress effects are still under debate, however, most authors consider that water stress primarily limits $gH_2O$, which in turn reduces $c_i$ and indirectly affects photosynthesis (for an overview see Lawlor 2002). However, in a pioneering field study, using unirrigated, fully sunlit cotton leaves, Björkman 1989) showed that as $\Psi_{leaf}$ decreased there was also a direct inhibition of photosynthetic capacity, such that photosynthetic rate decreased almost linearly with $gH_2O$, leaving $c_i$ almost constant. In our study ecosystem $c_i$ remained high, even though ecosystem $gH_2O$ was greatly reduced. Using a simple photosynthesis model (von Caemmerer 2000) we estimated that the indirect effect on $A_{eco}$ through stomata was about 7%. The observed reduction of 32% would require a much lower $c_i$ levels (275 p.p.m.) than observed (356 p.p.m.). We thus conclude that the drought reduced $A_{eco}$, in part due to a direct effect of drought on photosynthetic capacity.

We detected a significant reduction (average of 29%) in photosynthetic capacity, expressed as reduced $ETR_{max}$. Maximum potential photosynthetic electron transport ($ETR_{max}$) of most species was significantly reduced by the drought; similar responses have been reported for a Costa Rican seasonally dry forest (Brodribb, Holbrook & Gutiérrez 2002), where midday ETR, measured under high light intensities, was reduced during the dry seasons. $ETR_{max}$ was reduced even though photosynthesis at low light intensities was unaffected. This reduction of $ETR_{max}$ of leaves of the outer canopy could be used for remote sensing approaches. The photosynthetic reflectance index (PRI) derived from hyperspectral reflectance measurements (Gamon, Filella & Peñuelas 1993) potentially can quantify such drought-induced changes and could numerically be translated to changes in $A_{eco}$. This approach could complement thermal infrared remote sensing and the detection of surface energy fluxes (Anderson *et al*. 1997; Norman *et al*. 2003) and may be especially useful to quantify photosynthetic capacity of dense, multilayer canopies in which the normalized vegetation index (NDVI) fails (Danson & Plummer 1995; Gitelson 2004).

Reduced stomatal conductance to water vapour and/or leaf internal $CO_2$ concentration normally do not have a large effect on photosynthetic electron transport measured by gross $O_2$ exchange at ambient $CO_2$ and high light (Badger 1985). Additionally, there were no signs of photoinhibition, and non-photochemical quenching mechanisms, as well as the de-epoxidation state of the associated pigments remained constant during the imposed drought and may remain unaffected even despite more severe drought conditions (Cousins *et al*. 2002). Our findings point toward a direct drought effect on photosynthesis and may reflect a Rubisco-mediated inhibition of photosynthesis, which was shown using $A$–$c_i$ curves of drought-stressed herbaceous species. Water stress affects Rubisco activity, however, the underlying physiological mechanisms remain controversial (for review see Parry *et al*. 2002). Photosynthesis is $CO_2$ limited at high light intensities; thus it can be assumed, that a reduction of light-driven electron transport is associated with an equal reduction of $CO_2$ uptake rate. Using the whole ecosystem data and the same photosynthetic model, the reduced $c_i$ should only account for a 5% reduction of $ERT_{max}$ under light-saturating conditions. With an LAI of 4–5, we can assume that not more than 20–25% of the leaves operated at $ETR_{max}$; photosynthesis of most of the leaves operated at low light intensities, being unaffected by the slightly reduced $c_i$.

Thus, the drought-induced reduction of $A_{eco}$ of 32% is caused by three underlying effects: (1) the reduced leaf area accounts for about one-third of the reduction during drought and may be the main reason for the reduced after-drought NEE values and the slow recovery of $A_{eco}$ after rewatering resumed. (2) Reduced ETR of light reaction of photosynthesis at high irradiance ($ETR_{max}$) explains at least another one-fifth of the reduced $A_{eco}$. (3) Only one-sixth of the reduced $A_{eco}$ could be solely ascribed to stomata limited $CO_2$ uptake, which does not leave its signature in light reaction of photosynthesis.

Single-species responses were highly variable, showing different ecological strategies to face drought periods. *Ceiba pentandra*, on which most measurements were performed, maintained a high $\Psi_{leaf}$ during drought, and $ETR_{max}$ and RGR were not affected. The value of $\Psi_{leaf}$ of *Pt. indicus*, in contrast, decreased significantly. $ETR_{max}$ was reduced greatly, indicating a strong limitation of photosynthesis, including stomatal and non-stomatal effects. Functional diversity within tropical ecosystems may be significant and we do not know if the species, which were selected for this study, constitute a representative pool to scale leaf-level measurements to the ecosystem, however, we assume that the distribution of different functional groups is complex and may vary greatly. The impact of this heterogeneity on modelling and scaling is yet to be explored.

## ACKNOWLEDGMENTS

We thank A. Ponizovsky, S. Blagodatsky, V. Semenov, E. Demyanova and A. Bil from the Russian Academy of Science for their help with the soil sample analyses, K. Grieve for the sap flow measurements, Allen Wright and Jost van-Haren for technical assistance, Robert Arrington for climbing support, and Ramesh Murthy for help with the statistical analyses. Financial support was provided by Mr Edward Bass through a grant to Columbia University, by the Packard Foundation (grant DLP 998306 to G.L.) and by the Alexander von Humboldt Foundation (Feodor Lynen Fellowship to A.W.).

## REFERENCES

Amthor J.S. (1994) Scaling $CO_2$-photosynthesis relationships from the leaf to the canopy. *Photosynthesis Research* **39,** 321–350.

Anderson M.C., Norman J.M., Diak G.R., Kustas W.P. & Mecikalski J.R. (1997) A two-source time-integrated model for estimating surface fluxes using thermal infrared remote sensing. *Remote Sensing of Environment* **60,** 195–216.

Andreae M.O., Artaxo P., Brandao C., *et al.* (2002) Biogeochemical cycling of carbon, water, energy, trace gases, and aerosols in Amazonia: The LBA-EUSTACH experiments. *Journal of Geophysical Research – Atmospheres* **107,** 8066–8066.

Arain M.A., Shuttleworth W.J., Farnsworth B., Adams J. & Sen O.L. (2000) Comparing micrometeorology of rain forests in Biosphere-2 and Amazon Basin. *Agricultural and Forest Meteorology* **100,** 273–289.

Arneth A., Kelliher F.M., McSeveny T.M. & Byers A.N. (1999) Assessment of annual carbon exchange in a water-stressed *Pinus radiata* plantation: An analysis based on eddy covariance measurements and an integrated biophysical model. *Global Change Biology* **5,** 531–545.

Badger M.R. (1985) Photosynthetic oxygen exchange. *Annual Reviews of Plant Physiology* **36,** 27–53.

Bassi R. & Caffarri S. (2000) Lhc proteins and the regulation of photosynthetic light harvesting function by xanthophylls. *Photosynthesis Research* **64,** 243–256.

Bilger W. & Björkman O. (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynthesis Research* **25,** 173–185.

Bilger W., Schreiber U. & Bock M. (1995) Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. *Oecologia* **102,** 425–432.

Björkman O. (1989) Some viewpoints on photosynthetic response and adaptation to environmental stress. In: *Photosynthesis* (ed. W.R. Briggs), pp. 45–58. Alan R. Liss Inc., New-York, USA.

Bonal D., Sabatier D., Montpied P., Tremeaux D. & Guehl J.M. (2000b) Interspecific variability of δ13C among trees in rainforests of French Guiana: functional groups and canopy integration. *Oecologia* **124,** 454–468.

Bonal D., sBarigah T.S., Granier A. & Guehl J.M. (2000a) Late-stage canopy tree species with extremely low $\delta^{13}C$ and high stomatal sensitivity to seasonal soil drought in the tropical rainforest of French Guiana. *Plant, Cell and Environment* **23,** 445–459.

Brady N.C. & Weil R.R. (1996) *The Nature and Properties of Soils*, 11th edn. Prentice Hall, Upper Saddle River, NJ, USA.

Brodribb T.J., Holbrook N.M. & Gutiérrez M.V. (2002) Hydraulic and photosynthetic co-ordination in seasonally dry tropical forest trees. *Plant, Cell and Environment* **25,** 1435–1444.

von Caemmerer S. (2000) *Biochemical Models of Leaf Photosynthesis.* Techniques in Plant Sciences No. 2. 165 pp. CSIRO Publishing, Collingwood, Australia.

Chaves M.M., Maroco J.P. & Pereira J.S. (2003) Understanding plant responses to drought – from genes to the whole plant. *Functional Plant Biology* **30,** 239–264.

Clifton-Brown J.C., Lewandowski I., Bangerth F. & Jones M.B. (2002) Comparative responses to water stress in stay-green, rapid- and slow senescing genotypes of the biomass crop *Miscanthus*. *New Phytologist* **154,** 335–345.

Cornic G. & Fresneau C. (2002) Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during a mild drought. *Annals of Botany* **89,** 887–894.

Costa M.H. & Foley J.A. (2000) Combined effects of deforestation and doubled atmospheric $CO_2$ concentration on the climate of Amazonia. *Journal of Climate* **13,** 18–34.

Cousins A.B., Adam N.R., Wall G.W., Kimball B.A., Pinter P.J., Ottman M.J., Leavitt S.W. & Webber A.N. (2002) Photosystem II energy use, non-photochemical quenching and the xanthophyll cycle in *Sorghum bicolor* grown under drought and free-air $CO_2$ enrichment (FACE) conditions. *Plant, Cell and Environment* **25,** 1551–1559.

Cox P.M., Betts R.A., Joens C.D., Spall S.A. & Totterdell I.J. (2000) Accelerating of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* **408,** 184–187.

Danson F.M. & Plummer S.E. (1995) Red-edge response to forest leaf area index. *International Journal of Remote Sensing* **16,** 183–188.

Dawson T.E. (1996) Determining water use by trees and forests from isotopic, energy balance and transpiration analyses: the roles of tree size and the hydraulic lift. *Tree Physiology* **16,** 263–272.

Demmig B., Winter K., Krüger A. & Czygan F.C. (1987) Photoinhibition and zeaxanthin formation in intact leaves: a possible role of the xanthophyll cycle in the dissipation of excess light energy. *Plant Physiology* **84,** 218–224.

Dixon R.K., Brown S., Houghton R.A., Solomon A.M., Trexler M.C. & Wisniewski J. (1994) Carbon pools and flux of global forest ecosystems. *Science* **263,** 185–190.

Durand J.L., Onillon B., Schnyder H. & Rademacher I. (1995) Drought effects on cellular and spatial parameters of leaf growth in tall fescue. *Journal of Experimental Botany* **46,** 1147–1155.

Ehleringer J.R. & Field C.B. (eds) (1993) *Scaling Physiological Processes. Leaf to Globe*, 388 pp. Academic Press, San Diego, New York, Boston, London, Sydney, Tokyo, Toronto.

Enquist B.J., Economo E.P., Huxman T.E., Allen A.P., Ignace D.D. & Gillooly J.F. (2003) Scaling metabolism from organisms to ecosystems. *Nature* **423,** 639–642.

Fan S.M., Wofsy S.C., Bakwin P.S., Jacob D.J. & Fitzjarrald D.R. (1990) Atmosphere-Biosphere exchange of $CO_2$ and $O_3$ in the Central-Amazon-Forest. *Journal of Geophysical Research* **95,** 16851–16864.

Fernandez R.T., Perry R.L. & Flore J.A. (1997) Drought response of young apple trees on three rootstocks: growth and development. *Journal of the American Society for Horticultural Science* **122,** 14–19.

Field C.B., Jackson R.B. & Mooney H.A. (1995) Stomatal responses to increased $CO_2$ – implications from the plant to the global scale. *Plant, Cell and Environment* **18,** 1214–1225.

Fitter A. & Hay R.K.M. (2001) *Environmental Physiology of Plants*, 3rd edn, 367 pp. Academic Press, London, San Diego.

Gamon J.A., Filella I. & Peñuelas J. (1993) The dynamic 531-nm Ä reflectance signal: a survey of 20 angiosperm species. In *Photosynthetic Responses to the Environment* (eds H.Y. Yamamoto & C.M. Smith), pp. 172–177. American Society of Plant Physiologists, Rockville, MD, USA.

Genty B., Briantais J.M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990,** 87–92.

Gilmore A.M. & Yamamoto H.Y. (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C-18 high-performance liquid chromatographic column. *Journal of Chromatography* **543,** 137–145.

Gitelson A.A. (2004) Wide dynamic range vegetation index for remote quantification of biophysical characteristics of vegetation. *Journal of Plant Physiology* **161,** 165–173.

Grace J., Lloyd J., McIntyre J., *et al.* (1995) Fluxes of carbon-dioxide and water-vapor over an undisturbed tropical forest in south-west Amazonia. *Global Change Biology* **1,** 1–12.

Granier A. (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiology* **3,** 309–320.

Greitner C.S., Pell E.J. & Winner W.E. (1994) Analyses of aspen foliage exposed to multible stressors – ozone, nitrogen deficiency and drought. *New Phytologist* **127,** 579–589.

Grieu P., Robin C. & Guckert A. (1995) Effect of drought on photosynthesis in *Trifolium repens*: maintenance of photosystem

II efficiency photosynthesis. *Plant Physiology and Biochemistry* **33,** 19–24.
Heckenberger U., Roggatz U. & Schurr U. (1998) Effect of drought stress on cytological status of *Ricinus communis*. *Journal of Experimental Botany* **49,** 181–189.
Hogan K.P., Smith A.P. & Samaniego M. (1995) Gas exchange in six tropical semi-deciduous forest canopy tree species during the wet and dry seasons. *Biotropica* **27,** 324–333.
Hollinger D.Y., Kelliher F.M., Schulze E.D., *et al.* (1998) Forest-atmosphere carbon dioxide exchange in eastern Siberia. *Agricultural and Forest Meteorology* **90,** 291–306.
Horton J.L., Kolb T.E. & Hart S.C. (2001) Responses of riparian trees to interannual variation in ground water depth in a semi-arid river basin. *Plant, Cell and Environment* **24,** 293–304.
Hulme M. & Viner D. (1998) A climate change scenario for the tropics. *Global Change* **39,** 145–176.
Kitajima K., Mulkey S.S. & Wright S.J. (1997) Decline of photosynthetic capacity with leaf age in relation to leaf longevities for five tropical canopy tree species. *American Journal of Botany* **84,** 702–708.
Lai C.T., Katul G., Oren R., Ellsworth D. & Schafer K. (2000) Modeling $CO_2$ and water vapor turbulent flux distributions within a forest canopy. *Journal of Geophysical Research – Atmospheres* **105,** 26333–26351.
Langdon C., Broecker W.S., Hammond D.E., Glenn E., Fitzsimmons K., Nelson S.G., Peng T.H., Hajdas I. & Bonani G. (2003) Effect of elevated $CO_2$ on the community metabolism of an experimental coral reef. *Global Biogeochemical Cycles* **17,** 1–14.
Langdon C., Takahashi T., Sweeney C., Chipman D., Goddard J., Marubini F., Aceves H., Barnet H. & Atkinson M.J. (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochemical Cycles* **14,** 639–654.
Lawlor D.W. (2002) Limitation to photosynthesis in water-stressed leaves: stomata *vs.* metabolism and the role of ATP. *Annals of Botany* **87,** 871–885.
Lean J., Bunton C.B., Nobre C.A. & Rowntree P.R. (1996) The simulated impact of Amazonian deforestation on climate using ABRACOS vegetation characteristics. In *Amazonian Deforestation and Climate* (eds J.H.C. Gash, C.A. Nobre, J.M. Roberts & R.L. Victoria), pp. 549–576. John Wiley & Sons, New York, USA.
Leigh L.S. (1999) The Basis for Rainforest Diversity and Biosphere 2. PhD Dissertation. University of Florida, Gainesville, FL, USA.
Leigh L.S., Burgess T., Marino B.D.V. & Wei Y.D. (1999) Tropical rainforest biome of Biosphere 2: structure, composition and results of first two years of operation. *Ecological Engineering* **13,** 65–94.
Lin G.H., Adams J., Farnsworth B., Wei Y.D., Marino B.D.V. & Berry J.A. (1999) Ecosystem carbon exchange in two terrestrial ecosystem mesocosms under different atmospheric $CO_2$ concentrations. *Oecologia* **119,** 97–108.
Lin G.H., Berry J.A., Kaduk J., Griffin K., Southern A., Adams J., van Haren J. & Broecker W. (2002) Sensitivity of photosynthesis and carbon sinks in world tropical rainforests to projected atmospheric $CO_2$ and associated climate changes. In *Proceedings of 12th International Congress on Photosynthesis.* CSIRO Publishing, Melbourne, Australia.
Lin G.H., Marino B.D.V., Wei Y., Adams J., Tubiello F. & Berry J.A. (1998) An experimental and model study of the responses in ecosystem exchanges to increasing $CO_2$ concentrations using a tropical rainforest mesocosm. *Australian Journal of Plant Physiology* **25,** 547–556.
Löscher H.W., Oberbauer S.F., Gholz H.L. & Clark D.B. (2003) Environmental controls on net ecosystem-level carbon exchange and productivity in a Central American tropical wet forest. *Global Change Biology* **9,** 396–412.
Magnani F. & Grace J. (2000) Plants as self-organising systems. In *Leaf Development and Canopy Growth* (eds B. Marshall & J. Roberts), pp. 36–58. Sheffield Academic Press, Sheffield, UK.
Malhi Y., Nobre A.D., Grace J., Kruijt B., Pereira M.G.P., Culf A. & Scott S. (1998) Carbon dioxide transfer over a Central Amazonian rain forest: The Manaus LBA site. *Journal of Geophysical Research-Atmospheres* **103,** 31593–31612.
Martinez-Carrasco R., Sanchez-Rodriguez J. & Perez P. (2002) Changes in chlorophyll fluorescence during the course of photoperiod and in response to drought in *Casuarina equisetifolia* Forst. & Forst. *Photosynthetica* **40,** 363–368.
Matsubara S., Gilmore A.M. & Osmond C.B. (2001) Diurnal and acclimatory responses of violaxanthin and lutein epoxide in the Australian mistletoe *Amyema miquelii*. *Australian Journal of Plant Physiology* **28,** 793–800.
Maxwell K. & Johnson G.N. (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* **51,** 659–668.
Meinzer F.C., Andrade J.L., Goldstein G., Holbrook N.M., Cavelier J. & Wright S.J. (1999) Partitioning of soil water among canopy trees in a seasonally dry tropical forest. *Oecologia* **121,** 293–301.
Melillo J.M., McGuire A.D., Kicklighter D.W., Moore B., Vorosmarty C.J. & Schloss A.L. (1993) Global climate-change and terrestrial net primary production. *Nature* **363,** 234–240.
Moorcroft P.R. (2003) Recent advances in ecosystem–atmosphere interactions: an ecological perspective. *Proceedings of the Royal Society of London, B Biology Sciences* **270,** 1215–1227.
National Research Council (2004) *Implementing Climate and Global Change Research: A Review of the Final US Climate Change Science Program.* The National Academic Press, Washington, DC, USA. http://www.climatescience.gov.
Nepstad D.C., Decarvalho C.R., Davidson E.A., Jipp P.H., Lefebvre P.A., Negreiros G.H., Dasilva E.D., Stone T.A., Trumbore S.E. & Vieira S. (1994) The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. *Nature* **372,** 666–669.
Nepstad D.C., Moutinho P., Dias M.B., *et al.* (2002) The effects of partial throughfall exclusion on canopy processes, above-ground production, and biogeochemistry of an Amazon forest. *Journal of Geophysical Research – Atmosphere* **107,** article 8085.
Nobre C.A., Sellers P.J. & Shukla J. (1991) Amazonian deforestation and regional climate change. *Journal of Climate* **4,** 957–988.
Norman J.M., Anderson M.C., Kustas W.P., French A.N., Mecikalski J.R., Torn R., Diak G.R., Schmugge T.J. & Tanner B.C.W. (2003) Remote sensing of surface energy fluxes at the 10(1)-m pixel resolution. *Water Resources Research* **39,** article 1221.
Novick K.A., Stoy P.C., Katul G.G., Ellsworth D.S., Siqueira M.B.S., Juang J. & Oren R. (2004) Carbon dioxide and water vapor exchange in a warm temperate grassland. *Oecologia* **138,** 259–274.
Osmond C.B., Ananyev G., Berry J., *et al.* (2004) Changing the way we think about global change research: scaling up in experimental ecosystem science. *Global Change Biology* **10,** 393–407.
Parry M.A.J., Andralojc P.J., Khan S., Lea P.J. & Keys A.J. (2002) Rubisco activity: Effects of drought stress. *Annals of Botany* **89,** 833–839.
Rai S.N. & Proctor J. (1986) Ecological studies on four rain forests in Karnataka, India II. Litterfall. *Journal of Ecology* **74,** 439–454.
Rambal S., Ourcival J.M., Joffre R., Mouillot F., Nouvellon Y., Reichstein M. & Rocheteau A. (2003) Drought controls over conductance and assimilation of a Mediterranean evergreen

ecosystem: scaling from leaf to canopy. *Global Change Biology* **9,** 1813–1824.

Randerson J.T., Collatz G.J., Fessenden J.E., Munoz A.D., Still C.J., Berry J.A., Fung I.Y., Suits N. & Denning A.S. (2002) A possible global covariance between terrestrial gross primary production and C-13 discrimination: consequences for the atmospheric C-13 budget and its response to ENSO. *Global Biochemical Cycles* **16,** 1136.

Rascher U., Liebig M. & Lüttge U. (2000) Evaluation of instant light-response curves of chlorophyll-fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. *Plant, Cell and Environment* **23,** 1397–1405.

de Rosnay P., Polcher J., Bruen M. & Laval K. (2002) Impact of a physically based soil water flow and soil–plant interaction representation for modeling large-scale land surface processes. *Journal of Geophysical Research – Atmospheres* **107,** article 4118.

Running S.W. & Coughlan J.C. (1988) A general model for forest ecosystem processes for regional applications. I. Hydrologic balances, canopy gas exchange and primary production processes. *Ecological Modelling* **42,** 125–154.

San Jose J.J., Bracho R., Montes R. & Nikonova N. (2003) Comparative energy exchange from cowpeas (*Vigna unguiculata* (L.) Walp cvs. TC-9–6 and M-28-6–6) with differences in canopy architectures and growth durations at the Orinoco llanos. *Agricultural and Forest Meteorology* **116,** 197–219.

Schreiber U. & Bilger W. (1993) Progress in chlorophyll fluorescence research: major developments during the past years in retrospect. *Progress in Botany* **53,** 151–173.

Schreiber U., Bilger W. & Neubauer C. (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In *Ecophysiology of Photosynthesis* (eds E.D. Schulze & M.M. Caldwell), pp. 49–70. Springer Berlin, Heidelberg, New York, USA.

Schulze E.D. & Caldwell M.M. (1996) *Ecophysiology of Photosynthesis. Ecological Studies*, Vol. 100**.** Springer-Verlag, Berlin, Germany.

Schulze E.D. & Hall A.E. (1982) Stomatal responses, water loss and $CO_2$ assimilation rates of plants in contrasting environments. In *Physiological Plant Ecology II. Encycl Plant Phys, NS 12b* (eds O.L. Lang, P.S. Nobel, C.B. Osmond & H. Ziegler), pp. 181–230. Springer, Berlin, Heidelberg, New York.

Sellers P.J., Bounoua L., Collatz G.J., *et al.* (1996) Comparison of radiative and physiological effects of doubled atmospheric $CO_2$ on continental climate. *Science* **271,** 1402–1406.

Serpe M.D. & Matthews M.A. (1992) Rapid changes in cell wall yielding of elongating *Begonia argento-guttata* L. leaves in response to changes in plant water status. *Plant Physiology* **100,** 1852–1857.

Shackel K.A., Matthews M.A. & Morrison J.C. (1987) Dynamic relation between expansion and cellular turgor in growing grape (*Vitis vinifera* L.) leaves. *Plant Physiology* **198,** 1166–1171.

Sternberg L.S.L., Green L., Moreira M.Z., Nepstad D., Martinelli L.A. & Victória R. (1998) Root distribution in an Amazonian seasonal forest as derived from δ13C profiles. *Plant and Soil* **205,** 45–50.

Trenberth K.E. & Hoar T.J. (1997) El Niño and climate change. *Geophysical Research Letters* **24,** 3057–3060.

Valentini R., Epron D., Deangelis P., Matteucci G. & Dreyer E. (1995) In situ estimation of net $CO_2$ assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. Cerris* L.) leaves: diurnal cycles under different levels of water supply. *Plant, Cell and Environment* **18,** 631–640.

Walter A. & Schurr U. (1999) The modular character of growth in *Nicotiana tabacum* plants under steady state nutrition. *Journal of Experimental Botany* **50,** 1169–1177.

Wendler R. & Millard P. (1996) Impacts of water and nitrogen supplies on the physiology, leaf demography and nitrogen dynamics of *Betula pendula*. *Tree Physiology* **16,** 153–159.

Zotz G. & Winter K. (1994) Photosynthesis of a tropical canopy tree, *Ceiba pentandra*, in a lowland forest in Panama. *Tree Physiology* **14,** 1291–1301.

*Received 22 March 2004; received in revised form 11 June 2004; accepted for publication 14 June 2004*