Assimilação do carbono pelas árvores de uma floresta tropical húmida da Amazónia: sazonalidade, variação inter-anual e o impacto de secura induzida

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Resumo

As florestas tropicais húmidas constituem importantes ecossistemas terrestres. Os períodos de seca sazonal afectam o ciclo do carbono e prevê-se que aumentem no futuro, principalmente na Amazónia oriental.

Um ensaio de exclusão de chuva foi conduzido numa floresta tropical húmida da Amazónia de modo a estudar as respostas da floresta a uma redução da precipitação (cerca de 50%). Foram realizados estudos fisiológicos ao nível da folha durante cinco estações consecutivas (seca/húmida).

Observaram-se variações inter-anuais e sazonais e efeitos da exclusão de chuva na assimilação do carbono e na condutância estomática, que foram mais significativos nas estações secas. Não se observaram alterações nos parâmetros bioquímicos, sugerindo que a principal limitação na assimilação do carbono, em condições de secura induzida, foi estomática. A respiração foliar aumentou em resposta a reduzida disponibilidade hídrica.

Os baixos valores na assimilação do carbono sugerem que o fósforo poderá estar a limitar o potencial fotossintético desta floresta.

Carbon assimilation by trees of the Amazonian rain forest: seasonality, inter-annual variations and the impact of induced water deficits

Summary

Tropical rain forests are important terrestrial ecosystems. Seasonal drought affects the global carbon cycle and is predicted to increase in the future, particularly in the eastern Amazon.

A 'through fall exclusion' experiment (TFE) was conducted to promote drought stress in an Amazonian rain forest plot to investigate the forest responses to 50% through fall exclusion from the soil. Leaf-level measurements were performed in a Control and a TFE plots for five consecutive seasons (dry/wet).

Inter-annual and imposed seasonal variations were observed in carbon assimilation and stomatal conductance, which were reduced in the TFE plot particularly in the dry seasons. No alterations were observed in biochemical parameters, suggesting that the main limitation to carbon assimilation under reduced water availability was stomatal. Leaf dark respiration increased in response to reduced water availability.

The low values of carbon assimilation indicate that phosphorus may be limiting the photosynthetic potential of this forest.

Contents

Chapter 1

Introduction

The forest biome of Amazonia is one of the Earth´s greatest biological treasures and a major component of the Earth system (Malhi *et al.*, 2008).

The Amazon basin plays an important role in the global cycles of carbon, water and energy (Werth & Avissar, 2002) and is one of the most important areas of the biosphere-atmosphere interaction, influencing the functioning of the Earth system. However, the functioning of the Amazon basin itself is threatened as a result of climate change, deforestation and development (Fisher, 2005). Changes in precipitation, particularly in the dry season, are probably the most critical determinant of the climatic fate of the Amazon (Malhi *et al.*, 2008). For this reason, the functioning of Amazonian rain forest ecosystems during drought has become a scientific focal point, due to associated risks to forest integrity and climate (Meir *et al.*, 2009). And there is still considerable uncertainty about the impact of drought on forest ecosystem properties. This thesis is concerned with studying the impact of induced water deficits by rain exclusion, analyzing the factors that control leaf gas exchange in different tree species of an Amazonian Rain Forest and exploring the variability among seasons and years.

In 2001, the Amazonian rain forests covered about 5.4 million km^2, (Soares-Filho *et al.*, 2006), approximately 87% of their original extent, with 62% in Brazil. They account for about 15% of global terrestrial photosynthesis (Malhi *et al.*, 2008) and discharges 15-20% of all world's fresh water (Fisher, 2005). They host perhaps a quarter of the world's terrestrial species.

Amazonian forests have a substantial influence on regional and global climates. Hence, their removal by deforestation can itself be a driver of climate change and a positive feedback on externally forced climate change (Malhi *et al.*, 2008). Amazonian forests store 120 ± 30 Pg C in biomass carbon, of which 0.5 Pg C $year^{-1}$ were released through deforestation in the 1990s (Malhi *et al.*, 2008). In addition, forest plot studies suggest that intact forests are a carbon sink (~0.6 Pg C $year^{-1}$), particularly in more fertile western Amazonia.

Precipitation varies spatially and temporally in the Amazon basin (Asner & Alencar, 2010), but long-term station records indicate that annual rainfall is decreasing by an average of 0.32% yr^{-1} (Li *et al.*, 2008). There is evidence that Amazonian drought may become more frequent and more severe during this century (Meir *et al.*, 2009; Asner &

Alencar, 2010), especially in central and eastern Amazonia (IPCC, 2007). First, because El-Niño Southern Oscillation (ENSO) events and other sea surface temperature anomalies associated with intense, although episodic, droughts may take place in parts of Amazonia (Aragão *et al.*, 2007). Second, mean annual temperatures are projected to increase by 1.8 to 5.5ºC, resulting in rainfall reductions particularly during dry seasons (Malhi *et al.*, 2008). Finally, land use change is likely to exacerbate the effects of climate warming (Meir *et al.*, 2009).

Amazon forests appear vulnerable to increasing moisture stress, with the potential for large carbon losses to exert feedback on climate change (Phillips *et al.*, 2009) and there is some evidence for lagged impacts of drought, with mortality rates remaining elevated two years after the meteorological event is over (da Costa *et al.*, 2010; Phillips *et al.*, 2010). Thus, most drought impact is thought to be mediated by mortality and not by growth processes (Phillips 2009), with large trees appearing to be the most vulnerable to tropical forests drought (da Costa *et al.*, 2010).

Under these conditions (drought periods, warming), tropical rain forests may become a weak sink or even a source of CO_2 (Zeng *et al.*, 2005; Gullison *et al.*, 2007; Phillips *et al.*, 2009), thus reducing the global terrestrial carbon sink (Berthelot *et al.*, 2005). However, a high level of uncertainty associated with the model predictions is frequently acknowledged (Clark, 2004; Berthelot *et al.*, 2005; Huntingford *et al.*, 2008; Grant *et al.*, 2009; Malhi *et al.*, 2009; Galbraith *et al.*, 2010; Poulter *et al.*, 2010), mainly because the environmental factors that control tropical forest productivity are still relatively poorly understood, especially physiological processes (Baker *et al.*, 2003; Grant *et al.*, 2009; Galbraith *et al.*, 2010). It is also not clear how rainforest plant species might adapt to global climate changes, including increasing intensity and frequency of extreme meteorological events (Schurr *et al.*, 2006; Nepstad *et al.*, 2007; Borken & Matzner, 2009) and increasing tree mortality (da Costa *et al.*, 2010). This emphasises the need for gathering more ecophysiological field data to improve the models that simulate the forest response to drought (Clark, 2004; Meir *et al.*, 2006; Huntingford *et al.*, 2008; Meir *et al.*, 2008; Asner & Alencar, 2010; Galbraith *et al.*, 2010; Wu *et al.*, 2011).

Finally, the importance of including the physiological acclimation in climate change models, as well as the need of models capable of simulating phosphorus limitations

have been already acknowledged (Grant *et al.*, 2009; Galbraith *et al.*, 2010). Global change impacts on biosphere, both positive and negative, could be dampened more than previously assumed (Leuzinger *et al.*, 2011) and tropical rain forests might be more resilient to global change than expected (Leuzinger *et al.*, 2011).

1.1. Through-fall exclusion experiments

In order to investigate the role of drought in constraining forest gas exchange, experimental rainfall manipulations were implemented by the LBA (Large Scale Biosphere Atmosphere Experiment in Amazonia) program.

The method of physically excluding rainfall that penetrates the canopy ("through-fall exclusion", TFE) was replicated at Caxiuanã and Tapajós, the selected two sites in drought-threatened eastern Amazonia. It consisted of approximately six thousand 4.5 m^2 plastic panels and guttering placed at 2 m above the ground. The infrastructure (Fig. 1) removed approximately 50% of incoming precipitation (Fisher *et al.*, 2006) and was installed at Caxiuanã at the beginning of 2002. Each experiment comprised 1 ha of TFE forest and 1 ha of undisturbed (non-manipulated) control forest. The perimeter of the TFE plots was trenched to 1-2 m depth to prevent the horizontal ingress of water from adjacent normally watered soil, and the adjacent control plot perimeters were also trenched to avoid confounding treatment effects (Meir *et al.*, 2009). Full canopy access was provided using 30 m towers in all plots.

Figure 1. Infrastructure of Through-fall Exclusion experiment installed over 100x100 m plot at Caxiuanã.

The large scale of the manipulation was necessary because of substantial lateral extension of the surface roots of large trees. Treatment replication at both sites was

limited by financial resources, but pretreatment calibration were made in all plots to enable replication over time, and the method follows the design of other unreplicated large-scale manipulation experiments, whose strength is acknowledged, especially where large treatment effects are expected (Meir *et al.*, 2009).

Using this experimental set, we were able to impose on forest much more severe droughts than occur at the present (Fisher, 2005).

This thesis presents data collected within Caxiuanã through-fall exclusion experiment.

1.2. Ecophysiological studies in tropical rain forests

Most of the gas-exchange studies in tropical trees that are found in the literature have used seedlings (Huc *et al.*, 1994; Bonal & Guehl, 2001; Coste *et al.*, 2005) or were not focused in season (dry or wet) comparisons (Huc *et al.*, 1994; Carswell *et al.*, 2000; Coste *et al.*, 2005; Domingues *et al.*, 2007). Only in a few sites, drought has been experimentally imposed (Nepstad *et al.*, 2002; Fisher *et al.*, 2006). In one of these field studies also with through-fall exclusion, Nepstad *et al.* (2002) observed that photosynthetic capacity and growth declined in some species but no reduction in predawn leaf water potential was detected. Even so, the authors concluded that the net accumulation of carbon in mature Amazon forests could be very sensitive to small reductions in rainfall.

Studies in tropical rain forests focusing on the effects of drought in carbon assimilation are scarce (McWilliam *et al.*, 1996; Ishida *et al.*, 1999; Bonal *et al.*, 2000; Cao, 2000) and leaf dark respiration rarely measured. This thesis present data on ecophysiology and drought stress throughout the vertical canopy profile, for five consecutive seasons (dry/wet).

1.3. Methodological issues

Leaf physiological measurements were made on every tree accessible from a 30 m tall canopy access tower installed in each plot. Maximum accessible canopy height was approximately 32 m. Nine trees and eight trees were accessible from tower in the

Control and TFE plots, respectively, being 17 trees in total, corresponding to 16 species. All trees were systematically sampled at its highest accessible position in the canopy, and all trees were repeatedly sampled during the study period.

November 2001 was the end of dry season and was the pre-treatment measurement date. This first measurement date also served to adjust and control the methodologies for the next measurement dates. In this first field campaign, species were identified by its systematic position, shade tolerance strategy and position along the canopy. Trees were then grouped according to their position in the canopy: understory [0,10m[, mid-canopy [10,20m[and top-canopy [20,30m].

All measurements were made on fully expanded leaves without signs of senescence, throughout the vertical canopy profile, with a minimum of three leaf replicates by elevation above ground and by trees.

Data were recorded over a period of 2 years, from November 2001 to November 2003, on five measurement dates, at the end of the dry season (Nov 2001, Nov 2002 and Nov 2003) and at the end of the wet season (May 2002 and May 2003).

Leaf gas exchange measurements were performed with two cross-calibrated portable photosynthesis systems with a light source and a CO_2 injector system for controlled light and CO_2 concentrations (LI-6400, LI-COR Inc, Lincoln, NB, USA). Net CO_2 assimilation *vs.* photosynthetic photon flux density (*A*/*PPFD*) and net CO_2 assimilation vs. intercellular CO_2 concentration (*A/Ci*) response curves were obtained. Leaf temperature and relative humidity in the chamber were controlled and kept close to ambient. From *A/PPFD* response curves we determined the maximal rates of photosynthesis (A_{max}) (Chap. 2), maximal stomatal conductance (g_{smax}) (Chap. 2), dark respiration (R_d) (Chap. 4) and intrinsic water use efficiency (*IWUE*) (Chap. 2). In the beginning of the study were also used to determine the saturating light for A/C_i response curves. A/C_i response curves were used to fit the biochemical model of Farquhar *et al.* (1980) with modifications by Sharkey (1985), to estimate the maximum Rubisco CO_2 fixation capacity (V_{cmax}) (Chap. 2) and maximum electron transport rate (J_{max}) (Chap. 2) and the ratio between intercellular CO_2 concentration and atmospheric CO_2 concentration (C_i/C_a) (Chap. 2). The response curves were made between 0800 and 1400h local time to avoid stomatal closure that might occur thereafter. Additionally

daily courses of stomatal conductance (Chap. 6) were measured with a steady-state porometer (LI-1600; LI-COR Inc, Lincoln, NB, USA).

Leaf water potential was measured with a Scholander-type pressure chamber (Skye Instruments, Llandrindod Wells, UK) to evaluate leaf water status at predawn (Ψ_{Pd}) and midday (Ψ_{Md}) (Chap 2 and 6).

Specific leaf area (SLA) and nutrients (N, P, K) were determined at Embrapa Amazônia Oriental, Brazil, from leaf discs collected in the field. SLA was reported in chapters 2 and 4; nutrients only in chapter 2. We decided to present results of nutrients on a leaf area basis, because it is the general reference for use in terrestrial biosphere models (Kattge et al., 2009).

Leaf optical properties, reflectance, transmittance and the calculated absorbance (A=1-(R+T) of the upper leaf surface were determined using the LI-1800 spectroradiometer with an external integrating sphere (LI-1800-12S, LI-COR Inc., Lincoln, NE, USA) (Chap. 3). Reflectance was also used to calculate four reflectance based indices (Chap. 3), namely, Water Index (WI) (Penuelas et al., 1997), Red Edge Position (REP) (Poulos et al., 2007), a chlorophyll normalized difference index (chlNDI) (Gitelson & Merzlyak, 1994) and Photochemical Reflectance Index (PRI) (Gamon et al., 1997).

Chlorophylls were extracted at Embrapa Amazônia Oriental, Brazil, from leaves collected in the field and conserved freshly frozen (-18ºC) and chlorophyll *a, b, a+b* contents calculated and chlorophyll *a/b* ratio obtained (Chap. 3). Results are presented on a leaf area basis, which is the general reference for use in terrestrial biosphere models (Kattge *et al.*, 2009).

1.4. Overview of the thesis

The overall aim of this thesis is to use the results of the Caxiuanã through-fall exclusion experiment to improve our understanding of the impact of induced water deficits, by studying the factors that control leaf gas exchange in different tree species of an Amazonian rain forest and by exploring the variability among seasons and years.

Additionally, we believe that our data will contribute to reduce the level of uncertainty associated with the models currently used to predict climate change scenarios.

The thesis is designed as a series of five stand-alone chapters which have been prepared for publication as scientific papers.

1.4.1. Chapter 2. Effects of artificial drought on foliar carbon assimilation in an eastern Amazonian rainforest

The first paper is an analysis of gas-exchange data and nutrient concentrations used to support data interpretation. In this paper we investigate the responses of this tropical rain forest to the predicted drought in terms of carbon assimilation and the mechanisms behind these responses (diffusional and metabolic limitations to C assimilation). We also test the hypothesis that low rates of soil nutrient availability, especially N and P, limit the photosynthetic capacity of this Amazonian tropical rain forest. And, at the end, we examine how the different functional groups (defined according their positions along the canopy) acclimate or respond to a predicted rainfall reduction.

Key science questions

1. Do tropical rain forest trees respond to drought by reducing photosynthetic capacity?
2. Is undroughted photosynthetic capacity related to leaf nutrient levels?
3. Do trees in different functional groups have systematically different photosynthetic capacity and/or responses to drought?

We conclude that these tropical rain forest trees responded to decreased water availability mainly through stomatal closure and exhibited resilience in response to drought. At the end of the study period, after two years of drought imposition, we observed a recovery in carbon assimilation, independently of a continued decrease in leaf water potential. Additionally, trees from TFE plot showed decreased specific leaf area, compared to Control plot trees. This work pointed to an acclimation to drought with respect to leaf carbon assimilation, as well as a differential response to drought

throughout the vertical profile of the canopy. Our results also suggest that the photosynthetic potential of this forest might be limited by phosphorus availability.

1.4.2. Chapter 3. Leaf optical responses to light and drought in tropical rain forest trees

In this second paper, we use leaf optical properties and chlorophyll concentrations to describe leaf optical properties along the vertical canopy profile and to investigate leaf optical responses to decreased water availability. And, because leaf optical properties studies are very scarce in tropical rain forests, in this paper we provide field data for modelling and remote sensing techniques validation.

Key science questions:

1. Do leaf optical properties and chlorophyll concentrations change in response to decreased water availability?
2. Do leaf optical properties and chlorophyll concentrations differ throughout the vertical profile of the canopy?
3. Do leaf structural changes occur as an acclimation strategy to drought?
4. Are reflectance based indices good indicators of drought stress in tropical rain forests?

In the present paper we showed that leaf optical properties, as well as chlorophyll concentrations, change in response to decreased water availability and that those responses differ along the vertical canopy position. Our results indicate that leaf structural changes may occur as an acclimation strategy to drought, although more data is needed to confirm these findings. Finally, the significant correlations found with reflectance based indices indicate that measurements of leaf optical properties might be useful in practical application for estimation of the drought tolerance level.

1.4.3. Chapter 4. Impacts of experimentally imposed drought on leaf respiration and morphology in an Amazon rain forest

In the third paper we present measurements of leaf dark respiration and leaf morphology from different canopy heights over 5 years to assess the sensitivity to drought of leaf dark respiration, specific leaf area, leaf area index and foliar biomass per unit ground area. We used a "before-after-control-impact" approach to test for significant shifts in leaf dark respiration and specific leaf area both, over time - before and after the imposition of TFE treatment - and between dry and wet seasons; and between TFE treatment and Control for each individual measurement campaign. Finally, we use existing leaf area index data to upscale leaf dark respiration measurements to derive plot estimates of foliar night-time carbon effluxes.

Key science questions

1. Do leaf dark respiration and specific leaf area change in response to decreased water availability?
2. Do trees from different canopy heights have systematically different leaf dark respiration and specific leaf area and/or responses to drought?
3. What are the foliar night-time carbon effluxes?

This study evaluated the drought sensitivity of leaf dark respiration (R_d) at Caxiuanã. Partial rainfall exclusion of a 1-ha area of rain forest was associated with an estimated increase in night-time foliar C emissions of 1.4, 0.7 and 1.8 t ha^{-1} $year^{-1}$ compared to forest on a nearby Control plot 1.4, 1.9 and 5.1 years after rainfall exclusion respectively. This drought-induced physiological shift, if shown to occur more widely, might be sufficient to offset current estimates of the Amazon forest C sink, and alter model predictions of future changes in net C emissions from the Amazon basin. To build upon the key conclusions of this study more measurements are required to improve our understanding of the spatial and temporal variation in R_d, and of leaf respiration under light conditions.

1.4.4. Chapter 5. Evidence from Amazonian forests is consistent with isohydric control of leaf water potential.

The forth paper is an analysis of diurnal tree physiological data. In this paper we test the hypothesis that stomatal conductance (the main regulator of tree water use) is responsive to leaf water potential, so that, when leaf water potential reaches a critical minimum, stomata shut to avoid further water loss. This mechanism creates constant water potentials in the leaves under hydraulically stressed conditions, and therefore is referred to as the "isohydric hypothesis". We use a simulation model of the soil-plant-atmosphere continuum (the SPA model, Williams *et al.*, 1996) to represent the predictions of the isohydric hypothesis, and compare the predictions to diurnal cycles of leaf water potential, stomatal conductance, sap flow and stem water potential.

Key science questions

1. Are the leaf water potential, sap flow and stomatal conductance data consistent with the hypothesis that stomata function to maintain isohydric conditions within the plant under water stressed circumstances?
2. Are changes in soil-to-leaf water supply dominated by changes in soil water potential or soil-to-leaf water hydraulic resistance?
3. If there is a major change in soil-to-leaf hydraulic resistance between seasons, is the change in resistance located above or below ground?

We conclude that leaf water potential does appear to exert control over stomatal conductance on account of the existence of marked plateau leaf water potential throughout the day in the majority of trees studied. We found that soil-to-leaf hydraulic resistance increased by a factor of 7.6 between wet and dry seasons, while the change in soil-to-leaf water potential gradient increased by a factor of 2.2. The changes in tree water use simulated from these data were consistent with tree water use, leaf water potential and stomatal conductance data, and therefore we conclude that changes in soil-to-leaf hydraulic resistance are the main factor responsible for the changes in tree hydraulics between seasons. Measurements of stem water potential showed that the main increase in hydraulic resistance between seasons was located below ground.

1.4.5. Chapter 6. Shifts in plant respiration and carbon use efficiency at a large-scale drought experiment in the eastern Amazon

The overall purpose of the last paper was to examine the impacts of a large-scale through-fall exclusion (TFE) treatment in an eastern Amazon primary rainforest on ecosystem C cycling and partitioning. The analysis was centered on measurements made across one full seasonal cycle, 4 yr after imposition of the TFE treatment, in 2005, comparing data from the TFE and a nearby control plot. On both plots, for the focal period of 2005, we estimated and integrated all key ecosystem C fluxes to measure forest net primary productivity (NPP) and ecosystem respiration (R_{eco}). At the end, we examined the implications for the net change in tree C balance.

Key science questions

1. Will drought drive shifts in plant respiration and carbon use efficiency?

Drought caused by the TFE treatment appeared to drive fundamental shifts in ecosystem C cycling with potentially important consequences for long-term forest C storage. This study provides some of the first insights into ecosystem-level shifts in Amazon forest C metabolism associated with drought, which, although constrained by numerous uncertainties, provide a foundation for future modeling and experimental work testing questions and patterns arising from the data presented.

1.5. Publication status of thesis contents

Chapter 1 will be submitted to New Phytologist

Chapter 2 will be submitted, no defined journal yet

Chapter 3 published at Functional Ecology 2010

Chapter 4 published at Plant, Cell and Environment 2006

Chapter 5 published at New Phytologist 2010

1.6. References

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Chapter 2

Effects of artificial drought on foliar carbon assimilation in an eastern Amazonian rainforest

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Summary

Tropical rain forests are amongst the most important and still least monitored terrestrial ecosystems in terms of biodiversity and its role as sinks of atmospheric CO_2. Drought, associated with El Niño-like events, affects the global carbon cycle and is predicted to increase in the future, particularly in the eastern Amazon.

A 'through fall exclusion' experiment (TFE) was conducted to promote drought stress in an Amazonian rain forest plot to investigate the forest responses to 50% through fall exclusion from the soil. Gas exchange measurements were performed in a Control and a TFE plots for five consecutive seasons (dry/wet).

Inter-annual and imposed seasonal variations were observed in maximal rates of photosynthesis (A_{max}) as well as maximal stomatal conductance (g_{smax}), which were reduced in the TFE plot particularly in the dry seasons. No changes over time were observed in maximum Rubisco CO_2 fixation capacity (V_{cmax}) and maximum electron transport rate (J_{max}) parameters suggesting that the main limitation to carbon assimilation under reduced water availability was stomatal. Trees of different functional groups showed differential responses to drought.

The low values of A_{max} observed in all studied trees indicate that phosphorus may be limiting the photosynthetic potential of this forest.

Introduction

Tropical rain forests are amongst the most important and still least monitored terrestrial ecosystems in terms of biodiversity and its role as sinks of atmospheric CO_2. Several modelling studies showed that the fluxes of carbon and water from tropical rainforests may exhibit strong seasonal and inter-annual variation (Malhi *et al.*, 1998; Cox *et al.*, 2000), which can affect the global carbon cycle. Such variations are correlated with El Niño-like drought events (Malhi & Wright, 2004) and are predicted to intensify due to global climate change (Cox *et al.*, 2000; IPCC, 2007; Malhi *et al.*, 2008). Although there is no consistent trend in predicted annual precipitation, most climate models tend to show a reduction in the dry season rainfall, particularly in eastern Amazonia (Malhi et al. 2008). Under these conditions (drought periods), tropical rain forests may become a weak sink or even a source of CO_2 (Zeng *et al.*, 2005; Gullison *et al.*, 2007; Phillips *et al.*, 2009), thus reducing the global terrestrial carbon sink (Berthelot *et al.*, 2005). However, a high level of uncertainty associated with the model predictions is frequently acknowledged (Clark, 2004; Berthelot *et al.*, 2005; Huntingford *et al.*, 2008; Grant *et al.*, 2009; Malhi *et al.*, 2009; Galbraith *et al.*, 2010; Poulter *et al.*, 2010), mainly because the environmental factors that control tropical forest carbon balance and productivity are still largely unresolved (Baker *et al.*, 2003; Grant *et al.*, 2009; Galbraith *et al.*, 2010). It is also not clear how rainforest plant species might adapt to global climate changes, including increasing intensity and frequency of extreme meteorological events (Schurr *et al.*, 2006; Nepstad *et al.*, 2007; Borken & Matzner, 2009). This emphasises the need for gathering more ecophysiological field data to improve the models that simulate the forest response to drought (Clark, 2004; Meir *et al.*, 2006; Huntingford *et al.*, 2008; Meir *et al.*, 2008; Asner & Alencar, 2010; Galbraith *et al.*, 2010; Wu *et al.*, 2011).

At the leaf level, it is well known that stomata impose a large limitation on the rate of CO_2 assimilation and that this limitation is more severe when plants are water stressed (Farquhar & Sharkey, 1982; Chaves, 1991). Biochemical constraints may also take place under more prolonged drought conditions (Tezara *et al.*, 1999; Lawlor & Cornic, 2002; Lawlor & Tezara, 2009) but they are not so relevant until drought is severe (Chaves *et al.*, 2003; Bota *et al.*, 2004; Chaves *et al.*, 2009). At whole-plant level, reductions in growth are expected at early stages of water stress (Chaves, 1991). At a

stand level, reductions in leaf area index, and consequently of litter fall, and increased tree mortality are predicted and have been reported for this type of forests in response to drought (Lewis *et al.*, 2004; Fisher *et al.*, 2007; Nepstad *et al.*, 2007; Meir *et al.*, 2008; da Costa *et al.*, 2010).

Tropical forest soils are known to be nutrient-poor (Vitousek & Sanford, 1986; Marschner, 1995) containing very low amounts of available P, particularly in Amazonia (Vitousek & Sanford, 1986; Davidson *et al.*, 2007). Furthermore, soil drying may increase the phosphorus deficiency due to its poor mobility (Passioura, 2002). For this reason, the importance of phosphorus for carbon assimilation and growth in tropical forests is receiving increased attention (Raaimakers *et al.*, 1995; Mackensen *et al.*, 2000; Meir *et al.*, 2002; Meir *et al.*, 2007). Nevertheless, while carbon assimilation *vs.* nitrogen relationships have been extensively studied (Evans, 1989; Reich *et al.*, 1994; Carswell *et al.*, 2000; Domingues *et al.*, 2005; Posada *et al.*, 2009), there are still only few studies considering carbon assimilation *vs.* phosphorus relationships in tropical rain forests (Raaimakers *et al.*, 1995; Meir *et al.*, 2002; Meir *et al.*, 2007). Potassium is considered to play an important role in water relations of plants (Behboudian & Anderson, 1990), mainly due to its importance in stomatal control and osmoregulation (Ericsson & Kähr, 1993; Santiago & Wright, 2007) and consequently on carbon assimilation. However, as regarded to carbon assimilation studies in tropical forests, potassium is rarely referred (Santiago & Wright, 2007).

Functional groups were often considered regarding successional status (Raaimakers *et al.*, 1995; Reich *et al.*, 1995; Thomas & Bazzaz, 1999; Nogueira *et al.*, 2004; Mielke *et al.*, 2005; Meir *et al.*, 2007) or shade tolerance (Thompson *et al.*, 1992; Bonal *et al.*, 2000b; Rozendaal *et al.*, 2006; Poorter, 2009) and they proved to be important in the drought stress acclimation strategies (Huc *et al.*, 1994; Thomas & Bazzaz, 1999; Bonal *et al.*, 2000b; Baker *et al.*, 2003; Nogueira *et al.*, 2004). However, some uncertainty has been identified regarding this division (Poorter, 2009). Additionally, it was shown that there are differences in gas-exchange according to the canopy height (McWilliam *et al.*, 1996; Carswell *et al.*, 2000; Domingues *et al.*, 2007).

Most of the gas-exchange studies in tropical trees that are found in the literature have used seedlings (Huc *et al.*, 1994; Bonal & Guehl, 2001; Coste *et al.*, 2005) or were not focused in season (dry or wet) comparisons (Huc *et al.*, 1994; Carswell *et al.*, 2000;

Coste *et al.*, 2005; Domingues *et al.*, 2007). Only in a few sites, drought has been experimentally imposed (Nepstad *et al.*, 2002; Fisher *et al.*, 2006). In one of these field studies also with through-fall exclusion, Nepstad *et al.* (2002) observed that photosynthetic capacity and growth declined in some species but no reduction in predawn leaf water potential was detected. Even so, the authors concluded that the net accumulation of carbon in mature Amazon forests could be very sensitive to small reductions in rainfall.

Studies in tropical rain forests focusing on the effects of drought in carbon assimilation are scarce (McWilliam *et al.*, 1996; Ishida *et al.*, 1999; Bonal *et al.*, 2000a; Cao, 2000) and until the present date, and as far as we know, there have been no studies that report photosynthetic capacity and drought stress throughout the vertical canopy profile, as we report here.

Our experiment was part of the Large Scale Biosphere Atmosphere Experiment in Amazon (LBA). To investigate the mechanisms underlying the response of a tropical rain forest to reduced rainfall, a manipulation study was conducted on a 1 ha of land in Caxiuanã's, drought threatened eastern Amazonia.

The following key questions are addressed in this paper:

1. Tropical rain forest trees respond to drought by reducing photosynthetic capacity?

2. Is undroughted photosynthetic capacity related to leaf nutrient levels?

3. Trees in different functional groups have systematically different photosynthetic capacity and/or responses to drought?

We seek to answer these questions by measuring gas exchange and determining leaf nutrient concentrations over 5 consecutive seasons.

Materials and Methods

Site, species and sampling

The experimental site is located at Caxiuanã National Forest Reserve, Pará, Brazil (1º43'3.5''S, 51º27'36''W), an undisturbed and protected rain forest, representative of the type of lowland *terra firme* forest. The climate in Caxiuanã is characterized by a pronounced dry season between July and December and a wet season in the remaining months. Mean annual rainfall is 2272±193 mm, (with 555±116 mm rainfall recorded from 1999 to 2003 for the dry season) (Fisher *et al.*, 2006). The daily mean temperature of 25 ºC is almost constant over the year (diurnal variation is typically less than 3º C).

The soil of Caxiuanã is largely a yellow oxisol (Brazilian classification latosol), mostly well drained and nutrient-poor, as is typical for Amazonian lowlands (Ruivo *et al.*, 2002) and has a relatively high plant available water content of 0.200±0.032 m^3 m^{-3} (Fisher *et al.*, 2008). The site elevation is 15 m above river level in the dry season, and the water table has been observed at a depth of 10 m during the wet season (Fisher *et al.*, 2006).

An artificial soil drought was created by using through-fall exclusion (TFE) to investigate the limitation of soil water on forest gas exchange in drier conditions than those normally experienced. Two plots of 1ha were established, a Control and a treatment TFE plot, and the borders trenched to a depth of 1 m to reduce the lateral flow of water. In the TFE plot, a roof of transparent plastic sheeting and wooden guttering was installed at approximately 2 m height in January 2002, to keep the soil free from rainfall. So, rain passing through the canopy (through-fall) was intercepted by a system of plastic panels and then drained away from the plot, thereby artificially reducing soil moisture.

A 30m tall canopy access tower was installed in each plot. Nine trees and eight trees were accessible from tower in the Control and TFE plots, respectively, being 17 trees in total, corresponding to 16 species. Species were grouped according to their position in the canopy: understory [0,10m[, mid-canopy [10,20m[and top-canopy [20,30m]. It should be outlined that in the TFE plot no understory trees were available from the tower. Details of the distribution of the species throughout the vertical profile of the canopy are given in Table 1. Maximum canopy height was approximately 32 m.

Table 1. Species list of all trees studied in Control and TFE plots throughout the vertical profile of the canopy, with the functional group and systematic position.

Plot	Functional group	Family name	Species name	Height in the canopy (m)
Control	**Understory**	Quiinaceae	*Quiina florida*	2
		Sapotaceae	*Pouteria lateriflora*	2
		Burseraceae	*Protium heptaphyllum*	4
	Mid Canopy	Quiinaceae	*Quiina florida*	10
		Annonaceae	*Duguetia echinophora*	18
		Flacourtiaceae	*Hasseltia floribunda*	10
		Lauraceae	*Mezilaurus mahuba*	10
		Chrysobalanaceae	*Licania heteromorpha*	18
	Top Canopy	Sapotaceae	*Manilkara bidentata*	30
TFE	**Mid Canopy**	Annonaceae	*Duguetia echinophora*	14
		Chrysobalanaceae	*Licania canescens*	12
		Lauraceae	*Licaria armeniaca*	18
		Melastomataceae	*Mouriri duckeana*	16
	Top Canopy	Chrysobalanaceae	*Hirtela bicornis*	22
		Lecythidaceae	*Lecythis confertiflora*	28
		Sapotaceae	*Manilkara paraensis*	30
		Caesalpiniaceae	*Swartzia racemosa*	30

All measurements were made on fully expanded leaves without signs of senescence, throughout the vertical profile of the canopy, with a minimum of three leaf replicates. Data were recorded over a period of 2 years, from November 2001 to November 2003, in five measurement dates, at the end of the dry season (Nov 2001, Nov 2002 and Nov 2003) and at the end of the wet season (May 2002 and May 2003). November 2001 was the end of a long and severe dry season and was the pre-treatment measurement date. This first measurement date also served to adjust and control the methodologies for the next measurement dates.

Mean light environment

The light environment, measured as the average photosynthetic photon flux density (*PPFD*, μmol m^{-2} s^{-1}) at multiple heights throughout the canopy was determined using a LI-1800 spectroradiometer (LI-COR Inc., Lincoln, NE, USA). Measurements were performed in November 2001, November 2002, May 2003 and November 2003.

Gas exchange measurements

Leaf gas exchange measurements were performed with two cross-calibrated portable photosynthesis systems with a light source and a CO_2 injector system for controlled CO_2 concentrations (LI-6400, LI-COR Inc, Lincoln, NB, USA). Response curves of net CO_2 assimilation to photosynthetic photon flux density (*A*/*PPFD*) were obtained by increasing the *PPFD* (0, 25, 50, 100, 200, 400, 800, 1500 and 2000 μmol photons m^{-2} s^{-1}) using the light source in the sensor head of the LI-6400, at controlled CO_2 concentrations (360 μmol mol^{-1}). Gas-exchange from the fully expanded leaves was allowed to equilibrate at each *PPFD* before data was recorded. The obtained curves were fitted to a nonrectangular hyperbola according to Lambers *et al.* (1998), in order to determine the maximal rates of photosynthesis (A_{max}), as well as maximal stomatal conductance (g_{smax}). Net CO_2 assimilation *vs*. intercellular CO_2 concentration (A/C_i) response curves were obtained by increasing sequentially atmospheric CO_2 concentrations in the leaf cuvette (150, 250, 350, 500, 700, 1000, 1400, 2000 μmol mol^{-1}) at leaf temperature of 29.7±0.1º C and *PPFD* of 1000 μmol m^{-2} s^{-1} (which was found to be saturating for photosynthesis from the *A*/*PPFD* curves). The biochemical model of Farquhar *et al.* (1980) with modifications by Sharkey (1985) was fitted to A/C_i response curves using the NON-LIN procedure from SAS 9.1 (SAS Institute, Cary, NC, USA) in order to estimate the maximum Rubisco CO_2 fixation capacity (V_{cmax}) and maximum electron transport rate (J_{max}) as described in Maroco *et al.* (2002). Relative humidity in the chamber was manually controlled and kept close to ambient (73.3±0.2 %). Leaf temperature was recorded with a thermocouple sensor and the estimated parameters from *A*/*Ci* response curves were later corrected to a leaf temperature of 25ºC (based on temperature functions reported by Sharkey *et al* (2007) and Lloyd *et al.*,(1995)). The response curves were made between 0800 and 1400 h local time to avoid stomatal closure that might occur thereafter.

From the *A*/*PPFD* response curves, intrinsic water use efficiency (*IWUE*) was determined by the ratio of A_{max}/g_{smax}. From A/C_i response curves, the ratio between intercellular CO_2 concentration and atmospheric CO_2 concentration (C_i/C_a) was calculated.

Leaf water potential

Leaf water potential was measured to evaluate leaf water status. Predawn (Ψ_{Pd}) and midday (Ψ_{Md}) leaf water potentials were measured with a Scholander-type pressure chamber (Skye Instruments, Llandrindod Wells, UK) in three fully expanded leaves per tree similar to those used for gas exchange measurements. Measurement times were 0600 h (predawn) and 1200-1300 h (midday). For Ψ_{Pd}, leaves were covered with aluminium foil the night before excision.

Specific leaf area and nutrients

Three samples of three leaf discs (0.8 cm diameter) were taken from each studied tree and oven-dried at 80º C for specific leaf area (*SLA*) and nutrient determinations (nitrogen (N), phosphorus (P) and potassium (K)). *SLA* was calculated as the ratio of leaf area to dry weight. Samples were then gridded and an extract, obtained by sulphur digestion, was prepared to determine the total leaf nitrogen, phosphorus and potassium concentrations by colorimetric (for N and P) and flame photometry (for K) methods (Murphy & Riley, 1962; Salinas & Garcia, 1985; Mulvaney, 1996; EMBRAPA, 1999). Results of nutrients are presented on a leaf area basis, which is the general reference for use in terrestrial biosphere models (Kattge *et al.*, 2009).

Data analysis

For the analysis of the light profile inside the canopy, data was pooled by canopy height and a two-way ANOVA was used to distinguish between differences due to canopy level and treatment/plot effect.

To assess the impact of the TFE treatment on the parameters measured, two analyses were performed. First, we used a Repeated Measures Analysis of Variance (ANOVA-RM) as, in the five measurement dates, the same trees at the same canopy heights were repeatedly sampled. We examined the differences in measurement dates and treatment effect to see if there was an inter-annual variation (within-plot change over time) and if the treatment effect was the cause of such a variation (between-plot differences). Data

was then grouped by canopy position and season and analysed separately for each plot with a two-way ANOVA. When statistically significant differences were found, differences between group means were identified by post-hoc Tukey HSD tests. A principal component analysis (PCA) was performed to visualize variables association in an integrated picture and to check the relationships between the studied variables. Statistical analyses were carried out with SAS 9.1 (SAS Institute, Cary, NC, USA). Data were transformed with a natural logarithm when necessary, to meet the assumptions of parametric analyses, namely normal distribution of data and homogeneity of variances. For ANOVA-RM, the sphericity assumption was also checked with the Mauchly test. All statistical relationships were considered significant at $p<0.05$.

Results

Meteorology and mean light environment

November 2001 was the end of a long and severe dry season (Fig. 1). If we consider the amount of rain fall received by the plots prior to the first measurement date (between July and November 2001), the dry season of 2001 had the same amount of rain fall than the excluded through-fall on TFE plot in the next two dry seasons (~180 mm).

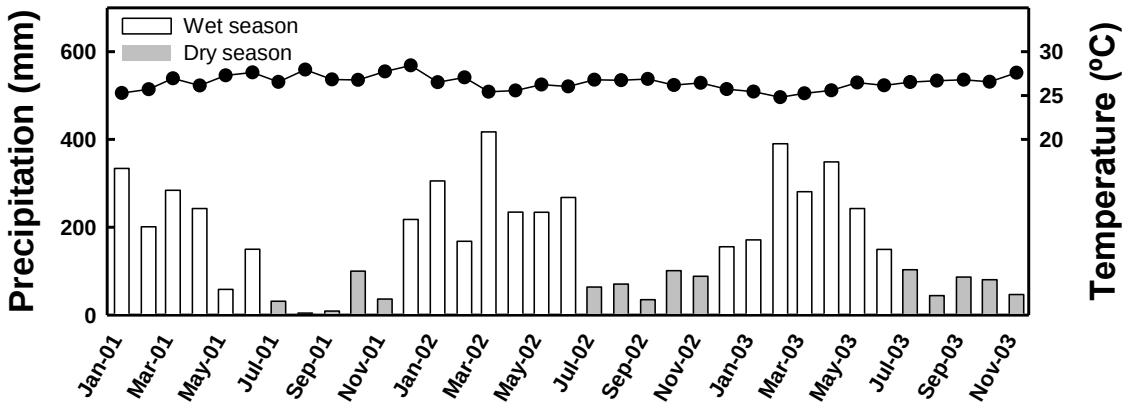

Figure 1. Precipitation (mm) and temperature (ºC) from 2001 to 2003. Grey bars represent the precipitation occurred during the dry seasons, prior to the measurement dates. In the dry season of 2001 the amount of rain was roughly half of the next two years.

The vertical profile of mean PPFD showed an exponential decrease with increasing depth into the canopy (Fig. 2). Light profile was similar in both plots, where no statistically significant effect was found for plot ($F_{(1,48)}$=1.39, p=0.244). However, PPFD was significantly reduced inside the canopy ($F_{(7,43)}$=68.36; p<0.001), reflecting statistically significant differences in light availability between the functional groups (p<0.001).

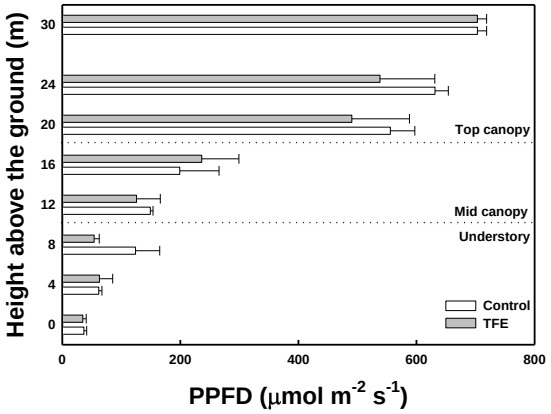

Figure 2. Light profile inside the canopy. Mean photosynthetic photon flux density (PPFD) throughout the vertical profile of the canopy in each plot. Values are mean ± SE of measurement dates. The irradiances corresponding to the functional groups (understory, mid canopy and top canopy) are separated by the horizontal dotted lines.

Inter-annual variation, seasonality and treatment effect

The pre-treatment measurements, in November 2001, showed very close similarity between Control and TFE plots (Fig. 3).

Predawn leaf water potential (Ψ_{Pd}, Fig. 3a) was reduced by the end of the first dry season of November 2001, recovering in the next wet season of May 2002, and steadily declined until the end of the study period. The overall mean of Ψ_{Pd} was similar in Nov 2001 and in Nov 2003 and significantly lower than the other measurement dates. However, the observed decline was much steeper in the TFE plot, leading to statistically significant differences between plots in the dry seasons after drought imposition (due to a statistically significant interaction in measurement date x plot, Table 2). At the end of the study period, Ψ_{Pd} was -0.44±0.02 and -0.77±0.08 MPa for Control and TFE plot, respectively. Overall, Ψ_{Md} changed significantly over time (Table 2), exhibiting a seasonal variation, with higher values in the wet seasons and lower values in the dry

seasons (Fig. 3b). The strong recovery observed in the Control plot in the 2nd wet season (May 2003), contrasted with a modest one in the TFE plot, resulting in decreasing values of Ψ_{Md}. At the end of the study period, Ψ_{Md} was -1.41±0.13 and -2.12±0.19 MPa for Control and TFE plot, respectively. Leaf water potentials, both Ψ_{Pd} and Ψ_{Md}, were always higher, on average, in the Control than in the TFE plot.

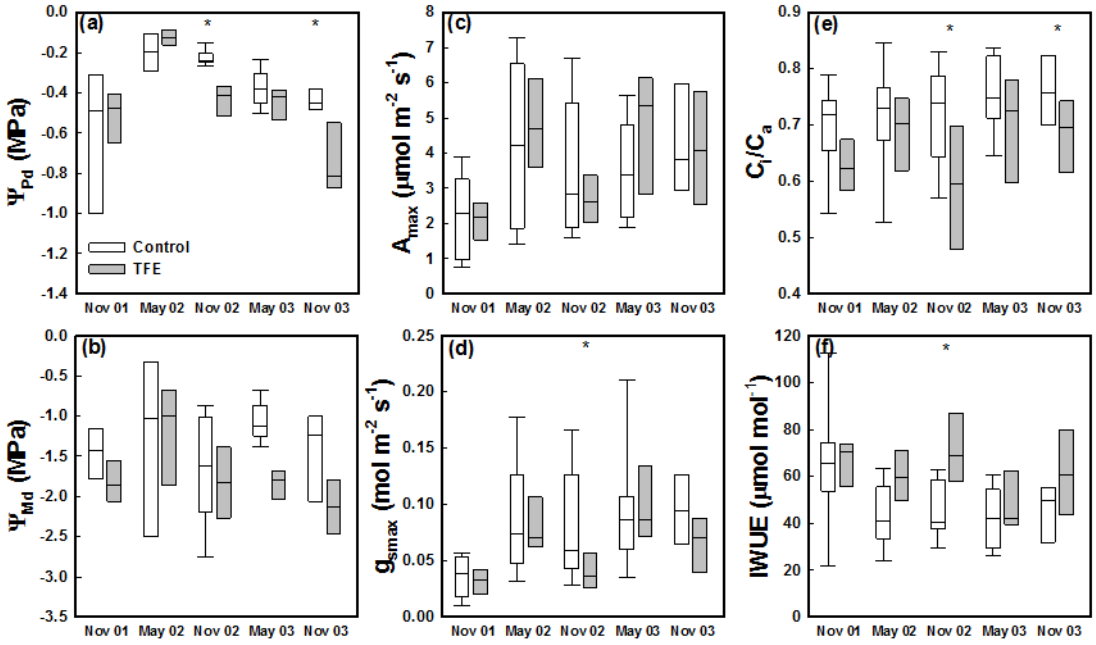

Figure 3. Box plots of (a) predawn leaf water potential (Ψ_{PD}), (b) midday leaf water potential (Ψ_{MD}), (c) maximal rates of photosynthesis (A_{max}), (d) maximal stomatal conductance (g_{smax}), (e) ratio of internal CO_2 concentration to ambient CO_2 concentration (C_i/C_a) and (f) intrinsic water use efficiency (*IWUE*, A_{max} / g_{smax}) for the different plots and measurement dates. The middle line in each box indicates the median of the observed distribution, the bottom and top parts the 25th and 75th percentiles, and the bottom and top "error bars" the 5th and 95th percentiles. Asteriscs indicate statistically significant difference between plots for a given measurement date (Tukey test after ANOVA-RM, $p < 0.05$)

Overall, there was a significant change over time in A_{max} and g_{smax} (Table 2) indicating a seasonal variation from November 2001, the end of the first dry season (the pre-treatment measurement date), to May 2003. A_{max} and g_{smax} were strongly inhibited in November 2001 (Fig. 3c,d) followed by a recovery in the next wet season of May 2002, and moderately inhibited in November 2002, the 2nd dry season. These two dry seasons

were significantly different from each other and significantly lower than the other measurement dates. In November 2003, the values of these parameters increased substantially, compared with the previous dry seasons. However, because in the Control plot, after November 2001, the values of these parameters remained similar, the seasonality observed was essentially determined by the variation observed in the TFE plot, where marked differences, mainly in g_{smax}, were observed between wet and dry seasons, leading to statistically significant differences between plots (due to a statistically significant interaction between measurement date and plot) in the 2nd dry season of November 2002 (Table 2). A_{max} ranged from 2.18±0.39 to 4.26±0.74 and from 2.06±0.21 to 4.91±0.52 μmol m^{-2} s^{-1}, for Control and TFE plot, respectively. g_{smax} ranged from 0.036±0.006 to 0.096±0.015 and from 0.031±0.004 to 0.094±0.013 mol m^{-2} s^{-1}, for the Control and TFE plot, respectively. At the end of the study period, A_{max} was 4.17±0.65 and 4.08±0.63 μmol m^{-2} s^{-1} for Control and TFE plot, respectively and gs_{max} was 0.096±0.015 and 0.069±0.009 mol m^{-2} s^{-1}.

Table 2. Summary of the results of repeated measures analyses of variance.

		P-values		
Variable	**n**	**Date**	**Plot**	**Date x Plot**
Ψ_{Pd}	81	<0.001	n.s.	<0.001
Ψ_{Md}	81	0.034	n.s.	n.s.
A_{max}	83	<0.001	n.s.	n.s.
g_{smax}	83	<0.001	n.s.	0.047
C_i/C_a	83	0.002	0.045	n.s.
IWUE	83	<0.001	0.008	n.s.
V_{cmax}	82	n.s.	n.s.	n.s.
J_{max}	82	n.s.	n.s.	n.s.
SLA	81	<0.001	n.s.	n.s.
N	81	<0.001	n.s.	<0.001
P	81	<0.001	n.s.	0.001
K	81	<0.002	n.s.	n.s.

n.s., not significant

The C_i/C_a ratio was affected by measurement date and plot (Table 2). C_i/C_a ratio remained stable in the Control plot and was always lower in the TFE plot, showing a marked seasonal variation in the latter, determining statistically significant differences

between treatments in the 2nd and 3rd dry seasons. C_i/C_a ratios were always higher than 0.7 in the Control plot and equal to or lower than 0.7 in the TFE plot. The *IWUE* was also affected by measurement date and plot (Table 2). As a consequence of lower variation in A_{max} than in g_{smax}, *IWUE* (Fig. 3f) was significantly lower in Control than in TFE plot, with higher intrinsic water use efficiencies being observed in the dry seasons.

The biochemical parameters (V_{cmax} and J_{max}) remained stable over time (Fig. 4) and no significant differences were observed between the Control plot and the TFE plot (Table 2).

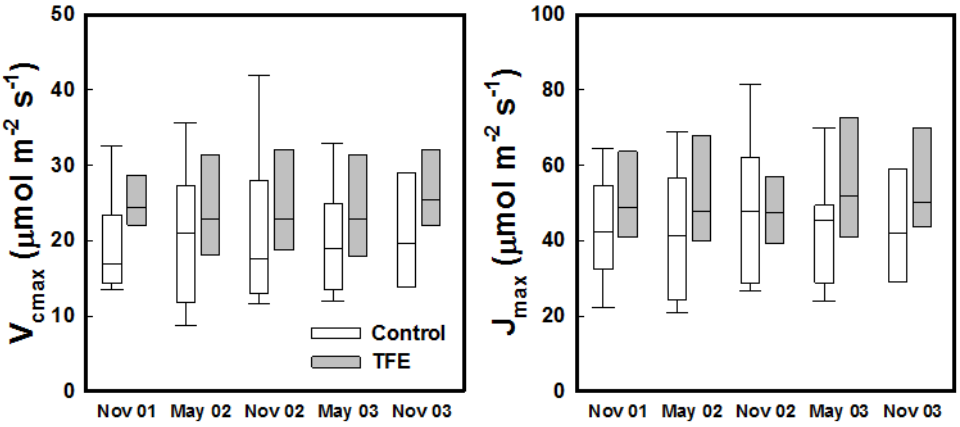

Figure 4. Box plots of (a) maximum Rubisco CO_2 fixation capacity (V_{cmax}) and (b) maximum electron transport rate (J_{max}), for the different plots and measurement dates. The middle line in each box indicates the median of the observed distribution, the bottom and top parts the 25th and 75th percentiles, and the bottom and top "error bars" the 5th and 95th percentiles.

SLA changed significantly over time (Table 2), with a decreasing trend from November 2001 to May 2003 (Table 3), this decrease being higher in the TFE plot. The dry season of November 2003 inverted the trend, where an increase in SLA was observed in both plots.

Considering foliar nutrient concentrations, a statistically significant effect of the measurement date on leaf N, P and K concentrations on an area basis was observed (Table 2) but the changes were not unidirectional over time (Table 3). A large variability was observed during the study period in leaf N concentration, which was in general lower in the Control plot that in the TFE plot, causing a significant plot effect

(due to a statistically significant interaction between measurement date and plot) in all measurement dates except May 2002. For the TFE plot there seems to be a seasonal variation in N content, with higher N concentrations in the dry seasons, except in November 2003. Overall, there was a significant seasonal variation from November 2001 to May 2003, with higher concentrations in the wet seasons of May 2002 and 2003. In November 2003, the values were similar in both plots. Leaf K concentration was generally similar between plots.

Table 3. Mean ± SE of specific leaf area (SLA), nitrogen (N), phosphorus (P) and potassium (K) concentrations measured during the different measurement dates.

		Nov 2001				May 2002				Nov 2002				May 2003				Nov 2003			
SLA ($cm^2\ g^{-1}$)	Control	127.69	±	10.67	ab	124.61	±	9.97	ab	118.02	±	8.94	b	112.20	±	10.9	b	150.44	±	17.8	a
	TFE	125.39	±	16.23	a	136.63	±	15.41	a	99.88	±	10.35	b	85.79	±	7.20	b	131.81	±	17.46	a
N ($g\ m^{-2}$)	Control	1.51	±	0.21	bB	1.05	±	0.11	cd	1.29	±	0.12	bcB	2.42	±	0.32	aA	0.84	±	0.13	dB
	TFE	2.65	±	0.49	aA	1.31	±	0.18	b	2.15	±	0.29	aA	1.49	±	0.23	bB	1.44	±	0.24	bA
P ($g\ m^{-2}$)	Control	0.04	±	0.01	c	0.06	±	0.01	ab	0.05	±	0.01	c	0.06	±	0.01	bB	0.07	±	0.01	a
	TFE	0.06	±	0.01	c	0.06	±	0.01	c	0.06	±	0.01	c	0.08	±	0.01	aA	0.07	±	0.01	b
K ($g\ m^{-2}$)	Control	0.13	±	0.03	b	0.15	±	0.02	b	0.21	±	0.03	a	0.20	±	0.07	ab	0.21	±	0.06	a
	TFE	0.15	±	0.02	b	0.13	±	0.02	b	0.26	±	0.07	a	0.20	±	0.03	ab	0.16	±	0.03	b

Different lower case letters represent statistically significant differences between measurement dates within the same plot (Control and TFE) and different capital letters represent statistically significant differences between treatments within the same measurement date.

Canopy position

Regarding the effects of drought over the functional groups, the analysis was done separately by plot and grouped by seasons (dry and wet) since, as observed in the previous analysis, the TFE treatment/plot showed seasonal variation on foliar C assimilation. The statistical analyses (Table 4) revealed some kind of complimentary responses with respect to season and functional groups effects in the studied plots.

Essentially, the impact of vertical structure on photosynthetic capacity was stronger under non-water limited conditions and when water was limiting, the functional group effect become obscured (Fig. 5).

An increase in the maximal photosynthetic rates (A_{max}) was observed with increasing canopy height (Fig. 5a). In other words, top canopy species tend to have higher maximal photosynthetic rates than mid canopy or understory species. However, when water was limiting, top canopy species suffer the most, as suggested by the strong inhibition in

carbon assimilation and stomatal conductance observed for the dry season in the TFE plot (Fig 5a,b). In fact, a reduction of about 60% in g_{smax} in the dry season as compared with the wet season was observed in top canopy species of the TFE plot, while in the Control plot was only 30%, a value close to the reduction observed for the Mid canopy species (about 26% and 36% for Control and TFE plots, respectively), and was higher than the observed reductions in A_{max} (less than a 20% decrease for all functional groups of Control plot and 34% and 43% less for Mid and Top canopy of TFE plot, respectively).

Table 4. Summary of the results of analyses of variance performed for each plot.

	CONTROL			TFE		
		P-values			P-values	
Variable	**n**	**Season**	**Functional group**	**n**	**Season**	**Functional group**
Ψ_{Pd}	42	n.s.	n.s.	39	<0.001	n.s.
Ψ_{Md}	42	n.s.	n.s.	39	0.019	n.s.
A_{max}	44	n.s.	0.044	39	<0.001	n.s.
g_{smax}	44	n.s.	n.s.	39	<0.001	n.s.
C_i/C_a	44	n.s.	<0.001	39	0.026	n.s.
IWUE	44	n.s.	n.s.	39	0.028	n.s.
V_{cmax}	44	n.s.	<0.001	39	n.s.	0.004
J_{max}	44	n.s.	<0.001	39	n.s.	0.007
SLA	42	n.s.	0.001	39	<0.001	0.027
N	42	n.s.	n.s.	39	0.026	n.s.
P	42	n.s.	<0.001	39	n.s.	0.003
K	42	n.s.	n.s.	39	n.s.	0.027

n.s., not significant

The photosynthetic capacity (V_{cmax} and J_{max}) increased with increasing canopy height (Fig. 5c,d) and remained stable between seasons, with the exception of a slight, although not significant, reduction in J_{max} in the dry season in Top canopy species.

SLA decreased with canopy height, with the lowest values belonging to the Top canopy species (Fig. 6a).

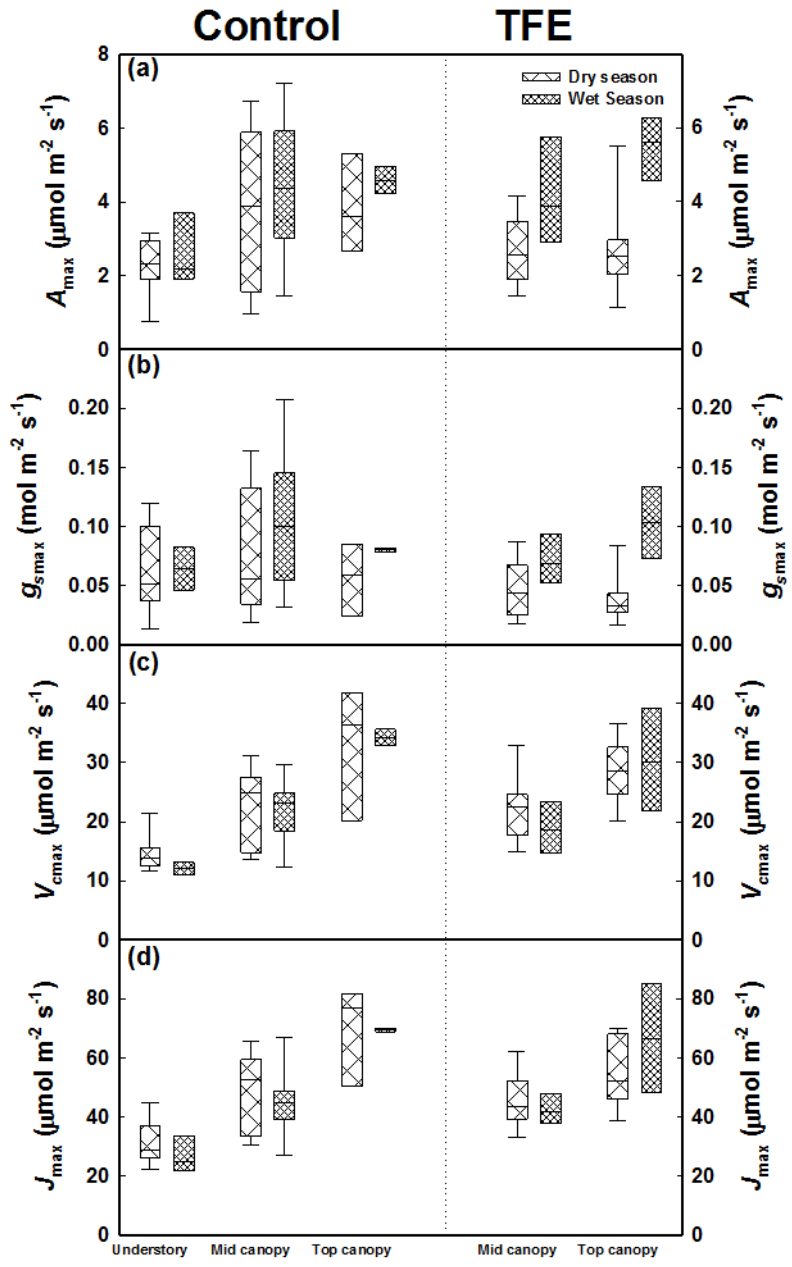

Figure 5. Box plots of (a) maximal rates of photosynthesis (A_{max}), (b) maximal stomatal conductance (g_{smax}), (c) the maximum Rubisco CO_2 fixation capacity (V_{cmax}) and (d) maximum electron transport rate (J_{max}), for the different functional groups and season. The middle line in each box indicates the median of the observed distribution, the bottom and top parts the 25th and 75th percentiles, and the bottom and top "error bars" the 5th and 95th percentiles.

For nutrient concentrations, variation between seasons was only observed in N content in the TFE plot, with higher concentrations in the dry season. There was a tendency for increasing leaf N concentration with canopy height, although not statistically significant (Fig. 6b). Leaf P concentration differed significantly between functional groups,

increasing with canopy height (Fig. 6c) independently of the plot considered. However, the K concentration was only significantly different from functional groups in the TFE plot (Fig. 6d).

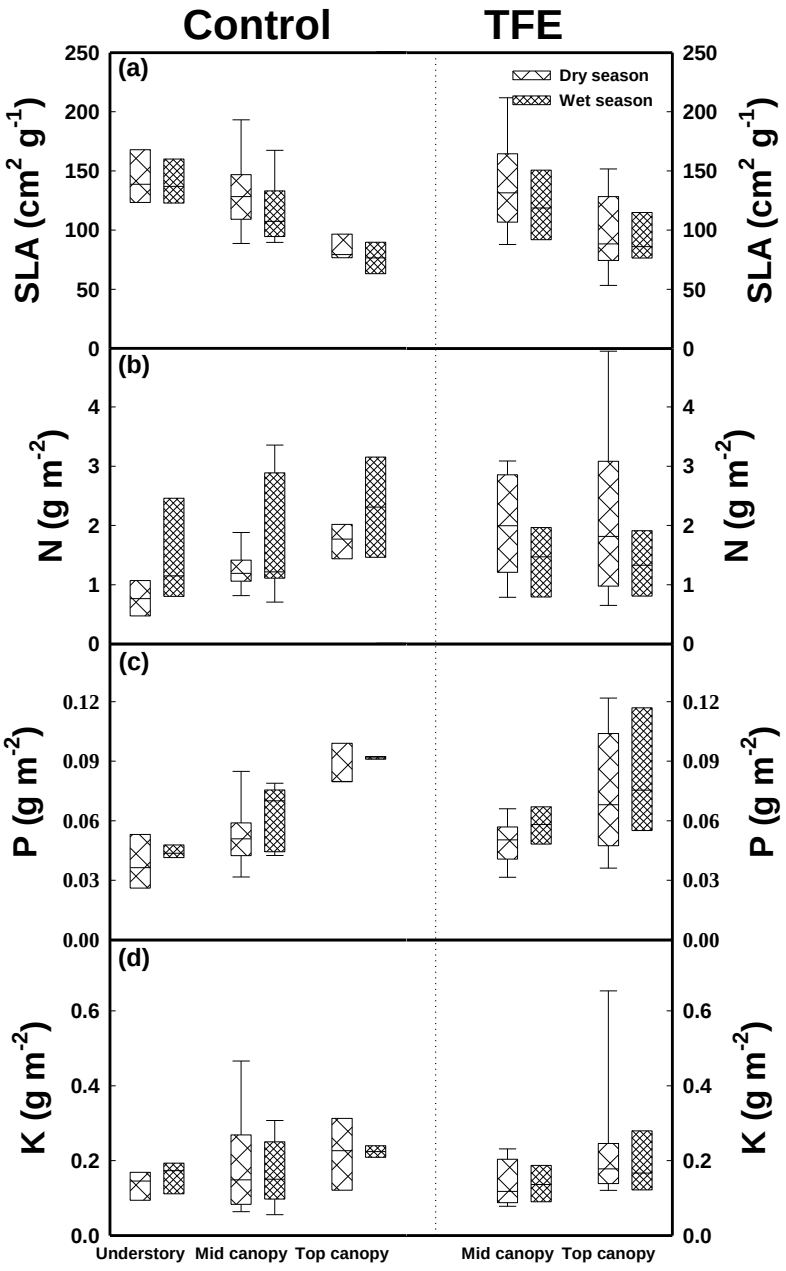

Figure 6. Box plots of (a) Specific leaf area (SLA), (b) nitrogen content (N), (c) phosphorus content (P) and (d) potassium content (K), for the different functional groups and season. The middle line in each box indicates the median of the observed distribution, the bottom and top parts the 25th and 75th percentiles, and the bottom and top "error bars" the 5th and 95th percentiles.

Relationships between variables

Principal component analysis (Fig. 7) was performed to all variables measured and showed that the first two axes explained 66% of the observed variability. The strongest correlations found were between the biochemical parameters themselves (V_{cmax} and J_{max}) and between the biochemical parameters and P concentration and, in a lesser extent, with N concentration. A_{max} was mainly related to g_{smax} and to K concentration and, in a lesser extent, to P or N concentrations. g_{smax} was closely related with Ψ_{leaf} and C_i/C_a ratio and C_i/C_a ratio was negatively correlated with *IWUE*. Amazingly, *IWUE* was not related with A_{max} because the axes are orthogonal.

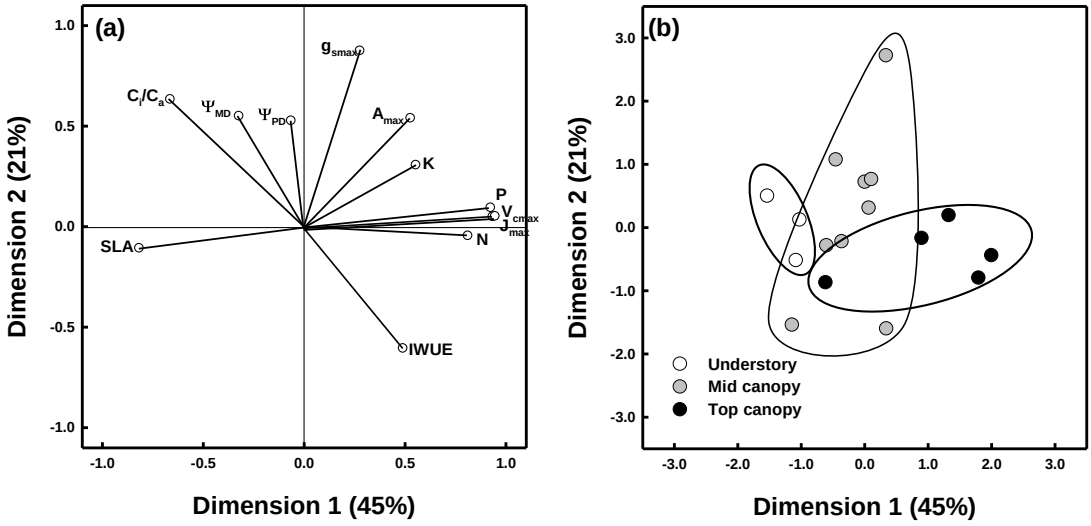

Figure 7. Principal components analysis of the studied trees, ordinated according the parameters measured: maximal rates of photosynthesis (A_{max}), maximal stomatal conductance (g_{smax}), maximum Rubisco CO_2 fixation capacity (V_{cmax}), maximum electron transport rate (J_{max}), specific leaf area (SLA), nitrogen concentration (N), phosphorus concentration (P), potassium concentration (K), intrinsic water use efficiency (*IWUE*), intercellular to ambient CO_2 concentration (C_i/C_a) ratio, predawn leaf water potential (Ψ_{Pd}) and midday leaf water potential (Ψ_{Md}). Projection of the variables on the factor planes (1x2) (a) and projection of the measured trees on the same plane (b) are shown. Circle envelopes point from species belonging to the three considered functional groups: Understory, Mid- and Top- canopy.

Regarding the functional groups, top canopy species are mostly linked with higher biochemical capacity, and N and P concentrations, whereas the mid canopy species seem to control better stomatal processes, which correlated more strongly with A_{max} and g_{smax}. Understory species were best characterized by leaf properties (*SLA*).

Discussion

The year of 2001 was much drier and warmer than the following two years (Fig. 1), explaining the strong inhibition on leaf carbon assimilation observed in November 2001 in both plots (Fig. 3c). This natural inhibition was not repeated in either plot until the end of the study period, reflecting a natural inter-annual variation in drought stress, as predicted of by some carbon exchange models for the Amazon (Cox *et al.*, 2000; Malhi *et al.*, 2008). It is in accordance with data on net ecosystem exchange from this site (Carswell *et al.*, 2002) and with gas exchange data elsewhere in the Amazon (McWilliam *et al.*, 1996; Domingues *et al.*, 2005). An imposed seasonal variation in carbon assimilation was observed in the TFE plot (Fig. 3c). In fact, we observed an inhibition of the maximal photosynthetic rates in the second dry season (November 2002) in the TFE plot that was accompanied by a reduction in $\Psi_{leaf,}$ g_{smax} and, consequently, C_i/C_a ratio (Fig. 3b-e). On the other hand, no treatment induced change was found for the biochemical parameters (V_{cmax} and J_{max}, Fig. 4), suggesting that the inhibition of carbon assimilation was mostly dictated by stomatal closure. This is consistent with observations that biochemical effects due to water deficits are hardly observed in adult trees with fully developed root systems (Chaves *et al.*, 2002; Chaves *et al.*, 2003; Bota *et al.*, 2004). In fact, roots have been detected at 10 m depth in Caxiuanã (Fisher *et al.*, 2008). The greatest reduction in carbon assimilation was found to be around 46%, for the top canopy species, not being severe enough to impact leaf biochemistry. We conclude that biochemical adaptation to water stress may also be present in tropical trees.

The C_i/C_a ratio was significantly lower in the TFE plot compared to the Control plot (Fig. 3e), indicating that reduced stomatal conductance led to a decrease in CO_2 availability near the chloroplast, which was enhanced by the maintenance of the photosynthetic capacity through the drought periods (Farquhar & Sharkey, 1982; Jones, 1985; Brodribb, 1996). g_{smax} was strongly correlated with A_{max} (Fig. 7). We therefore conclude that stomatal limitation was the main driver of the response to the observed drought stress. The continuous decrease in Ψ_{leaf} (both predawn and midday), in the TFE plot, from May 2002 to November 2003 without a concomitant decrease in g_{smax}, and consequently in A_{max}, may be interpreted as an acclimation to drought.

Comparing the gas exchange data from this study, where A_{max} ranged from 0.77 to 7.36 μmol m^{-2} s^{-1} and g_{smax} from 0.01 to 0.21 mol m^{-2} s^{-1}, with the literature, the values found here were generally lower than those reported for other mature Amazon trees (Domingues *et al.*, 2007; Meir *et al.*, 2007), saplings (Bonal *et al.*, 2000a) or seedlings (Huc *et al.*, 1994; Nogueira *et al.*, 2004). They are however in the same range of a study performed at the end of the dry season (Carswell *et al.*, 2000). The values found for V_{cmax} and J_{max} are in the same range of the ones reported in other studies (Carswell *et al.*, 2000; Coste *et al.*, 2005; Domingues *et al.*, 2007; Meir *et al.*, 2007) but, contrary to what was observed in water stressed tropical seedlings (Centritto, 2005), we did not observe any significant decline in V_{cmax} or J_{max} as a result of water deficits.

The low CO_2 assimilation rates that we observed in the trees of the Caxiuanã forest may indicate that there are limitations to the photosynthetic capacity in this forest. These limitations may be associated with low nutrient availability. N does not seem to be limiting for C assimilation but both P and K concentrations are very low, according to Vitousek & Sanford (1986), and this may be influencing forest performance in the studied site. Previous works reported that N availability in the lowland tropics is usually above plant demand (Martinelli *et al.*, 1999; Ometto *et al.*, 2006). In this study, we found a weak correlation between C assimilation and N and a strong correlation with P, as well as a very strong correlation between P concentration and the biochemical parameters (Fig. 7). This indicates a strong limitation of P on tree metabolism, as observed by others (Meir *et al.*, 2001; Meir *et al.*, 2007). Thus, photosynthetic responses can be partitioned into biochemical status (the 1st axis) and stomatal functioning (the 2nd axis). P is strongly associated with biochemical status and, since Amazon soils are generally poor in P, this may constitute a stronger limitation to photosynthetic potential than long-term drought effects over the photosynthetic apparatus. Some reasons may explain these observations: i) in tropical forests, where N is not limiting and leaves can last for several years (Poorter, 2009), N will be used for other purposes than photosynthesis (Meir *et al.*, 2007), such as physical structure or defence (Wullschleger, 1993); thus a smaller proportion than the usually reported 50% (Evans, 1989) will be allocated to the photosynthetic apparatus; ii) soils in the Eastern Amazon are known to be nutrient poor (Quesada *et al.*, 2010) and become limited in P with age (LeBauer & Treseder, 2008). This is in accordance with the consideration that leaf P content may be a major determinant of A (Kirschbaum & Tompkins, 1990; Raaimakers *et al.*, 1995;

Meir *et al.*, 2001). With regard to K, the values found here suggest that the plants may be K limited, but no direct effect of this limitation was observed as stomatal conductance was recovered during the wet seasons. Anyway, K showed a strong association with stomatal functioning (as evaluated by A_{max} and g_{smax}). Probably K deficiency was not severe enough and also the importance of K for leaf function may be obscured by the complex interplay between plant adaptation to nutrient stress (Santiago & Wright, 2007). Malhi *et al.*(2009) also reported that Caxiuanã leaves had significantly lower K content than other Amazonian sites. As Kaspari *et al.* (2008) suggested, some of the Amazonian forests, and here specifically Caxiuanã, may be in a non-Liebig condition, where multiple nutrients co-limit productivity. Nutrient limitation may also mask the drought effects on photosynthetic capacity in the sense that the photosynthetic apparatus may be already limited by low nutrient availability and this will make the effects of drought less visible. K deficiency may add to the short-term drought effects by acting as co-limitation to stomatal functioning under drought stress. Nevertheless, P and K concentrations increased with canopy position.

Specific leaf area data was strongly correlated with light availability (Fig. 7b), Allocation patterns seem to have optimized SLA and hence, growth (Santiago & Wright, 2007). An increase in SLA has been interpreted as a mechanism to optimize light harvesting under low light environments (Aranda *et al.*, 2005). High SLA and low N per unit area reflect low investment costs per unit area and more efficient (shade) leaves (Turner, 2001). The range found here is wider than in another studies (Carswell *et al.*, 2000; Nogueira *et al.*, 2004; Domingues *et al.*, 2007). In both plots, there was a decline in SLA until May 2003, followed by an increase in Nov 2003, which was great in the TFE plot, but not significantly (Table 3). This might be related to leaf lifespan, which range from few months to several years (Reich *et al.*, 1995; Poorter, 2009), being 2 years on average for top canopy species (Reich & Oleksyn, 2004). In November 2003 we probably measured and collected newly formed (although mature) leaves. So, we argue that SLA decreased "naturally" from November 2001 to May 2003, due to the leaf lifespan (Cavaleri *et al.*, 2010), as well as in response to drought stress in TFE plot, where the decrease was higher. SLA increased again in November 2003, with new leaves, with leaves of TFE being thicker, or denser, as compared to Control plot. This might be interpreted as an adaptation to drought (Chaves *et al.*, 2004), as in the pre-treatment collection averaged SLA was similar in both plots.

Taking into account different functional groups will allow a degree of simplification that may reveal general patterns and facilitate prediction about forest processes (Nogueira *et al.*, 2004).The present study shows differences in species photosynthetic characteristics from different functional groups.

Photosynthetic capacity decreased from the top to the bottom of the canopy as expected due to the gradient in light availability through the canopy (Thomas & Bazzaz, 1999). Strong vertical gradients of stomatal conductance and maximum photosynthetic rate in Amazonian forests have also been reported elsewhere (Roberts et al. 1990, McWilliam et al. 1996). The reductions observed in g_{smax} in the dry season as compared with the wet season, (Fig. 5b) were higher in the Top canopy species and were also higher than A_{max} reductions (Fig. 5a).This may be partly due to stomatal closure in response to the higher leaf-to-air vapour pressure deficit observed in the top of the canopy as compared to the mid-canopy and understory, which may have exacerbated the water stress effects and reduced water flow to the top, demonstrated by a decline in sap flow observed in some of the studied trees (Fisher *et al.*, 2006). These effects are mainly stomatal since no significant effects of TFE were observed in biochemical capacity (V_{cmax} and J_{max}, Fig. 5c,d) between seasons, although a slight reduction in J_{max} was observed in the top canopy species during the dry season. Other studies at this experimental site, for the same time period, have also shown that stomata act to prevent leaf water potential from dropping below a critical threshold level (Fisher *et al.*, 2006). Moreover, *WUE* usually increases with reduced (moderate) water supply (Osório *et al.*, 1998), as we observed during the dry seasons mostly in the TFE plot because drought-induced stomatal closure restricted water loss more than CO_2 uptake (Chaves *et al.*, 2004).

Studies at the same site also showed an increase in leaf respiration in response to drought (Metcalfe *et al.*, 2010) and an increase in tree mortality (da Costa *et al.*, 2010), mainly in large trees, after a longer period than we report here (5-7 years).The parameters measured in this study proved to be important for use in scaling up models aiming at the prediction of the effects of global change on tropical rain forests. It should be noted that within the Amazon region there is a great diversity of soils and vegetation (Quesada *et al.*, 2010), thus differential responses to drought may be expected (Sombroek, 1996). The importance of associating species according to particular edaphic or climatic conditions was recognized by some authors (Baker *et al.*, 2003) to be useful for categorizing important variation in the ecology of species in tropical

forests and was considered to be central to future research on tropical rain forest ecology. The importance of including the physiological acclimation in climate change models, as well as the need of models capable of simulating P limitations have been already acknowledged (Grant *et al.*, 2009; Galbraith *et al.*, 2010). Global change impacts on biosphere, both positive and negative, could be dampened more than previously assumed (Leuzinger *et al.*, 2011) and tropical rain forests might be more resilient to global change than expected (Leuzinger *et al.*, 2011).

Considering that the TFE plot received roughly the same amount of rain in all the dry seasons, the same responses would be expected in the next dry seasons after the pre-treatment measurement date. In fact, what we observed was, besides a full recovery of leaf carbon assimilation in the following wet seasons, a decreased impact of the drought imposition over the time.

This work points to an acclimation to drought with respect to leaf carbon assimilation, as well as a differential response to drought throughout the vertical profile of the canopy.

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Chapter 3

Leaf optical responses to light and drought in tropical rain forest trees

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Abstract

Light, the driving force of photosynthesis, is highly variable in tropical rain forests and play a prominent role in determining the ecophysiological comportment of forest plants. Light intensity inside the canopy might change substantially as a result of the predicted reduction in precipitation, due to increased tree mortality.

A 'through-fall exclusion' experiment (TFE) was conducted in an Amazonian rain forest plot to investigate the forest responses to a reduction of approx 50% through-fall. Here we report leaf optical properties and chlorophyll concentrations in Control and TFE plots for the first 2 years of the experiment (five consecutive seasons; dry/wet).

Drought stress changed leaf optical properties by increasing leaf reflectance in the 500-600 nm range, by decreasing transmittance and increasing absorptance in the 700-1100 nm range (NIR) and by decreasing chlorophyll (*a* and *b*) concentration. The impact of drought stress increased along the canopy vertical gradient. The observed changes in leaf optical properties suggest a structural acclimation to drought, but more studies are needed.

Reflectance based indices proved to be useful in detecting drought stress in tropical rain forests, particularly the photochemical reflectance index.

Introduction

Growth of autotrophic plants is directly and dramatically influenced by the intensity of light, the driving force of photosynthesis, which provides nearly all the carbon and chemical energy needed for plant growth (Bjorkman, 1981). Although gradients of several different environmental factors are noticeable in tropical forests, light intensity is highly variable and appears to play a prominent role in determining the ecophysiological behaviour of forest plants (Lüttge, 1997). In primary tropical rain forests, which canopy is very dense, there is a huge attenuation of light intensity from the top to the bottom of the canopy, where only a few per cent average reaches the ground (Lüttge, 1997).

Climate change models (Cox *et al.*, 2000; Betts *et al.*, 2004) predict a reduction in the dry season rainfall, particularly in eastern Amazonia (Malhi et al. 2008). In addition, large-scale deforestation could result in reduced precipitation over Amazonia as well (Costa & Foley, 1999). As a consequence of reduced precipitation, an increase in tree mortality is likely to occur, particularly in the top of canopy trees (da Costa *et al.*, 2010), driving substantial changes in the light environment inside the canopy. An experimental test of light limitation by cloud cover during tropical rainy seasons revealed that light, rather than water, temperature, or leaf nitrogen, was the primary factor limiting CO_2 uptake during the rainy season (Graham *et al.*, 2003).

Photosynthetically active radiation (PAR, 400-700 nm) intercepted by the photosynthetic apparatus is the basic factor that controls the activity of photosynthesis (Valladares *et al.*, 1997). Near-infrared radiation (NIR, 700-1100 nm) is responsible for signalling processes and tissues temperature (Grzesiak *et al.*, 2010). Irradiation reaching plants is subjected to physical processes of reflectance (R), transmittance (T) and absorbance (A). Two important regions emerge from the reflectance of a leaf, at the visible wavelengths (400-700 nm) that is predominantly due to light absorption by pigments (mainly chlorophylls and carotenoids) (Peñuelas & Filella, 1998; Castro-Esau *et al.*, 2006), thus determined by composition and concentration of chlorophyll *a*, chlorophyll *b* and carotenoids (Chappelle *et al.*, 1992; Cochrane, 2000). At the NIR wavelengths (700-1100 nm) is influenced by light scattering at air-cell interfaces from the internal leaf structure and is characterized by high reflectance and transmittance (Peñuelas & Filella, 1998; Castro-Esau *et al.*, 2006). So, leaf absorptance is determined

by the photosynthetic pigments, in the PAR range, and by water in the NIR range (Grzesiak *et al.*, 2010). Transmittance is the part of irradiance which is not absorbed or reflected by plant organs and is transmitted to deeply situated cells or escape outside (Grzesiak *et al.*, 2010).

Measurements of leaf absorptance in the PAR range are of interest because it is only these wavelengths that are useful for photosynthesis, and nearly 50% of the sun's irradiance and 80% of the solar radiation absorbed by leaves is in this waveband (Ehleringer, 1980). A change in leaf absorption will directly affect photosynthesis and leaf energy balance (leaf temperature). Indirectly, a change in leaf temperature will affect rates of transpiration and photosynthesis (Ehleringer, 1980). Despite the importance of leaf absorptance, leaf reflectance has been studied more extensively than transmittance or absorptance, largely as a result of interests in remote sensing (Carter & Knapp, 2001; Baltzer & Thomas, 2005) and several reflectance based indices have been proposed for diagnosing the physiological status of the plant, as reviewed by Ollinger (2011).

A number of studies have linked responses in leaf spectral reflectance, transmittance, or absorptance to physiological stress (e.g. Carter, 1993; Carter & Knapp, 2001; Poulos *et al.*, 2007; Grzesiak *et al.*, 2010; Serrano *et al.*, 2010; Liu *et al.*, 2011). Compared to other environments, there are significantly less studies regarding leaf optical properties in tropical environments, and those were mainly performed in tropical dry forests (Lee & Graham, 1986; Lee *et al.*, 1990; Avalos *et al.*, 1999; Cochrane, 2000; Clark *et al.*, 2005; Castro-Esau *et al.*, 2006; Zhang *et al.*, 2006; Sánchez-Azofeifa *et al.*, 2009; Göttlicher *et al.*, 2011). Only a few studies addressed physiological issues (Poorter *et al.*, 1995; Gamon *et al.*, 2005).

Results from leaf-level studies are often used as a basis for establishing techniques for retrieving information from canopy reflectance by remote sensors (Castro-Esau *et al.*, 2006; Peñuelas *et al.*, 2011). So, understanding the optical behavior of leaves, through field measurements, is critically important in remote sensing, and needed to narrow the uncertainty of the Amazon basin-level responses to drought and climate change (Sánchez-Azofeifa *et al.*, 2009; Asner & Alencar, 2010; Ollinger, 2011).

Pigments are integrally related to the physiological function of leaves (Sims & Gamon, 2002) and are known to vary along the vertical profile of the canopies (Givnish, 1988;

Poorter *et al.*, 1995). Chlorophylls are responsible for absorbing light energy and transferring it into the photosynthetic electron transport apparatus. Carotenoids can contribute to the photosynthetic light capturing system as well but, when incident light energy exceeds that needed for photosynthesis, the carotenoids that compose the xanthophyll cycle dissipate excess energy, thus avoiding damage to the photosynthetic system (Demmig-Adams & Adams, 1996).

Variations in chl *a* and *b* between leaves growing under different irradiance availabilities are commonly observed (Poorter *et al.*, 1995; Lei *et al.*, 1996). Chl *b* is found exclusively in the light harvesting chlorophyll protein complex, primarily associated with photosystem II (Lei *et al.*, 1996). It is suggested to be important in the maintenance of the energy balance between the photosystems by capturing more efficiently the (red) light in the forest shade (Bjorkman, 1981). Chl *a/b* ratio appears to decrease from the top to the bottom of the canopies and is associated with the diminishing availability of red light under forest shade (Bjorkman, 1981).

There are numerous structural traits that, in conjunction to physiological traits, determine the leaf light-harvesting capacity and photosynthetic potential (Niinemets & Sack, 2006). Knowledge on how the different traits co-vary is important for understanding plant functioning in changing environmental conditions (Niinemets & Sack, 2006). Several structural characteristics affect light interception per unit leaf area (Niinemets & Sack, 2006): at tissue scale, structural modifications in response to changing environment alter the amount of light intercepted by unit chlorophyll, while at a leaf scale, structural changes modify the exposure of single leaves. Finally, leaf arrangement and aggregation on the shoot may further alter the average irradiance on the leaf surface. Structural characteristics, such as leaf thickness, density, number of air water interfaces, cuticle thickness, and pubescence may have significant effects on leaf optical properties (Sims & Gamon, 2002). Leaf mass per area (LMA, or 1/LMA, specific leaf area, SLA) is a general and easily measured variable used to describe the effect of leaf structure on leaf photosynthesis (Niinemets & Sack, 2006).

In our earlier paper (Lobo-do-Vale *et al.*, in review), we showed that these tropical rain forest trees responded to decreased water availability mainly through stomatal closure and exhibited resilience in response to drought. At the end of the study period, after two years of drought imposition, we observed a recovery in carbon assimilation,

independently of a continued decrease in leaf water potential. Additionally, trees from TFE plot showed decreased in SLA, compared to Control plot trees. This work pointed to an acclimation to drought with respect to leaf carbon assimilation, as well as a differential response to drought throughout the vertical profile of the canopy.

In the present paper, we used leaf optical properties and chlorophyll concentrations to: 1) describe leaf optical properties along the vertical profile of the canopy; 2) investigate leaf optical responses to decreased water availability and; 3) provide field data for modelling validation.

Specifically, we seek the answers to the following questions:

1. Do leaf optical properties and chlorophyll concentrations change in response to decreased water availability?
2. Do leaf optical properties and chlorophyll concentrations differ throughout the vertical profile of the canopy?
3. Do leaf structural changes occur as an acclimation strategy to drought?
4. Are reflectance based indices good indicators of drought stress in tropical rain forests?

We tried to answer to these questions by studying spectral reflectance measured between 400 and 1100 nm and chlorophyll concentrations.

Material and Methods

Site, species and sampling

The experimental site is located at Caxiuanã National Forest Reserve, Pará, Brazil (1º43'3.5''S, 51º27'36''W), an undisturbed and protected rain forest, representative of the type of lowland *terra firme* forest. The climate in Caxiuanã is characterized by a pronounced dry season between July and December and a wet season in the remaining months. Mean annual rainfall is 2272±193 mm, (with 555±116 mm rainfall recorded from 1999 to 2003 for the dry season) (Fisher *et al.*, 2006). The daily mean temperature of 25 ºC is almost constant over the year (diurnal variation is typically less than 3º C).

The soil of Caxiuanã is vetic acrisol (World Reference Base soil classification), highly weathered and nutrient-poor (Quesada *et al.*, 2010) and has a relatively high plant available water content of 0.200±0.032 $m^3 m^{-3}$ (Fisher *et al.*, 2008). The site elevation is 15 m above river level in the dry season, and the water table has been observed at a depth of 10 m during the wet season (Fisher *et al.*, 2006).

An artificial soil drought was created by using trough-fall exclusion (TFE) to investigate the limitation of soil water on tree physiology in drier conditions than those normally experienced. Two plots of 1ha were established, a Control and a treatment TFE plot, the latter with the borders trenched to a depth of 1 m to reduce the lateral flow of water. In the TFE plot, a roof of transparent plastic sheeting and wooden guttering was installed at approximately 2 m height in January 2002, to keep the soil free from rainfall. So, rain passing through the canopy (through-fall) was intercepted by a system of plastic panels and then drained away from the plot, thereby artificially reducing soil moisture.

A 30-m-tall canopy access tower was installed in each plot. Nine trees and eight trees were accessible from tower in the Control and TFE plots, respectively, being 17 trees in total, corresponding to 16 species. Species were grouped according to their position in the canopy (understory [0;10 m[, mid-canopy [10;20 m[and top-canopy [20;30m]). Details of the distribution of the species throughout the vertical profile of the canopy are given in Table 1. Maximum accessible canopy height was approximately 32 m.

Table 1. Species list of all trees studied in Control and TFE plots throughout the vertical profile of the canopy, with the functional group and systematic position.

Plot	Functional group	Family name	Species name	Height in the canopy (m)
Control	**Understory**	Quiinaceae	*Quiina florida*	2
		Sapotaceae	*Pouteria lateriflora*	2
		Burseraceae	*Protium heptaphyllum*	4
	Mid Canopy	Quiinaceae	*Quiina florida*	10
		Annonaceae	*Duguetia echinophora*	18
		Flacourtiaceae	*Hasseltia floribunda*	10
		Lauraceae	*Mezilaurus mahuba*	10
		Chrysobalanaceae	*Licania heteromorpha*	18
	Top Canopy	Sapotaceae	*Manilkara bidentata*	30
TFE	**Mid Canopy**	Annonaceae	*Duguetia echinophora*	14
		Chrysobalanaceae	*Licania canescens*	12
		Lauraceae	*Licaria armeniaca*	18
		Melastomataceae	*Mouriri duckeana*	16
	Top Canopy	Chrysobalanaceae	*Hirtela bicornis*	22
		Lecythidaceae	*Lecythis confertiflora*	28
		Sapotaceae	*Manilkara paraensis*	30
		Caesalpiniaceae	*Swartzia racemosa*	30

Mean light environment

Photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}), throughout the canopy was determined using a LI-1800 spectroradiometer (LI-COR Inc., Lincoln, NE, USA). Measurements were performed in November 2001, November 2002, May 2003 and November 2003.

Leaf optical properties

Reflectance, transmittance and the calculated absorbance (A=1-(R+T) of the upper leaf surface were determined using the LI-1800 spectroradiometer with an external integrating sphere (LI-1800-12S, LI-COR Inc., Lincoln, NE, USA). The spectroradiometer has a detection range of 330-1100 nm, with a wavelength accuracy of 2 nm, spectral resolution of 6 nm and scanning speed 20 nm s^{-1}.

Four reflectance based indices were calculated as follows: 1) Water index (WI), a measure of plant water content, was calculated as WI = R_{900}/R_{970} (Penuelas *et al.*, 1997); 2) Red edge position (REP) as REP=700+40*[(R_{re}–R_{700})/(R_{740}–R_{700})] (Poulos *et al.*, 2007), where R_{re}=(R_{670}+R_{780})/2, and R is the spectral reflectance; 3) a chlorophyll normalized difference index ($_{chl}$NDI), that is sensitive to chlorophyll *a* concentrations, as $_{chl}$NDVI = (R_{750}-R_{705})/(R_{750}+R_{705}), (Gitelson & Merzlyak, 1994); 4) Photochemical reflectance index (PRI), an optical indicator for detecting epoxidation and de-epoxidation changes of xanthophyll related to heat dissipation (Gamon *et al.*, 1997), corresponding to the carotenoid:chlorophyll pigment ratio (Sims & Gamon, 2002), as PRI = (R_{531}-R_{570})/(R_{531}+R_{570});. For all indices, R_x indicates spectral reflectance at *x* nm.

Chlorophyll content

Leaves collected for chlorophyll determination were identical to those used for gas-exchange measurements and leaf optical properties. Chlorophyll was extracted from freshly frozen (-18ºC) leaves using the using the DMSO method based on Barnes *et al.*(1992). Weighted tissues (ca 0.30 mg per leaf) were placed in test tubes with 5.0 ml of 99% DMSO. The tubes were sealed with rubber caps, heated in a water bath at 70ºC and centrifuged (3600 rpm) for 2 hours, for chlorophyll solubilisation. The extraction process was considered complete when, by visual examination, the samples were transparent (Arnon, 1949). Chlorophyll absorption in a 15 ml aliquot from extract was determined using a spectrophotometer (Shimadzu UV-601, Kyoto, Japan). Chlorophyll a, b, a+b contents were calculated following the equations taken from Barnes *et al* (1992) and chlorophyll a/b ratio obtained. Results are presented on a leaf area basis, which is the general reference for use in terrestrial biosphere models (Kattge *et al.*, 2009).

All measurements were made on fully expanded leaves without signs of senescence, throughout the vertical profile of the canopy, with a minimum of two leaf replicates. Data were recorded over a period of 2 years, from November 2001 to November 2003, in five measurement dates, at the end of the dry season (Nov 2001, Nov 2002 and Nov 2003, for leaf optical properties and chlorophyll concentration) and at the end of the wet season (May 2002 and May 2003, only for chlorophyll concentration). November 2001 was the pre-treatment measurement date.

Data analysis

For the analysis of the light profile inside the canopy, data was pooled by canopy height and a two-way ANOVA was used to distinguish between differences due to canopy level and treatment/plot effect.

To assess the impact of the TFE treatment on the parameters measured, two analyses were performed. First, we used generalized Linear Model (GLM) to examine the differences in measurement dates and treatment effect to see if there was an inter-annual variation (within-plot change over time) and if the treatment effect was the cause of such a variation (between-plot differences). In these analyses, in addition to the measurement date and treatment factors, canopy leaf position (height above ground) was used as a random effect to control for the potentially confounding effect of sampling differences between plots. Data was then grouped by canopy position and analysed separately for each measurement date. When statistically significant differences were found, differences between group means were identified by post-hoc Tukey HSD tests. In addition, the links between parameters were assessed with a Spearman´s Rank Correlation. Statistical analyses were carried out with SAS 9.1 (SAS Institute, Cary, NC, USA). Data were transformed with a natural logarithm when necessary, to meet the assumptions of parametric analyses, namely normal distribution of data and homogeneity of variances. All statistical relationships were considered significant at $p<0.05$.

Results

Meteorology and mean light environment

November 2001 was the end of a long and severe dry season (Fig. 1). If we consider the amount of rain fall received by the plots prior to the first measurement date (between July and November 2001), the dry season of 2001 had the same amount of rain fall than the excluded through-fall on TFE plot in the next two dry seasons (~180 mm).

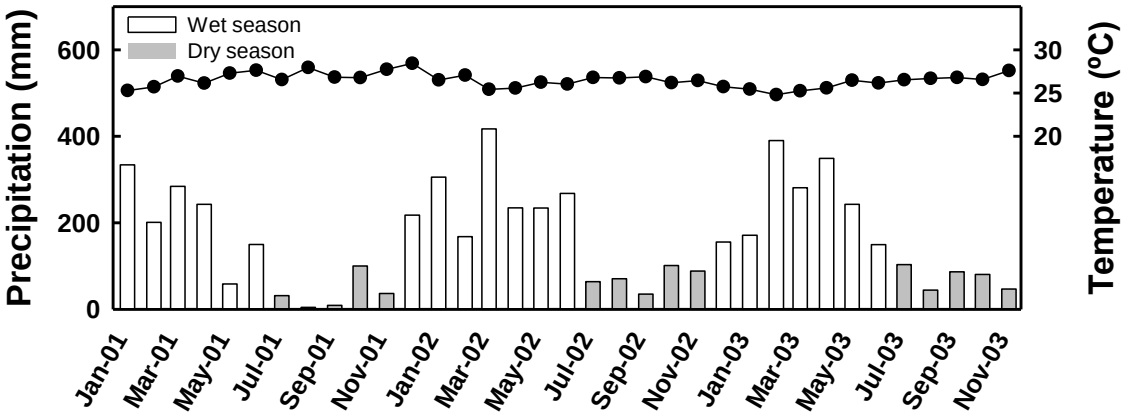

Figure 1. Precipitation (mm) and temperature (ºC) from 2001 to 2003. Grey bars represent the precipitation occurred during the dry seasons, prior to the measurement dates. In the dry season of 2001 the amount of rain was roughly half of the next two years These data are reproduced from Lobo-do-Vale *et al*. (in review).

The vertical profile of mean PPFD showed an exponential decrease with increasing depth into the canopy (Fig. 2). Light profile was similar in both plots, where no statistically significant effect was found for plot ($p=0.244$). However, PPFD was significantly reduced inside the canopy ($p<0.001$), reflecting statistically significant differences in light availability between the vertical gradient groups considered ($p<0.001$).

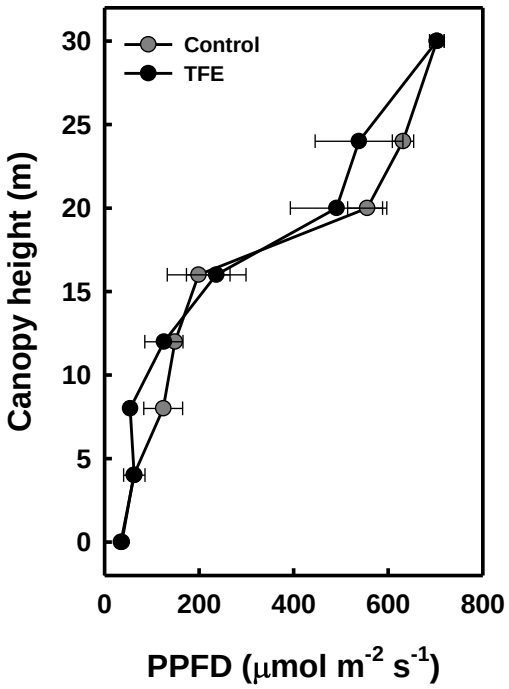

Figure 2. Light profile inside the canopy. Mean photosynthetic photon flux density (PPFD) throughout the vertical profile of the canopy in each plot. Values are mean ± SE of measurement dates.

Leaf optical properties

The spectral shape of the tropical rain forest trees studied showed a typical pattern, with mean absorptance (500-600 nm) values between 0.75 and 0.91 (Fig. 3). The largest difference in absorption spectra was found in the 550-556 nm range and the spectra converged in the regions below 500 nm and above 680 nm.

Leaf optical properties showed very close similarity between Control and TFE plots in the pre-treatment measurement date, November 2001 (Fig. 3).

Leaf reflectance, in the 500-600 nm range, differed among plots ($p=0.003$) at the end of the study period, in Nov 2003, due to a steady increase in the TFE plot over time, compared to stable values in the Control plot (Fig. 3a). A decrease in water availability caused an increase in leaf reflectance of the trees of the TFE plot (Fig 4), which was more pronounced at the top canopy trees (Fig. 4i). Nevertheless, the vertical gradient groups were not statistically different from each other. Considering all measurement dates together, reflectance was higher in the TFE plot and significantly different from

the Control plot (p=0.006). In the near infrared range (NIR, 700-1100), water availability had no influence on leaf reflectance of the studied trees, as well as vertical gradient grouping, where no statistically significant effects of the factors were observed. Considering all data together, no changes over time or plot effect were found in the NIR range.

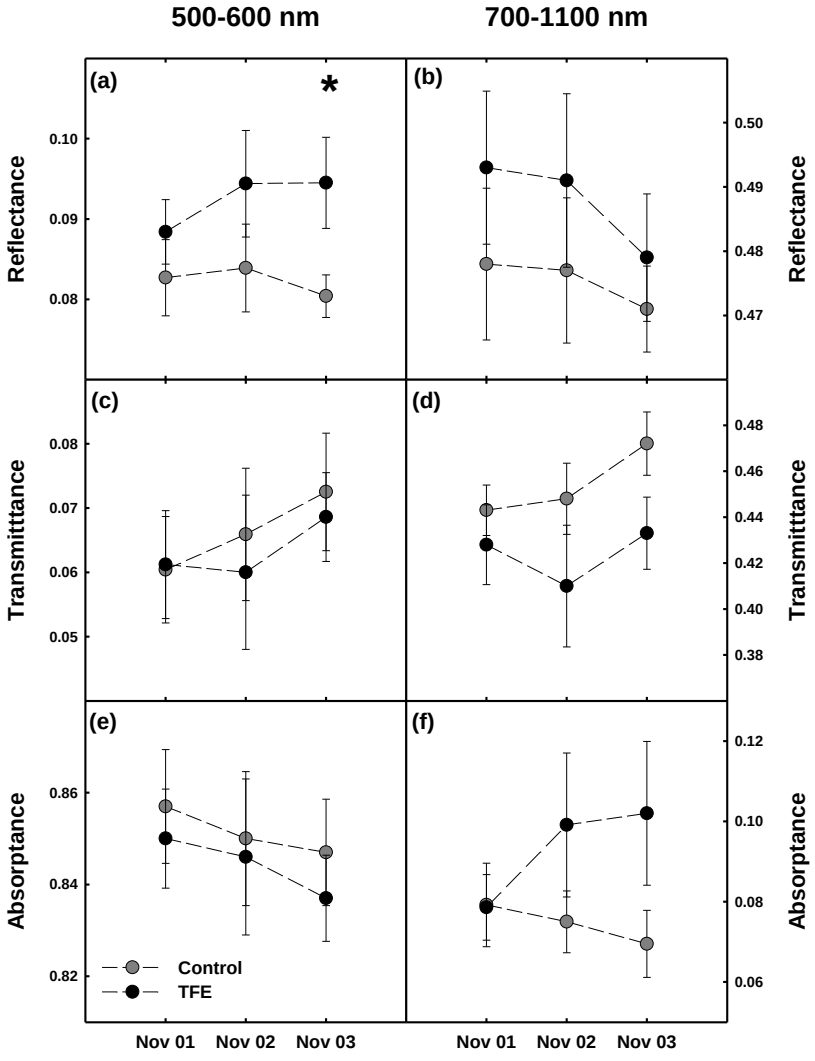

Figure 3. Leaf optical properties measured in Control and TFE plots during the dry seasons of November 2001, November 2002 and November 2003. Reflectance in the 500-600 nm range (a), in the NIR range (b); Transmittance in the 500-600 nm range (c), in the NIR range (d) and Absorptance in the 500-600 nm range (e), in the NIR range (f)

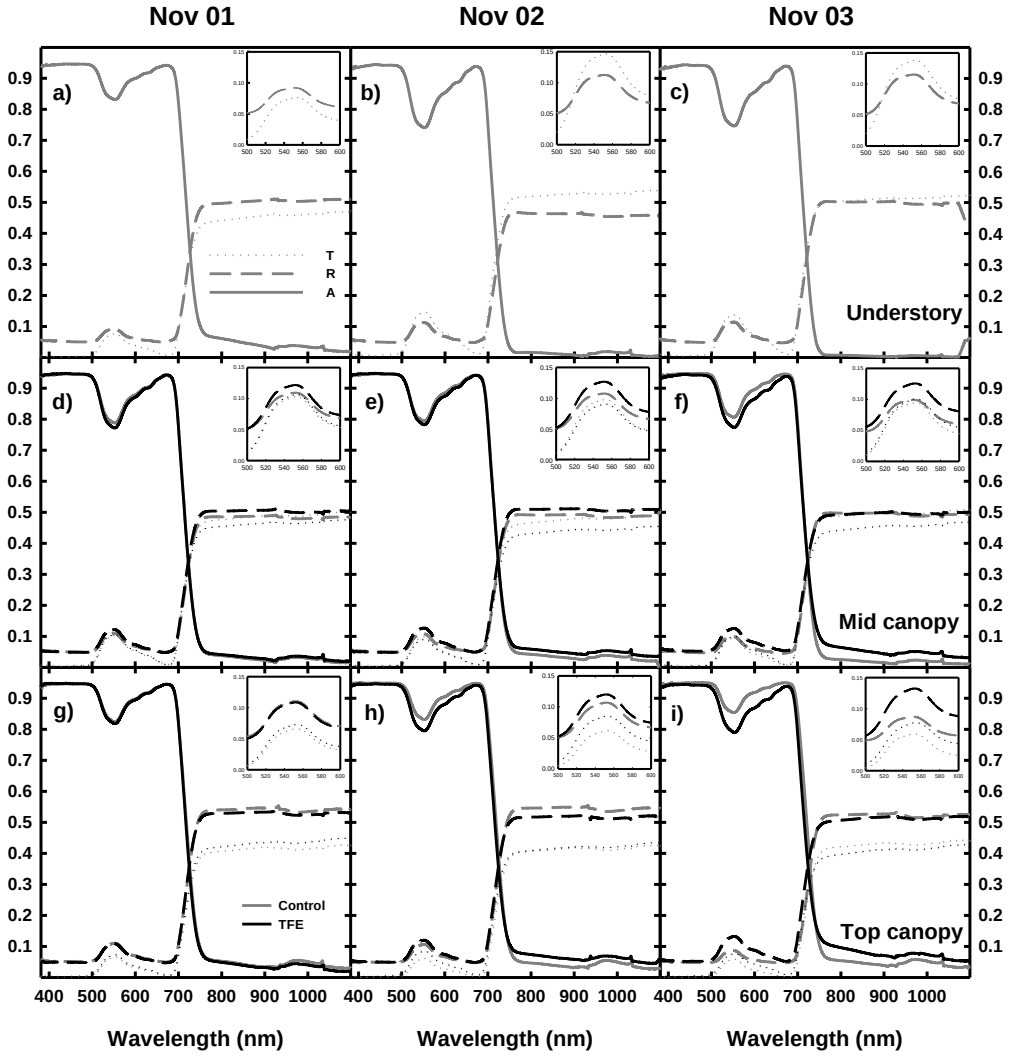

Figure 4. Leaf optical properties in the different canopy position groups, measured in Control and TFE plots during the dry seasons of November 2001, November 2002 and November 2003. The entire studied spectra is shown. Reflectance are dashed lines, transmittance are dotted lines, and absorptance are solid lines. The inserts shown reflectance and transmittance in the 500-600 nm range.

Transmittance, in the range of 500-600 nm, was not significantly affected by water availability (Fig. 3c), but differed substantially across the vertical gradient groups ($p=0.017$). Transmittance was higher in the understory trees compared with top canopy trees (Fig 4). However, by the end of the study period, top canopy trees of the TFE plot showed higher transmittance compared to those from the Control plot (Fig. 4i). Overall, this response was similar between plots and groups, where no effects of measurement date or vertical gradient grouping were found. In the NIR range (Fig. 3d), transmittance was not affected by drought stress but differed significantly among vertical gradient

groups (p=0.032). Top canopy trees had the lowest transmittances (Fig. 4i). Considering all data together, no changes over time were observed in transmittance, but drought stress caused differences between plots (p=0.0046), with a lower transmittance in the TFE plot.

As a result of higher reflectance and lower transmittance in TFE trees, in the 500-600 nm range, in comparison to Control plot (Fig 3), leaf absorptance, at the end of the study period, was only affected by vertical gradient grouping (p=0.027) (Fig. 3e), with top canopy trees exhibiting higher light absorption than understory trees. There was, however, a trend toward decreased absorptance in mid and top canopy trees under drought stress (Fig, 4f, i). Considering all measurement dates together, no significant changes over time or vertical grouping differences were observed in the absorptance in the 500-600 nm range. In the NIR range, differences in absorptance were only found when measurement dates were considered together, showing a significant increase of leaf absorptance on the TFE trees (Fig. 3f) (p=0.034) in response to drought stress.

Reflectance indices

Reflectance based indices showed very close similarity between Control and TFE plots in the pre-treatment measurement date, in November 2001 (Fig. 5), with the exception of WI (Fig. 5a), which differed significantly among vertical gradient groups.

Water index (WI) was reduced in both plots by Nov 01 (Fig. 5a), the pre-treatment measurement date, maintaining similar values in Nov 02 in the TFE plot and decreasing in Nov 03, contrasting with the Control plot, which increased in Nov 02 and maintained similar values in Nov 03. *WI* was significantly affected by drought stress in Nov 02 (p=0.02) and in Nov 03 (p=0.007), overlapping the vertical gradient differences observed in Nov 01 (p=0.010) (Fig. 6a, b, c). Considering all data together, there were no changes over time in *WI* but water availability caused differences between plots (p=0.001), strongly reducing WI in the TFE plot (Fig. 5a).

Red edge position (REP) moved towards shorter wavelengths in the TFE plot (Fig. 5b) in response to water stress, as compared to the Control plot that remained unchanged over time. The increased differences between plots led to significant differences among plots at the end of the study period (p=0.018). Despite the increasing difference between

top canopy trees (Fig. 6d, e, f), no effect of the vertical gradient grouping was found. No change over time or plot effect was found for the pooled data.

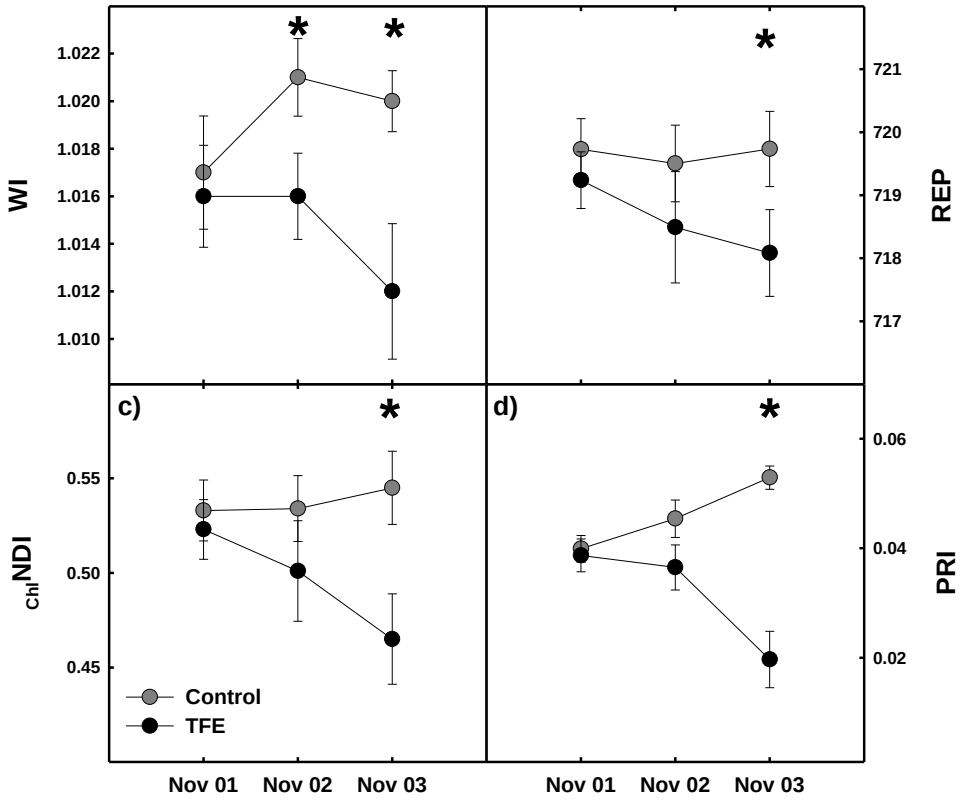

Figure 5. Reflectance based indices measured in Control and TFE plots during the dry seasons of November 2001, November 2002 and November 2003. a) Water Index (WI); b) Red edge position (REP); c) chlorophyll normalized difference index ($_{chl}$NDI); d) Photochemical reflectance index (PRI).

Chlorophyll normalized difference index ($_{chl}$NDI) was affected by drought stress as well (p=0.004) (Fig. 5c) and was similar in the different vertical gradient groups (Fig. 6g, h, i), although a trend to an increase with canopy height could be discerned. Increased water limitation caused decreased $_{chl}$*NDI* values in the TFE plot (Fig. 5c). When considering all data together, no changes over time could be observed nor did water availability affect this index.

Photochemical reflectance index (PRI) was strongly reduced by water stress in Nov 03 (Fig. 5d), whereas an increase was found in the Control plot over the time, determining significant differences among plots in Nov 03 (<0.001) and for all measurement dates

(p=0.003). Regarding vertical gradient grouping, the values found for this index were similar between groups. There was, however, a trend toward decreased PRI at the top canopy trees (Fig. 6i).

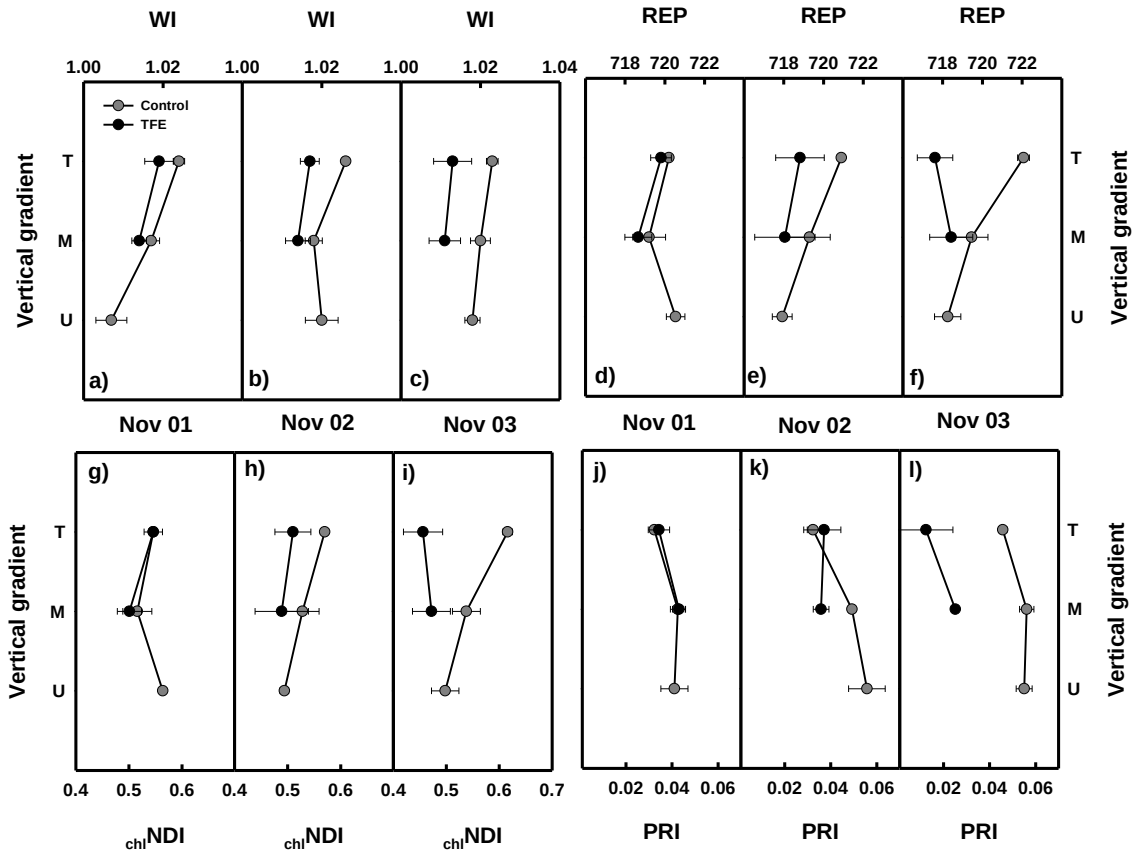

Figure 6. Reflectance based indices comparison between vertical gradient groups shown separately for each measurement date, the Control and TFE plots. Water Index (WI) (a, b, c), Red edge position (REP) (d, e, f), chlorophyll normalized difference index ($_{chl}$NDI) (g, h, i), Photochemical reflectance index (PRI) (j, k, l).

Chlorophyll concentration

Chlorophyll (*Chl*) concentrations were measured during five consecutive seasons: in the dry seasons of Nov 01, Nov 02 and Nov 03 and the wet seasons in-between, in May 02 and May 03. Chlorophyll concentrations showed very close similarity between Control and TFE plots in the pre-treatment measurement date, in November 2001 (Fig. 7).

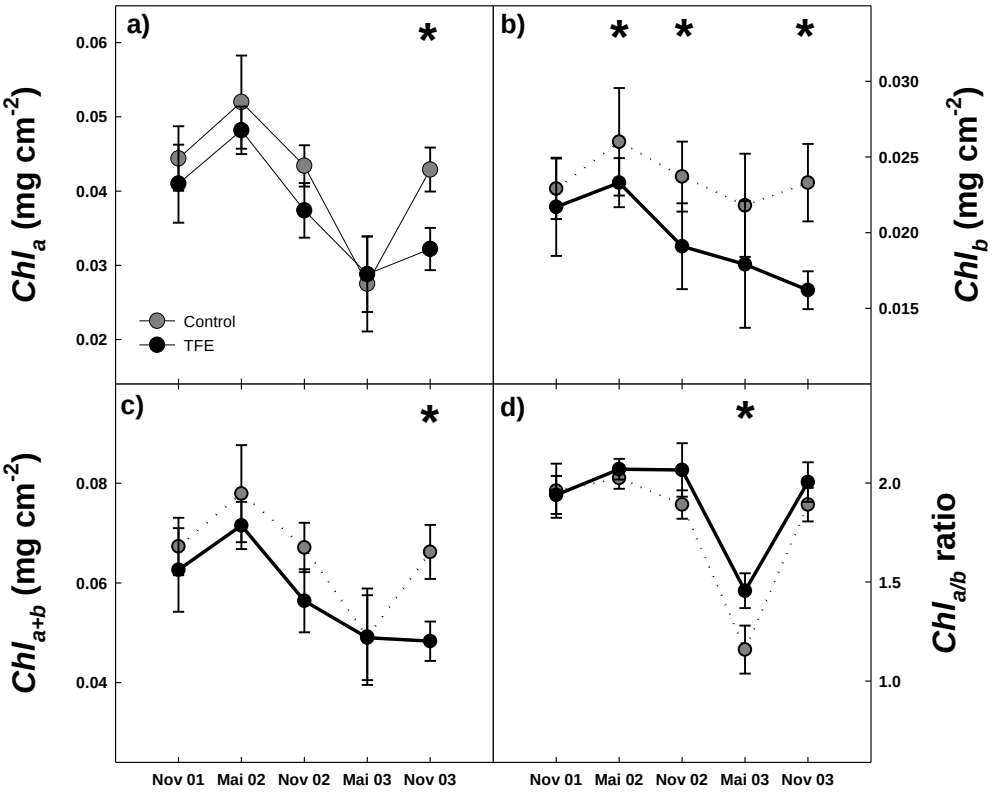

Figure 7. Chlorophyll concentrations (mg cm^{-2}) in Control and TFE plots during the dry seasons of November 2001, November 2002 and November 2003. a) chl_a, b) chl_b, c) chl_{a+b}, and d) $chl_{a/b}$

Overall, *chl* concentrations (*a*, *b*, *total*, mg cm^{-2}) were lower in the TFE plot compared with the Control plot (Fig. 7). *Chl* concentrations decreased steadily in the TFE plot over time, after a slight increase in the wet season of May 02, compared with a sort of seasonal variation in the Control plot, where the observed similar values of *chl* in the dry seasons (Nov 01, 02 and 03) were interspersed by an increase and a decrease of concentrations in the wet seasons of May 02 and May 03, respectively (Fig. 7). The pooled data show a change over time in chl_a and chl_{a+b} ($p<0.001$ and $p=0.001$, respectively) and a significant treatment effect in chl_a, chl_b and chl_{a+b} ($p=0.014$, $p<0.001$ and $p=0.004$, respectively). When comparisons were done separately for each measurement date, by plot and vertical gradient groups, drought stress affected primarily chl_b concentrations (Fig. 7b), which were substantially reduced in the TFE plot from May 02 onwards ($p=0.028$, $p=0.041$, n.s. and $p=0.002$ in May 02, Nov 02, May 03 and Nov 03, respectively) and only later, by the end of the study period, caused

a reduction of chl_a concentrations (p=0.007 in Nov 03) (Fig. 7a). Total chlorophyll concentration was mostly determined by chl_a, with a similar pattern of chl_a (p=0.003 in Nov 03).

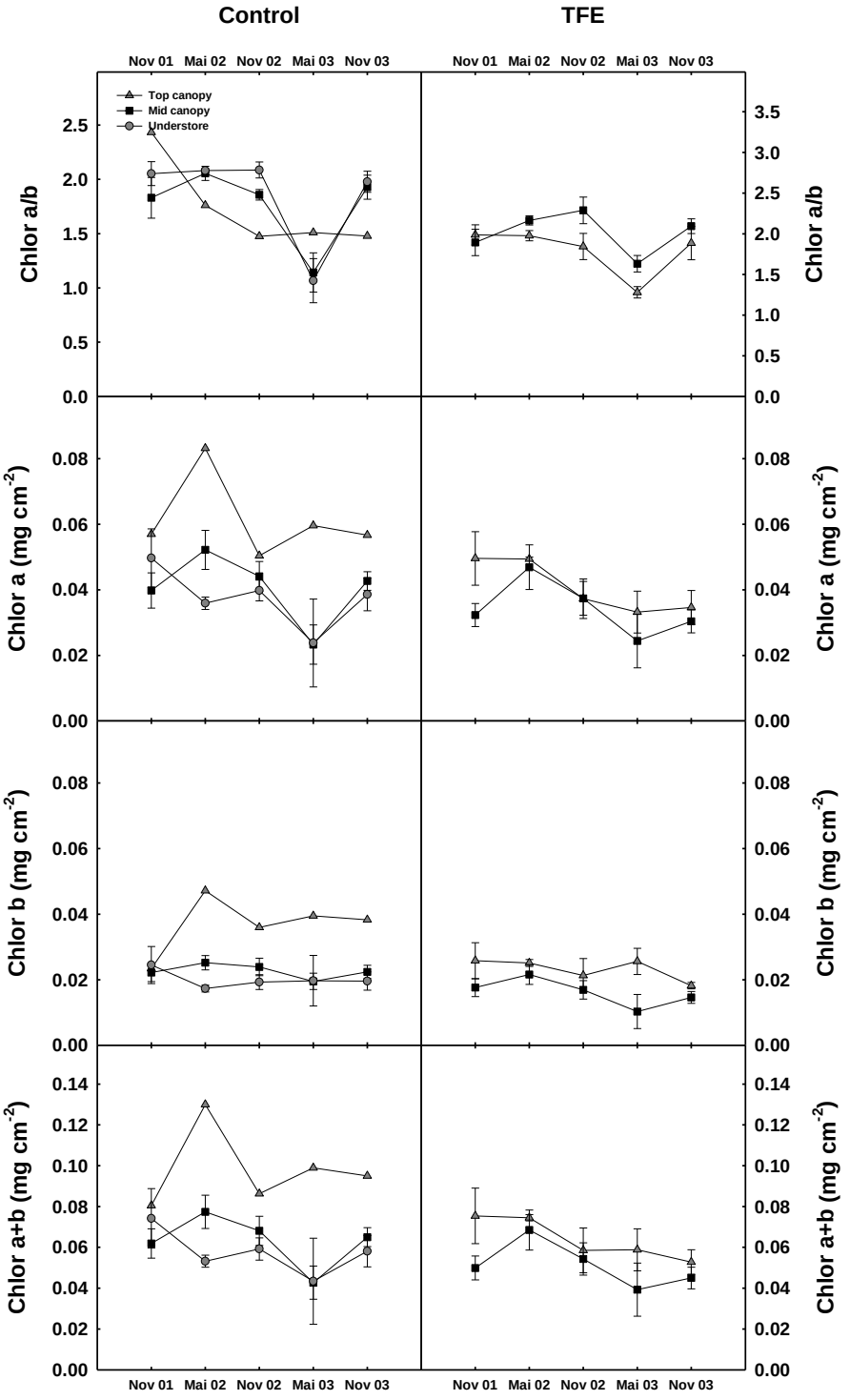

Figure 8. Chlorophyll concentrations (mg cm^{-2}) evolution shown separately in Control and TFE plots, by vertical gradient groups. chl_a, (a, b) chl_b, (c,d) chl_{a+b}, (e, f) and $chl_{a/b}$ (g, h)

Vertical gradient grouping revealed that the highest reductions caused by decreased water availability were observed on the top canopy trees (Fig. 8) and especially regarding chl_b concentrations (p=0.015, p=0.017, p=0.022 and p=0.027 in May 02, Nov 02, May 03 and Nov 03, respectively). Besides the high reductions observed, mean chl_b concentration of top canopy trees was significantly higher than understory trees (Fig 8c, d). $chl_{a/b}$ ratio was not affected by drought stress (Fig. 7d), with overall observed changes over time (p<0.001), determined by a high reduction in this ratio in May 03 in both plots. Nevertheless, TFE plot had higher $chl_{a/b}$ ratios and the analysis by measurement date revealed a significant effect of water availability in Nov 02 (p=0.009), as well as differences between vertical gradient groups (p=0.013) with the lowest ratios belonging to the top canopy trees (Fig. 8g, h).

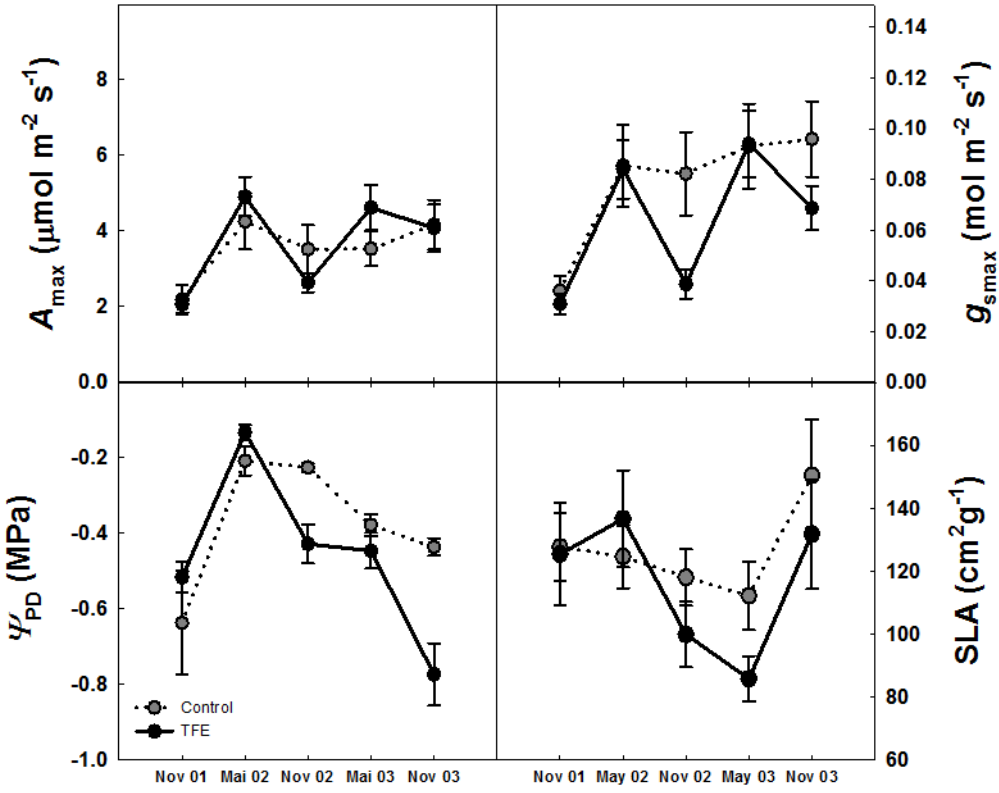

Figure 9. Seasonal trends of a) maximal rates of photosynthesis corrected to 25ºC (A_{max}), (b) maximal stomatal conductance corrected to 25ºC (g_{smax}), c) predawn leaf water potential (Ψ_{PD}) and d) specific leaf area (SLA). Values are mean ± SE. TFE, trough-fall exclusion. These data are reproduced from Lobo-do-Vale *et al*. (in review).

Discussion

The studied tropical rain forest trees responded to drought stress mainly by increasing leaf *R* in the visible wavelengths (Fig. 3a). The differences found for *T* (Fig 3d) and *A* (Fig. 3f) in the NIR range were mostly determined by variations in the Control plot rather than substantial changes in *T* and *A* in the TFE plot. However, the different responses across the canopy groups (Fig. 4), associated with a high variability, may have obscured the drought effect in both wavelengths considered. In fact, when comparing the canopy groups at the end of the study (Nov 03), from TFE and Control plots, we observed a difference of about 6% in leaf absorptance (500-600 nm) in the top canopy trees and about 3% in the mid canopy trees. *T*, in 500-600 nm range, was increased by 43% and 10% in top and mid canopy trees, respectively, and *R* in the same range increased by 50% and 27%, respectively. On the other hand, in the NIR range, no changes were observed in leaf *R*, but *T* decreased around -4% in the top canopy trees and -8% in mid canopy trees, respectively, leading to increases in *A* of 18% and 46% in leaf, in TFE plot compared to Control plot. So, the impact of drought stress was higher for top canopy trees and contradicts previous observations indicating similar leaf optical properties across the vertical profile of the canopy (Lee & Graham, 1986; Knapp & Carter, 1998). Nevertheless, the significant effects of canopy position in *A* and *T* in the visible range were already reported (Poorter *et al.*, 1995).

An increased leaf reflectance at visible wavelengths is a common response to drought stress (Carter, 1993; Grzesiak *et al.*, 2010) as a result of decreased absorption by pigments (Carter, 1993). Chlorophyll concentrations also decreased with water availability, with higher reductions in top canopy trees, as observed in other studies (Poulos *et al.*, 2007; Grzesiak *et al.*, 2010). Increasing leaf reflectance and decreasing chlorophyll content also reduces light harvesting in drought stressed plants (Carter & Knapp, 2001). Pigments responsible for light capture are favored under shade, while those responsible for protection against over-excitation may be down-regulated (Levizou & Manetas, 2007). So, we would expect chlorophyll *a/b* ratios to be lower at the bottom of the canopy, due to the high ratios of light harvesting complexes per reaction centers (Levizou & Manetas, 2007).

Top canopy trees are exposed to high irradiance, whereas understory trees depend on lightflecks for the majority of their carbon gain (Santiago & Wright, 2007). Leaf

absorption affects directly carbon assimilation and leaf energy balance (Ehleringer, 1980), but decreasing water availability makes less water to be available for heat transfer from leaves (Ehleringer, 1980), leading to changes in leaf temperature that will affect, by its turn, rates of transpiration and photosynthesis (Ehleringer, 1980). In this context, acclimation to high light often results in increases in cuticle thickness, whose primary function is prevention of water loss (Baltzer & Thomas, 2005). Such cuticular changes may also alter leaf surface reflectance patterns (Grant *et al.*, 1993). Therefore, if reflectance does not increase with light, this response may be partially attributed to cuticular changes (Baltzer & Thomas, 2005). In our case, reflectance increased in response to reduced water availability.

Specific leaf area (or leaf mass per area) commonly decreases in response to drought stress (Niinemets & Sack, 2006). However, it is clear from our observations (Fig. 9d, Lobo-do-Vale et al, in review) that SLA was significantly affected by water availability, when comparing TFE and Control plot at the end of the study period. And these changes were considered as an acclimation to drought (Chaves *et al.*, 2004). Nevertheless, when compared SLA observed in the TFE plot in the pre-treatment measurement date with those at the end of the study period, the values are similar. This may indicate that other structural changes might have occurred. Or distribution of chlorophyll alone could account for changes in light propagation? If not, internal leaf anatomy may play a significant role (Brodersen & Vogelmann, 2010).

Reflectance in the NIR range depends on leaf water content, due to the fact that water is a major absorber of light (Grzesiak *et al.*, 2010) and is known to be strongly correlated with internal light scattering (Knapp & Carter, 1998) that, by its turn, is influenced by leaf thickness (Knapp & Carter, 1998). Here we found no significant changes in leaf reflectance in the NIR range. This might indicate that leaf thickness did not change but other changes in leaf structure could have taken place. Differences in leaf longevity and growth form within a habitat may help explain the lack of consistent patterns in leaf reflectance (Knapp & Carter, 1998).

It is widely recognized that the visible, rather than infrared reflectance is the most reliable indicator of plant stress (Carter, 1993) because reflectance changes in the visible spectrum by stress conditions result from the sensitivity of leaf chlorophyll concentrations to metabolic disturbance (Knipling, 1970). Indeed, several studies have

shown that indices based on reflectance in the far-red can estimate leaf chlorophyll concentration (Chappelle *et al.*, 1992; Gitelson & Merzlyak, 1994; Gamon *et al.*, 1997; Carter & Knapp, 2001; Filella *et al.*, 2004; Penuelas *et al.*, 2004; Gamon *et al.*, 2005; Poulos *et al.*, 2007; Zhang *et al.*, 2007; Serrano *et al.*, 2010; Paradiso *et al.*, 2011). Thus, leaf optical properties in a relatively narrow spectral band near 700 nm are crucial for plant stress detection and the estimation of leaf chlorophyll concentration. (Carter & Knapp, 2001). Photosynthetic pigment composition can then be used as an indicator of the physiological status of a plant (Peñuelas & Filella, 1998; Sims & Gamon, 2002). Several studies have used leaf optical properties to assess physiological stresses at leaf or canopy level (Poulos *et al.*, 2007; Serrano *et al.*, 2010), most of them by the use of reflectance indices (Serrano *et al.*, 2010). Reflectance based indices are considered to be advantageous, compared to reflectances at particular wavelengths, by partly removing disturbances caused by external factors (Peñuelas & Filella, 1998). However, these studies were mostly performed in dry environments, such as Mediterranean or temperate forests or crops (Carter, 1993; Penuelas *et al.*, 1998; Lewis *et al.*, 2000; Evain *et al.*, 2004; Penuelas *et al.*, 2004; Serrano *et al.*, 2010; Liu *et al.*, 2011; Paradiso *et al.*, 2011).

Here we used four reflectance based indices to evaluate the applicability in tropical rain forests, namely, water index (WI), red edge position (REP), chlorophyll normalized difference index ($_{chl}$NDI) and photochemical reflectance index (PRI). All of them changed significantly with water availability (Fig. 5). Nevertheless, only $_{chl}$NDI and PRI proved to be clearly suited for tropical rain forests, in predicting chlorophyll concentrations and/or drought stress.

Water index is derived from the near infrared water absorption band centered at 970nm and has been shown to be effective in the measuring water concentration of whole plants or canopies (Peñuelas & Filella, 1998). Taking into account that at predawn, leaf water potential reflects whole tree water potential, we would expect WI to be strongly related with Ψ_{PD}. Indeed no correlation was found. We found only a significant correlation between WI and maximal stomatal conductance (g_{smax}) in the Control plot ($r=0.48$, $p=0.020$). When considered TFE plot or pooled data, the correlation was not significant. In a study with drought stress in a Mediterranean vineyard, Serrano *et al*. (2010) found a negative close correlation between WI and g_s, although the relationship was treatment dependent. May be this index is only effective for lower leaf water

potentials. Nevertheless, the values found in this study are in the same range than those found for vineyard (Serrano *et al.*, 2010)

The `red edge' is the name given to the abrupt reflectance change in the 680±740 nm region of vegetation spectra that is caused by the combined effects of strong chlorophyll absorption and leaf internal scattering (Dawson & Curran, 1998). Red edge position (REP) represents the point of maximum slope on the reflectance spectrum of vegetation between red and near-infrared wavelengths (Dawson & Curran, 1998). Dawson & Curran (1998) have presented several techniques for interpolating the red edge position. We have chosen the simplest one to access the applicability of this reflectance based index in tropical rain forests. *REP* is usually strongly correlated with foliar chlorophyll content and provides a very sensitive indicator of vegetation stress. Stress causes a movement of the point of maximum slope to lower wavelengths. *REP* was significantly affected by water stress, only at the end of the study period (Fig. 5b) and showed a trend toward higher reductions in top canopy trees (Fig. 6i), although not significant. The *REP* range was lower compared to another study (Poulos *et al.*, 2007), but was significantly correlated with chlorophyll concentrations ($r=0.717$, $p<0.001$; $r=0.625$, $p<0.001$; $r=0.691$, $p<0.001$, for chl_a, chl_b and chl_{a+b}., mg cm^{-2}, respectively).

$_{chl}NDI$ is known to be sensitive to chlorophyll *a* concentrations (Gitelson & Merzlyak, 1994). $_{chl}NDI$ showed a very similar pattern to *REP* (Fig. 5c and Fig. 6g-i). High chlorophyll levels were associated with high $_{chl}$NDI (Levizou & Manetas, 2007). A significant reduction in $_{chl}$NDI with reduced water availability was also found in other woody species in response to drought stress (Zhang *et al.*, 2007). The range $_{chl}$NDI was narrower than found elsewhere (Levizou & Manetas, 2007; Poulos *et al.*, 2007; Zhang *et al.*, 2007). $_{chl}$NDI was significantly correlated with, not only chl_a concentration, but also with chl_b and chl_{a+b} concentrations on an area basis ($r=0.654$, $p<0.001$; $r=0.558$, $p<0.001$; $r=0.638$, $p<0.001$, for chl_a, chl_b and chl_{a+b}, respectively).

PRI is considered to be an index of the chlorophyll/carotenoid ratios (Gamon *et al.*, 1997; Sims & Gamon, 2002). *PRI* was, like the other indices, strongly reduced by water availability (Fig. 5d). Although we did not find significant differences between groups, top canopy trees showed the highest reduction at the end of the study period (73% in TFE compared to Control plot). Trees from the TFE plot increased the carotenoid/chlorophyll ratio in response to drought stress that is a response to stress

(Sims & Gamon, 2002). High carotenoid/chlorophyll ratios are associated with low *PRI* (Levizou & Manetas, 2007). This may indicate both a variation in xanthophyll cycle pigments between plants with different capacities for photosynthetic utilization of the photon flux density or variation in chlorophyll/carotenoid ratios that have developed in response to longer term acclimation of the plants to their local environment (Sims & Gamon, 2002). Total carotenoid to chlorophyll ratios are reduced in leaves under low light (Levizou & Manetas, 2007). Chlorophyll tends to decline more rapidly than carotenoids when plants are under stress (Gitelson & Merzlyak, 1994). *PRI* values are often reported as negative, probably because most of the studies are performed in dry environments. It was reported that PRI was not suited for use across wide ranges in illumination from deep shade to full sun (Gamon *et al.*, 1997) but was significantly correlated with net CO_2 uptake (Gamon *et al.*, 1997). The data suggest adequacy of this index but data on carotenoid concentration are needed to quantitatively evaluate this index. PRI was significantly correlated with g_{smax}, when considered plots separately ($r=0.443$, $p=0.345$ and $r=-0.622$, $p=0.002$, for Control and TFE plots, respectively), but not correlated with pooled data.

Leaves demonstrate a considerable capacity to acclimate morphological, anatomical, and physiological traits to different environments (Craven *et al.*, 2011). In the present paper we showed that leaf optical properties, as well as chlorophyll concentrations, changed in response to decreased water availability and that those responses differ along the vertical canopy position. Our results indicate that leaf structural changes may occur as an acclimation strategy to drought, although more data is needed to confirm these findings. Finally, the significant correlations found with reflectance based indices indicate that measurements of leaf optical properties might be useful in practical application for estimation of the drought tolerance level.

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Chapter 4

Impacts of experimentally imposed drought on leaf respiration and morphology in an Amazon rain forest

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Impacts of experimentally imposed drought on leaf respiration and morphology in an Amazon rain forest

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Summary

1. The Amazon region may experience increasing moisture limitation over this century. Leaf dark respiration (R) is a key component of the Amazon rain forest carbon (C) cycle, but relatively little is known about its sensitivity to drought.

2. Here, we present measurements of R standardized to 25 °C and leaf morphology from different canopy heights over 5 years at a rain forest subject to a large-scale through-fall reduction (TFR) experiment, and nearby, unmodified Control forest, at the Caxiuanã reserve in the eastern Amazon.

3. In all five post-treatment measurement campaigns, mean R at 25 °C was elevated in the TFR forest compared to the Control forest experiencing normal rainfall. After 5 years of the TFR treatment, R per unit leaf area and mass had increased by 65% and 42%, respectively, relative to pre-treatment means. In contrast, leaf area index (L) in the TFR forest was consistently lower than the Control, falling by 23% compared to the pre-treatment mean, largely because of a decline in specific leaf area (S).

4. The consistent and significant effects of the TFR treatment on R, L and S suggest that severe drought events in the Amazon, of the kind that may occur more frequently in future, could cause a substantial increase in canopy carbon dioxide emissions from this ecosystem to the atmosphere.

Key-words: tropical forest, climate change, moisture deficit, leaf dark respiration, night-time foliar carbon emissions, specific leaf area, leaf area index, through-fall exclusion experiment

Introduction

Leaf dark respiration (R) of carbon dioxide (CO_2) is a key component of the Amazon rain forest ecosystem carbon (C) cycle but remains poorly understood and rarely measured, compared to other ecosystem fluxes such as photosynthesis and soil CO_2 efflux (Malhi, Baldocchi & Jarvis 1999; Chambers *et al.* 2004b; Meir *et al.* 2008). This lack of knowledge impedes attempts to predict the impacts of current and future environmental change upon C cycling in the Amazon rain forest.

In the case of the Amazon, of particular interest is the effect of water availability upon R because the region may experience increasingly frequent and severe drought events associated with global climate change, fire and deforestation over the next 100 years (Werth & Avissar 2002; Christensen *et al.* 2007; Cox *et al.* 2008; Harris, Huntingford & Cox 2008; Malhi *et al.* 2008). Previous periods of drought during El Niño events have appeared to cause a shift in regional scale C exchange across the entire Amazon forest from a net C sink to a source of up to $1{\cdot}5 \times 10^9$ t C year^{-1}

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(Roedenbeck *et al*. 2003; Zeng, Mariotti & Wetzel 2005). Models that simulate the interactions between forest and atmosphere have been able to approximate this inter-annual pattern of regional C fluxes by simulating a simultaneous decline in C uptake via photosynthesis and a rise in ecosystem respiration during drier and warmer years (Tian *et al*. 1998; Peylin *et al*. 2005; Zeng, Mariotti & Wetzel 2005). Several studies have attempted to experimentally corroborate these model predictions. While there is reasonable supporting evidence for a drought-induced reduction in photosynthesis (Chaves & Oliveira 2004; Flexas *et al*. 2004; Fisher *et al*. 2007), most available data on ecosystem respiration suggest that it decreases under dry conditions (Saleska *et al*. 2003; Vourlitis *et al*. 2005; Hutyra *et al*. 2007; Meir *et al*. 2008). Much of this drought-induced inhibition may be attributable to a decline in the largest single component of ecosystem respiration-soil CO_2 efflux (Davidson *et al*. 2000; Schwendenmann *et al*. 2003; Sotta *et al*. 2004, 2007; Metcalfe *et al*. 2007).

However, ecosystem respiration is a composite flux derived from not only soil CO_2 efflux but also *R* and other sources, which may each respond to environmental change in different ways. Foliar C emissions per unit ground area are the integrated product of *R*, leaf area index (*L*) and the vertical distribution of *R* and *L* through the forest canopy. *L*, in turn, is the product of foliar biomass per unit ground area (*M*) and specific leaf area (*S*). All of these parameters are potentially sensitive to changes in water availability (Nepstad *et al*. 2002; Hanson & Wullschleger 2003; Miranda *et al*. 2005; Wright *et al*. 2006; Fisher *et al*. 2007; Myneni *et al*. 2007; Brando *et al*. 2008). There currently exists relatively little information about the individual responses of *R*, *S*, *L* and *M* to drought in the Amazon and even fewer data on the net effect of drought-induced changes in these parameters upon stand-scale foliar C emissions. Thus, it remains unclear to what extent measured responses of Amazon forest soil CO_2 efflux may be offset or accentuated by simultaneous shifts in foliar C emissions.

The overall objective of this study, therefore, was to assess the sensitivity to drought of *R*, *S*, *L* and *M* at an eastern Amazon rain forest site. The impact of sustained drought was estimated by comparing measurements made in a 1-ha plot where *c*. 50% of incident rainfall had been excluded (through-fall reduction or TFR plot) to an adjacent, similar but unmodified, Control plot. While the TFR treatment was not replicated (Hurlbert 1984, 2004) because of logistical and financial constraints, it did provide insights into ecosystem processes that would otherwise have been impossible to capture in smaller scale, more easily replicated experiments (Reviews: Carpenter 1996; Sullivan 1997; Osmond *et al*. 2004; Stokstad 2005; Field studies: Nepstad *et al*. 2002; Davidson, Ishida & Nepstad 2004; Fisher *et al*. 2007; Metcalfe *et al*. 2007; Sotta *et al*. 2007; Brando *et al*. 2008). Specifically, we used a 'before-after-control-impact' (Underwood 1997; Rasmussen *et al*. 2001; Gotelli & Ellison 2004) approach to test for significant shifts in *R* and *S* both (i) Over time- before and after the imposition of the TFR treatment and between dry and wet seasons, and (ii) Between the TFR treatment and Control for each individual measurement campaign. Finally, we use existing *L* data to upscale leaf-level *R* measurements to derive plot estimates of foliar night-time C effluxes.

Materials and methods

STUDY SITE

The experimental site is located in the Caxiuanã National Forest, Pará State, north-eastern Brazil (1°43′3·5″S, 51°27′36″W). The forest is a lowland *terra firme* rain forest situated on a level plain 10–15 m above river water level, with a high annual rainfall (~2500 mm) and a pronounced seasonality in leaf fall which peaks during the strong dry season (see Table 1 for additional plot details). Plant species diversity is high at around 100 species per hectare, of which over half are *Sapotaceae*, *Fabaceae*, *Violaceae* and *Chrysobalanacae*, and less than 1% are lianas. Mean annual air temperature is *c*. 25 °C and the diurnal variation is typically less than 3 °C. The most widespread soil type is a highly weathered yellow Oxisol (US Department of Agriculture soil taxonomy). In January 2002, a 1-ha area of forest was modified with the installation of plastic panels at 2 m height to exclude *c*. 50% of incident rainfall (TFR plot). This reduction in rainfall is similar to a key early long-term climate prediction for the region (Cox *et al*. 2000). The perimeter of the TFR plot was trenched to a mean depth of 1 m and lined with plastic sheeting to minimize lateral flow of water into the site. Intercepted water was channelled away to a run-off area 50 m away from the plot. An adjacent 1-ha Control plot with similar topography, soil type and vegetation structure (Fisher *et al*. 2007) was used to assess natural patterns of *L*, *S* and *R*, in the absence of any TFR treatment. Supplementary measurements during the first 3 years of the TFR treatment demonstrated that soil water potential, tree stem sapflow, stomatal conductance and photosynthesis were all substantially reduced in the TFR plot compared to the Control, particularly during the dry season (Fisher *et al*. 2007). At the beginning of the experiment in January 2001, 30 m tall canopy access towers were installed near the centre of both plots. All measurements were

Table 1. Key vegetation and soil features for each plot surveyed

Plot characteristics	Control	TFR
Vegetation		
Tree number ha^{-1}	434	421
Stem basal area (m^2 ha^{-1})	23·9	24·0
Tree species ha^{-1}	118	113
Soil 0–10 cm		
Clay content (%)	18	13
Silt content (%)	5	4
Sand content (%)	77	83
pH	4	4
Carbon content (g kg^{-1})	9	12
Nitrogen content (g kg^{-1})	0·4	0·3
Phosphorus content (mg dm^{-3})	3	3
Carbon : nitrogen ratio	23	35
Soil cation exchange (cmol dm^{-3})	0·8	0·7

TFR, through-fall reduction. Tree number and basal area represents all individuals over 10 cm diameter at breast height, measured in January 2005. Soil values are collated from data in Sotta *et al*. (2007).

taken at least 20 m inside the perimeter of each plot to minimize edge effects.

MEASUREMENT OF LEAF DARK RESPIRATION AND SPECIFIC LEAF AREA

R and *S* from trees on both plots were recorded on six occasions between November 2001 and January 2007: once before and five times after imposition of the TFR treatment. *L* data are also available from the same periods on both plots (Fisher *et al.* 2007). All measurement campaigns sampled fully expanded, non-senescent, un-diseased leaves, and recorded additional information about the height and tree species of the sampled leaves. Thus, *R* measurements from these leaves should primarily reflect 'maintenance' respiration rather than 'growth' respiration associated with metabolic costs of constructing new plant tissue (McCree 1970). All leaves were sampled during the daytime (08.30–15.00 h) and kept in the dark until CO_2 gas exchange had stabilized (usually after 5–10 min) before *R* at ambient air CO_2 concentration (360–380 p.p.m.) and humidity (60–80%) was recorded, thus minimizing biases potentially introduced by light-enhanced dark respiration and the photorespiratory post-illumination burst (Atkin, Evans & Siebke 1998).

The first five measurement campaigns (conducted between November 2001 and 2003) used the following methodology: 17–26 leaves from nine trees, and 18–26 leaves from eight trees were sampled around the canopy access towers on the Control and TFR plots respectively (Table 2). Measurements were taken from the same trees and from leaves at the same canopy heights in each measurement campaign. *R* was measured *in situ* from un-excised leaves with an infra-red gas analyser (IRGA) connected to a leaf measurement cuvette (LI-COR 6400 portable photosynthesis system with 6400-02B leaf cuvette; Lincoln, NE, USA). Leaf discs of a known area were cut from leaves on the same branch as leaves used for *R* measurement, dried at 70 °C until constant mass and weighed. *S* was calculated for each leaf disc sampled by dividing dry mass by one-sided area.

The final measurement campaign (in January 2007) sampled a total of 33 and 28 individual leaves from the Control and TFR plots, respectively, from 15 separate trees per plot. Of these trees sampled, 10 were randomly selected as the closest tree to every 10 m intersection point along two 40 m long transects in the centre of the plot. A branch from the outer canopy of each selected tree was excised at between one and three different canopy heights. No attempt was made to cut and re-cut branches under water because this would not have guaranteed that gas exchange remained unaltered (Santiago & Mulkey 2003). Instead, we designed an experiment to quantify and, if necessary, correct for any impacts of branch excision (see text in Methods section). To facilitate sampling of leaves higher up in the emergent canopy and to replicate measurements on individual trees made earlier, an additional five trees per plot were similarly sampled around the canopy access towers on each plot. *R* was measured for most leaves within 3 h of branch excision, using an IRGA connected to a leaf measurement cuvette (CIRAS-1 IRGA with PLC6 leaf cuvette; PP Systems, Hitchen, UK). The interval of time between branch excision and *R* measurement was noted for each leaf sampled. There was no significant difference in the mean time between excision and *R* measurement on the plots.

Table 2. Tree species sampled on the plots

Control	TFR
Duguetia echinophora	*Duguetia echinophora*
Hasseltia floribunda	*Hirtela bicornis*
Licania heteromorpha	*Lecythis confertiflora*
Manilkara bidentata	*Licaria armeniaca*
Mezilaurus lindawiana	*Licania canescens*
Pouteria lateriflora	*Manilkara paraensis*
Protium heptaphyllum	*Mouriri duckeana*
Quiina florida	*Swartzia racemosa*

TFR, through-fall reduction. An individual representative of each species (two individuals of *Quiina florida* on the Control plot) was repeatedly sampled between November 2001 and 2003. In the final measurement campaign (January 2007) trees sampled were not identified to species level.

After measurement, the same leaves were photographed to calculate leaf area with digital image analysis, and then dried at 70 °C until constant mass and weighed. *S* was calculated for each entire leaf sampled (including petioles) by dividing dry mass by one-sided area.

Measurements made with the LI-COR 6400 IRGA maintained a flow rate of 500 μmol s^{-1}, with a mean ± standard error (SE) difference between C_r and C_s of 0·52 ± 0·07 p.p.m. The CIRAS-1 IRGA was set to a lower flow rate of 200 μmol s^{-1}, and consequently the observed mean ± SE CO_2 difference was 0·91 ± 0·06 p.p.m. The inward diffusion of respired CO_2 from leaf material clamped under the cuvette gasket (Pons & Welschen 2002) and the diluting effect of water vapour produced by the leaf was corrected for.

Leaf temperatures recorded automatically by the IRGA systems during *R* measurement varied between 22 and 31 °C. Species-specific *R* temperature response functions were not available for all of the trees sampled, so measurements were standardized to a reference temperature of 25 °C (R_{25}) with the following formula that describes the average *R* temperature response across 116 terrestrial plant species (Atkin & Tjoelker 2003; Atkin, Bruhn & Tjoelker 2005):

$$R_{25} = R_\alpha\{3{\cdot}09 - 0{\cdot}0435[25 + T_\alpha]/2\}^{[(25-T_\alpha)/10]}$$

where R_a is *R* recorded at ambient temperature (T_a).

To investigate the potential confounding influences of branch excision on the *R* values recorded in January 2007, the following experiment was devised. An un-excised leaf was placed within the IRGA cuvette and *R* was measured every minute for 1 h. After this period, the branch attached to the leaf within the cuvette was excised, but *R* measurement was continued at the same temporal frequency for 5 h, to observe whether there was any change in *R* with time since branch excision. Over this period, the sensor was regularly automatically calibrated with air passed through a molecular sieve to remove all CO_2. Before and during measurements the molecular sieve was frequently checked to ensure that it was not exhausted. This procedure was repeated three times, on consecutive days from three individual leaves each on separate trees of different species. All leaves sampled showed no change in *R* over the hour prior to excision, but after excision *R* rose gradually over time, approximately doubling after 5 h compared to the pre-excision mean value (data not shown). A third-order polynomial model was fitted to the mean trend of *R* over time since branch excision (R^2 = 0·77). This model was not chosen as a realistic mechanistic simulation of plant gas exchange, but purely for limited predictive purposes over the duration of the measurements because it provided the best fit to the data. This equation, together with data collected on the interval of time between branch excision and *R* measurements for each leaf, was used to correct for the confounding effect of excision and storage on January 2007 measurements by calculating *R* at time since excision = 0 for each leaf sampled. No immediate effect of excision itself on *R* was apparent.

MEASUREMENT OF LEAF AREA INDEX AND FOLIAGE MASS

Mean plot L estimated during the first five measurement campaigns (conducted between November 2001 and 2003) is presented in Fisher *et al.* (2007). These data were derived from canopy images captured at 100 points per plot with LAI-2000 plant canopy analysers (LI-COR Inc.). For this study, additional L data were collected in January 2007 based upon canopy images per plot collected at 25 locations along a with a digital camera and fish-eye lens (Nikon Coolpix 900; Nikon Corporation, Melville,USA) and subsequently analysed with digital image analysis software (Hemiview 2.1 SR1; Delta-T Devices Ltd, Cambridge, UK). All images of the canopy were recorded in the early morning or late afternoon, during periods of fully diffuse incoming radiation along a regular grid within both plots (following the methodology of Aragão *et al.* (2005). The distribution of L with height above the ground on both plots was estimated once – in November 2001 – by recording L with the LAI-2000 plant canopy analysers (LI-COR Inc.) every 2 m up each of the plot canopy access towers. Plot-level M was estimated for each measurement campaign by multiplying mean plot leaf mass per unit leaf area ($1/S$) by L.

ESTIMATING STAND-SCALE NIGHT-TIME FOLIAR CARBON EFFLUX

To illustrate how R, S and L interact, and to facilitate direct comparison of our leaf-level R measurements with other ecosystem C fluxes, we derived approximate estimates of stand-scale foliar C efflux. To do so, we calculated mean ± 95% confidence intervals of L and R per unit area separately for three canopy height layers (≤10, 11–20, ≥21 m). Night-time foliar C emissions per unit ground area were estimated for each canopy layer as the product of R per unit leaf area multiplied by L. Given the low temporal frequency of our direct measurements and the focus on between-plot (rather than seasonal or annual) differences we opted for the relatively simple, more transparent, up-scaling approach of assuming constant night-time air temperature of 25 °C and 12 h of dark conditions each day throughout the year. For the purposes of this analysis, we also assumed that dark-equilibrated R recorded during the day in this study was representative of night-time leaf respiration (Chambers *et al.* 2009; but see Hubbard, Ryan & Lukens 1995). Means from each canopy layer were summed to derive total plot estimates. Where necessary, 95% confidence intervals were propagated by quadrature of absolute errors for addition and subtraction, and quadrature of relative errors for multiplication and division (Mood, Graybill & Boes 1974; Cavaleri, Oberhauer & Ryan 2008). This assumes that errors are independent and normally distributed.

DATA ANALYSIS

To assess the impact of the TFR treatment on S and R the following two statistical analyses were performed. (i) Within-plot change over time since the imposition of the TFR treatment was quantified with a repeated-measures analysis of variance (RM-ANOVA). Data from the final measurement campaign were not included in the RM-ANOVA because a different set of trees were sampled with a different methodology, whereas the previous five campaigns repeatedly sampled leaves from the same trees and same canopy heights. To examine specifically which time periods differed from each other in terms of S and R, pairwise comparisons between measurement campaigns were conducted within the RM-ANOVA analysis. (ii) Between-plot differences in R and S over all measurement campaigns were quantified with a Generalized Linear Model (GLM) with plot as a fixed-effects factor, and leaf height, tree family and sampling time specified as random-effects factors to control for the potentially confounding effect of sampling differences between plots. Using this method, plot differences were examined both for all data at each measurement campaign, and for all data in different canopy height categories (≤10, 11–20, ≥21 m). In addition, the links between leaf height, R and S were assessed with a Spearman's Rank Correlation. Statistical analyses were carried out with SPSS 14·0 for Windows (SPSS Inc., Chicago, IL, USA). Key outputs of the analyses were an F-statistic (for the RM-ANOVA and GLM), a correlation coefficient (r, for the Spearman's Rank Correlation analysis) and a significance P-value for all tests. Data were transformed with a natural logarithm, where necessary, to conform to the assumptions of parametric analysis.

Results

PLOT TRENDS IN STAND-SCALE NIGHT-TIME FOLIAR CARBON EFFLUX

R per unit area together with L data, apportioned into canopy height categories (≤10, 11–20, ≥21 m), were used to estimate total ± 95% confidence intervals foliar night-time C emissions prior to the TFR treatment of 3·4 ±0·1 and 3·4 ±0·3 t ha^{-1} year^{-1} on the Control and TFR plots respectively (Table 3). According to these estimates, elevated R on the TFR plot relative to the Control was not fully offset by the drought-associated decline in L, such that night-time foliar C emissions on the TFR plot were 0·7 ± 0·4 and 1·8 ± 0·9 t C ha^{-1} year^{-1} greater than the Control plot based upon measurements in November 2003 and January 2007 respectively (Table 3).

PLOT TRENDS IN LEAF DARK RESPIRATION

Plot mean R values were comparable to existing data from other studies in the Amazon forest (Table 4). In the Control plot, there was no significant overall change in R per unit area (RM-ANOVA, d.f. = 4, F = 0·77, P = 0·56) and mass (RM-ANOVA, d.f. = 4, F = 1·78, P = 0·17) between November 2001 and 2003 (Table 5, Fig. 1c,d). In contrast,

Table 3. Plot estimates of night-time stand-scale foliar C efflux (t C ha^{-1} year^{-1})

	November 2001	May 2002	November 2002	May 2003	November 2003	January 2007
Control	3·4 ± 0·1	4·0 ± 0·4	4·2 ± 1·9	3·5 ± 0·6	4·6 ± 0·2	4·1 ± 0·4
TFR	3·4 ± 0·3	4·2 ± 0·4	3·1 ± 0·2	4·8 ± 1·1	5·3 ± 0·3	5·9 ± 0·8
Difference TFR − Control	0·0 ± 0·4	0·2 ± 0·6	−1·1 ± 1·9	1·4 ± 1·2	0·7 ± 0·4	1·8 ± 0·9

TFR, through-fall reduction. Values are estimated from instantaneous measurements at each date extrapolated over a year assuming constant air temperature of 25 °C and 12 h of dark conditions every night.

Table 4. A summary of available data on respiration per unit area at 25 °C from leaves at primary lowland *terra firme* rain forest sites in the Amazon

Source	Coordinates	Respiration (μmol m^{-2} s^{-1})	Notes
Reich *et al.* (1998)	1°56′N, 67°03′W	0·91 ± 0·23*	
Meir, Grace & Miranda (2001)	10°05′S, 61°55′W	0·36 ± 0·20†	
Domingues *et al.* (2005)	3°33′S, 55°83′W	0·43 ± 0·36‡	Wet season
		0·57 ± 0·39‡	Dry season
Miranda *et al.* (2005)	11°25′S, 55°20′W	0·33 ± 0·17‡	Wet season
		0·66 ± 0·37‡	Dry season
Cavaleri, Oberhauer & Ryan (2008)	10°20′N, 83°50′W	0·59 ± 0·44†	
This study	1°43′3·5″S, 51°27′36″W	0·32 ± 0·13‡	Control plot, November 2001
		0·33 ± 0·17‡	TFR plot, November 2001
		0·41 ± 0·20‡	Control plot, January 2007
		0·55 ± 0·27‡	TFR plot, January 2007

TFR, through-fall reduction. Respiration values represent mean ± standard deviation. Respiration data collected in this study in November 2001 and January 2007 used different methods and sampling strategies.
*Measured at 25 °C.
†Corrected to 25 °C using site-specific temperature response curves.
‡Corrected to 25 °C using a generic temperature response curve (Atkin & Tjoelker 2003; Atkin, Bruhn and Tjoelker 2005).

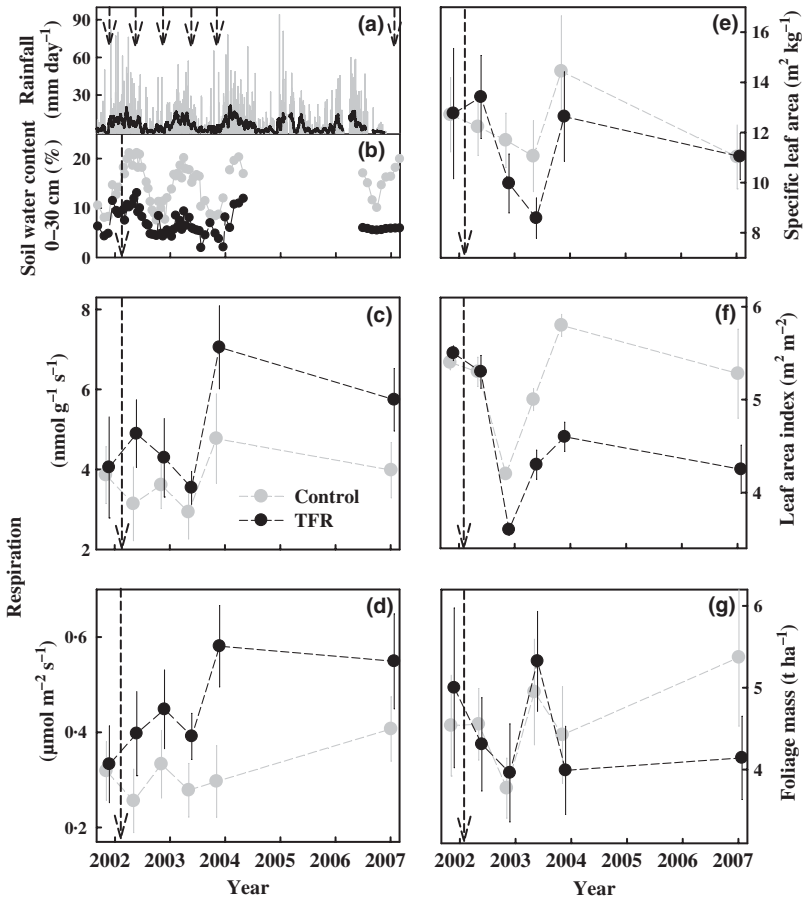

Fig. 1. Seasonal trends in site rainfall (a), plot surface soil water content (b), respiration per unit mass (c) and area (d), specific leaf area (e), leaf area index (f) and foliage mass (g) over the study period. Values denote plot means ± 95% confidence intervals. TFR, through-fall reduction. Rainfall is presented both on a daily basis (grey) and as a 15-day running average (black). The vertical dashed arrows in (a) mark leaf sampling times, and in (b)–(g) mark the date of TFR treatment establishment. *L* data for the measurement campaigns between November 2001 and 2003 are reproduced from Fisher *et al.* (2007).

over the same period on the TFR plot, *R* per unit area (RM-ANOVA, d.f. = 4, F = 5·24, P = 0·004) and mass (RM-ANOVA, d.f. = 4, F = 5·14, P = 0·004) increased significantly (Table 5, Fig. 1c,d). Specifically, while *R* per unit area on the TFR plot began to rise immediately after imposition of the treatment (Table 5, Fig. 1d), it only became significantly higher compared to pre-treatment values during the peaks of the dry season in November 2002 (RM-ANOVA pairwise comparison, P = 0·011) and 2003 (RM-ANOVA pairwise comparison, P = 0·003). *R* per unit mass showed a slightly different temporal trend following the TFR treatment (Table 5, Fig. 1c) becoming significantly higher compared to

Table 5. Significance *P*-values for differences in respiration and specific area from branches re-sampled over five successive measurement campaigns in both plots

November 2001	May 2002	November 2002	May 2003	November 2003
Respiration per unit area				
Control plot				
November 2001				
May 2002	**0·21**			
November 2002	**0·94**	0·08		
May 2003	**0·28**	0·88	0·40	
November 2003	**0·89**	0·44	0·89	0·22
TFR plot				
November 2001				
May 2002	**0·31**			
November 2002	**0·011***	0·19		
May 2003	**0·21**	0·75	0·28	
November 2003	**0·003****	0·01**	0·27	0·06
Respiration per unit mass				
Control plot				
November 2001				
May 2002	**0·16**			
November 2002	**0·45**	0·13		
May 2003	**0·31**	0·98	0·64	
November 2003	**0·28**	0·13	0·30	0·042*
TFR plot				
November 2001				
May 2002	**0·25**			
November 2002	**0·48**	0·89		
May 2003	**0·52**	0·038*	0·17	
November 2003	**0·027***	0·045*	0·08	0·004**
Specific leaf area				
Control plot				
November 2001				
May 2002	**0·60**			
November 2002	**0·049***	0·25		
May 2003	**0·42**	0·74	0·63	
November 2003	**0·23**	0·12	0·047*	0·014*
TFR plot				
November 2001				
May 2002	**0·51**			
November 2002	**0·24**	0·016*		
May 2003	**0·07**	0·002**	0·039*	
November 2003	**0·93**	0·31	0·08	

TFR, through-fall reduction. Values are derived from an RM-ANOVA pairwise comparison. Significant ($P < 0·05$) and very significant ($P < 0·01$) differences are marked with single and double asterisks respectively. Values in bold highlight differences compared to data collected prior to imposition of the TFR treatment (November 2001).

the pre-treatment mean only by November 2003 (RM-ANOVA pairwise comparison, $P = 0·027$).

Directly comparing plot R estimates from each measurement campaign and controlling for plot sampling differences in tree family, leaf height and sampling time showed that there was no significant plot difference in R per unit area (GLM, d.f. = 1, $F = 0·30$, $P = 0·68$) and mass (GLM, d.f. = 1, $F = 4·34$, $P = 0·28$) prior to imposition of the TFR treatment (Fig. 1c,d). However, after the TFR treatment, measured R on the TFR plot increased (Table 5, Fig. 1c,d) until it became significantly higher than the Control in January 2007 (GLM, d.f. = 1, Per unit mass: $F = 6·12$, $P = 0·035$; Per unit area: $F = 16·91$, $P = 0·002$). This plot difference in R was mainly attributable to a significantly lower rate of R per unit mass (GLM, d.f. = 1, $F = 28·38$, $P < 0·001$) and area (GLM, d.f. = 1, $F = 45·77$, $P < 0·001$) in lower canopy leaves (≤10 m above the ground surface) on the TFR plot compared to the Control (Fig. 2a,b). No significant plot difference was found in upper canopy leaves (≥21 m above the ground surface).

There was a highly significant positive correlation between leaf height above the ground and R per unit area in the Control plot (Fig. 2b, Spearman's Rank Correlation, $n = 147$, $r = 0·48$, $P < 0·001$), while on the TFR plot the trend was weaker but still present (Fig. 2b, Spearman's Rank Correlation, $n = 141$, $r = 0·16$, $P = 0·054$). However, when quantified on a per unit mass basis, R in the Control plot ceased to show any significant pattern of change with leaf height above the ground (Fig. 2a).

PLOT TRENDS IN SPECIFIC LEAF AREA

Overall, S showed significant change over time in both the Control (Table 5, Fig. 1e, RM-ANOVA, d.f. = 4, $F = 3·16$, $P = 0·036$) and TFR plots (Table 5, Fig. 1e, RM-ANOVA, d.f. = 4, $F = 4·34$, $P = 0·009$). However, this change reflected bidirectional seasonal and annual variation (Fig. 1e) rather than progressive unidirectional change over time: in the Control plot only mean S measured in November 2002 was significantly different from the pre-treatment mean (Table 5, RM-ANOVA pairwise comparison, $P = 0·049$) while none of the S measurements in the TFR plot diverged significantly from initial values recorded in November 2001 (Table 5). Once variation derived from sampling differences was excluded, no significant difference in S between plots during any individual measurement campaign was found. Neither was there any significant plot difference in mean S in the different canopy height categories (Fig. 2), although on both plots, leaves in the upper canopy tended to have lower S compared to understorey leaves (Fig. 3c, Control plot: Spearman's Rank Correlation, $n = 135$, $r = -0·56$, $P < 0·001$; TFR plot: Spearman's Rank Correlation, $n = 129$, $r = -0·54$, $P < 0·001$).

PLOT TRENDS IN LEAF AREA INDEX

Before imposition of the TFR treatment both plots had similar L (~5·5 m^2 m^{-2}), but after the treatment L on the TFR plot declined, reaching a value of *c.* 4·5 m^2 m^{-2} (~1 m^2 m^{-2} or 20% lower than the Control) after almost 2 years of the TFR treatment (Fig. 1f). On both plots, there was an abrupt and substantial decline in L measured during November 2002 which was gradually recovered over subsequent years (Fig. 1f).

According to tower L height profile measurements in November 2001, slightly less L was located in the lower canopy on the TFR plot compared to the Control, such that 65% and 71% of total L occurred above 20 m on the Control and TFR plots respectively (Fig. 3d).

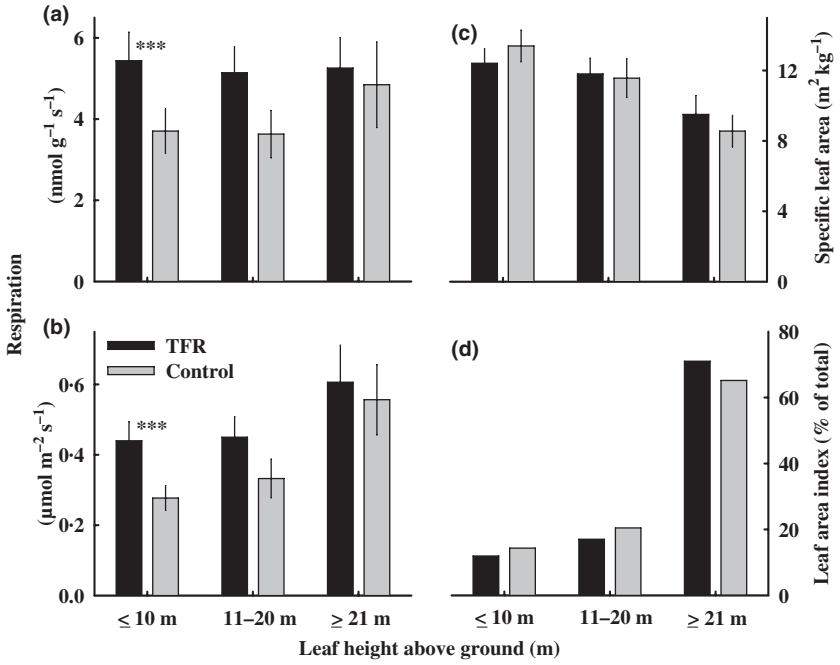

Fig. 2. Plot differences in respiration per unit mass (a) and area (b), specific leaf area (c) and leaf area index (d) amongst canopy height categories (≤10, 11–20, ≥21 m). TFR, through-fall reduction. Values denote plot means ± 95% confidence intervals from data pooled across all post-treatment sampling sessions, with the exception of the *L* canopy profile which was measured only once, before the drought treatment in November 2001. ***Significant plot differences ($P < 0{\cdot}001$).

PLOT TREND IN FOLIAGE MASS

On both plots over all measurement campaigns, *M* varied between 4 and 5·5 t ha^{-1} (Fig. 1g). There was no clear difference in *M* between the plots over the first few years of the TFR treatment, because lower TFR plot *L* (Fig. 1f) was offset by higher *S* (Fig. 1e) compared to the Control. This changed by the last measurement campaign (January 2007) when the TFR plot *S* fell back to levels similar to that of the Control, but *L* remained relatively low, with the consequence that estimated *M* was 1·2 ± 0·7 lower on the TFR plot compared to the Control (Fig. 1e–g).

Discussion

ESTIMATING STAND-SCALE NIGHT-TIME FOLIAR CARBON EFFLUX

Combining leaf-level *R* and *S* data with *L*, to produce estimates of stand-scale foliar respiration, illustrates how their individual responses interact to determine ecosystem foliar C emissions, and how this differs between plots. The results indicate that the effect of the decline in the amount of respiring leaf tissue in the TFR plot, measured as *L*, was outweighed by the simultaneous increase in *R* per unit area. The estimated net consequence of these opposing changes was that night-time foliar C emissions from the TFR plot increased by 1·4 ± 1·2, 0·7 ± 0·4 and 1·8 ± 0·9 t ha^{-1} year^{-1} compared to the Control in the last three measurement campaigns 1·4, 1·9 and 5·1 years after imposition of the TFR treatment respectively (Table 3). The three previous measurements (before, 0·4 and 0·9 years after the TFR treatment) showed no clear difference in night-time foliar C emissions between plots (Table 3). By comparison, the largest single ecosystem respiration term – soil CO_2 efflux – was estimated to be 4·2 and 0·7 t C ha^{-1} year^{-1} lower on the TFR plot relative to the Control in 2003 (Sotta *et al.* 2007) and 2006 (Metcalfe *et al.* 2007) respectively. Thus, incorporating the plot differences in night-time foliar C emissions estimated in this study offsets a large portion of the estimated drought-induced reduction in soil CO_2 efflux. Given that atmospheric CO_2 fertilization of Amazon vegetation is unlikely to stimulate net ecosystem uptake far in excess of 0·5 t C ha^{-1} year^{-1} (Phillips *et al.* 1998; Chambers *et al.* 2001, 2004a; Baker *et al.* 2004) we suggest that inclusion, and improved quantification, of night-time foliar C emissions in atmosphere-biosphere models could be vital for accurate prediction of changes in Amazon forest C exchange in response to climate change.

The approach taken in this study to up-scaling leaf-level *R* measurements makes a number of assumptions which deserve examination. First, it is unclear how confidently *R* measured during the day (albeit equilibrated to dark conditions) may be extrapolated to night-time conditions (Hubbard, Ryan & Lukens 1995), although at a similar Amazon rain forest type to our study site, *R* measured from 27 trees showed no clear diurnal variation in *R*, and no significant difference between night- and day-time values (Chambers *et al.* 2009). Secondly, our assumption of constant temperature (25 °C) on both plots and 12 h of darkness each day throughout the year is clearly simplistic though any error introduced is minor compared to natural intra- and inter-species variation, and affects both plots similarly. Thus, this method facilitates direct comparison of plots and, in the absence of more detailed process-level data, provides a transparent basis for up-scaling instantaneous measurements.

DROUGHT EFFECTS ON LEAF RESPIRATION AND MORPHOLOGY

Estimates of *R* from this study are consistent with previous estimates from other lowland Amazon rain forest ecosystems

(Table 4). While there were significant differences in R between the plots after several years of the TFR treatment, these differences were relatively minor compared to global variation amongst studies, sites and biomes (Wright *et al*. 2006). In this study, evidence for enhancement of R by drought comes from (i) the increase in R over time since imposition of the TFR treatment, (ii) the higher mean R compared to the Control during each individual measurement campaign and (iii) the slight but consistent increase in R on both plots during the dry seasons (Fig. 1, Table 5). While only some of these differences were statistically significant individually, the effect was consistent across all post-treatment measurement campaigns. Leaf nitrogen content did not differ between plots, neither was there a clear correlation between leaf nitrogen content and R (R. Lobo-do-Vale, unpublished data). Other studies in the Amazon have also recorded an increase in R in the dry season compared to the wet season (Table 4). In addition, a comprehensive survey across 208 woody plant species from 20, mainly temperate, sites showed that, for a given S, R was higher at low-rainfall sites compared to higher rainfall sites (Wright *et al*. 2006; but see Flexas *et al*. 2005; Atkin & Macherel 2008). The change in R in all these studies could either reflect a shift in R for a given S, or altered S with little concomitant change in R, or some combination of these two processes. Potential mechanisms for enhanced R at a given S under moisture stress include: increased energy demand for the maintenance of vacuolar solute gradients, repair of water-stress-induced cell damage and/or increased wastage respiration via futile cycles (Hue 1982; Lambers 1997; Lambers, Chapin & Pons 1998; Cannell & Thornley 2000; Flexas *et al*. 2005; Würth *et al*. 2005; Wright *et al*. 2006; Atkin & Macherel 2009). Determining which of these processes dominate will be important for modelling the pattern and magnitude of change in R across the Amazon in the face of future climate changes.

The reduction in L on the TFR plot (Fig. 1f) was consistent with existing data on Amazon forest responses to soil moisture deficit (Nepstad *et al*. 2002; Fisher *et al*. 2007; Myneni *et al*. 2007) and over the first 3 years of the TFR treatment was largely caused by declining S, because total plot M remained remarkably similar between the plots despite substantial seasonal variation (Fig. 1g). In January 2007, lower L on the TFR plot could not be attributed to S and was therefore most likely a product of an imbalance between leaf growth and shedding (Fig. 1g). Changes in S may reflect adaptation to drought on the part of TFR plot trees by developing thicker and/or denser leaves (Witkowski & Lamont 1991). The abrupt drop in L during November 2002 followed by a sharp rise in M (Fig. 1f,g) on both plots was also attributable to a decline in S. The reason for this change is not known. The meteorological conditions during this period do not appear to have been anomalous while the equipment and sampling strategy used to quantify L and S remained the same over this period. Taken together this suggests that the decline in L and S over this period was a real biological pattern perhaps linked to seasonal phenology rather than an artefact of methodology.

The pattern of change in R through the canopy was strikingly different between plots: with significantly higher rates of R (Fig. 2a,b) in foliage below 20 m on the TFR plot compared to the Control. It is unlikely that the infrastructure of the TFR plot itself could account for these differences because the panels diverting rainfall were installed to a maximum height of 2 m, while all R measurements were recorded in tree canopies above this height. We suggest that the plot differences in the pattern of change in R through the canopy more likely reflect the fact that most of the lower leaves sampled came from smaller stature trees with shallower root systems, which were likely to suffer more from surface soil moisture limitation.

Conclusion

This study evaluated the drought sensitivity of R at an eastern Amazon rain forest site. Partial rainfall exclusion of a 1-ha area of rain forest was associated with an estimated increase in night-time foliar C emissions of 1·4, 0·7 and 1·8 t ha^{-1} $year^{-1}$ compared to forest on a nearby Control plot 1·4, 1·9 and 5·1 years after rainfall exclusion respectively. This drought-induced physiological shift, if shown to occur more widely, might be sufficient to offset current estimates of the Amazon forest C sink, and alter model predictions of future changes in net C emissions from the Amazon basin. To build upon the key conclusions of this study more measurements are required to improve our understanding of the spatial and temporal variation in R, and of leaf respiration under light conditions.

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Chapter 5

Evidence from Amazonian forests is consistent with isohydric control of leaf water potential

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Evidence from Amazonian forests is consistent with isohydric control of leaf water potential

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ABSTRACT

Climate modelling studies predict that the rain forests of the Eastern Amazon basin are likely to experience reductions in rainfall of up to 50% over the next 50–100 years. Efforts to predict the effects of changing climate, especially drought stress, on forest gas exchange are currently limited by uncertainty about the mechanism that controls stomatal closure in response to low soil moisture. At a through-fall exclusion experiment in Eastern Amazonia where water was experimentally excluded from the soil, we tested the hypothesis that plants are isohydric, that is, when water is scarce, the stomata act to prevent leaf water potential from dropping below a critical threshold level. We made diurnal measurements of leaf water potential (Ψ_l), stomatal conductance (g_s), sap flow and stem water potential (Ψ_{stem}) in the wet and dry seasons. We compared the data with the predictions of the soil–plant–atmosphere (SPA) model, which embeds the isohydric hypothesis within its stomatal conductance algorithm. The model inputs for meteorology, leaf area index (LAI), soil water potential and soil-to-leaf hydraulic resistance (R) were altered between seasons in accordance with measured values. No optimization parameters were used to adjust the model. This 'mechanistic' model of stomatal function was able to explain the individual tree-level seasonal changes in water relations ($r^2 = 0.85$, 0.90 and 0.58 for Ψ_l, sap flow and g_s, respectively). The model indicated that the measured increase in R was the dominant cause of restricted water use during the dry season, resulting in a modelled restriction of sap flow four times greater than that caused by reduced soil water potential. Higher resistance during the dry season resulted from an increase in below-ground resistance (including root and soil-to-root resistance) to water flow.

Key-words: drought; psychrometer; sap flow; SPA model; stem water potential; stomatal conductance.

INTRODUCTION

Over the next 50–100 years, most global climate models predict that increasingly El Niño-like climate conditions will cause reduced rainfall over Eastern Amazonia (Cox *et al*. 2000; Cubasch *et al* 2001; Cowling *et al*. 2004; Cramer *et al*. 2004). Cox *et al*. (2004) suggested that the reduction in rainfall over Amazonia may reach as high as 50% by 2100. Reductions in rain forest evapotranspiration during the dry season have been observed at Manaus, in relatively wet and humid Central Amazonia, by using the eddy covariance technique (Malhi *et al*. 1998, 2002). However, more recent eddy covariance studies in Eastern Amazonia have not shown any limitation in forest evaporation during the dry season, even though these forests experience more intense dry seasons than the Manaus forest (Carswell *et al*. 2002; Saleska *et al*. 2003; Goulden *et al*. 2004; da Rocha *et al*. 2004). The lack of a simple correlation between the limitation of forest evapotranspiration and the intensity of the dry season implies that we cannot simply extrapolate the results of a small number of experiments uniformly across the Amazon region. A more sophisticated understanding of the drought physiology of rain forest trees is necessary before we can predict the responses to the predicted drier conditions.

To investigate the mechanisms underlying the response of forest evapotranspiration to reduced rainfall, we conducted a manipulation study on a 1 ha parcel of land in Caxiuanã, Eastern Amazonia. Rain passing through the canopy (through-fall) was intercepted by a system of plastic panels and then drained away from the plot, thereby artificially reducing soil moisture. Within this through-fall exclusion (TFE) site and an adjacent control site, we collected diurnal cycles of leaf water potential (Ψ_l), stem water potential (Ψ_{stem}), stomatal conductance (g_s) and sap flow from a sample of trees during the wet and dry seasons. We used the tree physiology data to investigate three areas of uncertainty: (1) the mechanism by which the stomata sense and respond to soil water status; (2) whether leaf water supply is controlled by changes in soil water potential (Ψ_s) or in soil-to-leaf hydraulic resistance (R); (3) and whether major resistance to water uptake is located above or below ground.

The first part of our investigation involved testing the hypothesis that the stomata sense reduced soil moisture via 'hydraulic signalling' between soil water potential and leaf water potential (Jones 1998; Salleo *et al*. 2000; Chaves, Maroco & Pereira 2003). In this hypothesis, low soil mois-

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ture affects stomatal conductance and gas exchange via its influence on leaf water potential. It has been repeatedly observed that some plants act to maintain leaf water potential above a critical minimum value, or 'isohydric' conditions, under hydraulically stressed circumstances (Field & Holbrook 1989; Tardieu 1993; Saliendra, Sperry & Comstock 1995; Cochard, Breda & Granier 1996; Comstock & Mencuccini 1998; Sperry *et al*. 1998, 2002; Oren *et al*. 1999; Bonal *et al*. 2000; Salleo *et al*. 2000; Hubbard *et al*. 2001). However, this does not mean that the water potential is constant at all times; rather, it suggests that because of stomatal closure, there exists a definite minimum value which the water potential does not exceed. The supposed primary role of this mechanism is the avoidance of low water potential, which leads to xylem cavitation (Jones & Sutherland 1991) and the possibility of runaway cavitation (Tyree & Sperry 1988). It has yet to be demonstrated that this isohydric behaviour is common in tropical forest trees, or that it can solely explain altered water use in whole trees between seasons.

As a complement to empirical testing, we used a modelling approach to investigate the ability of the isohydric hypothesis to explain the physiology of whole trees. In the field, stomatal conductance is high when both energy and water are abundant and stomata close due to either due to a lack of water, or due to a lack of energy (when the increase in photosynthesis to be gained by opening stomata is very small) (Farquhar *et al*. 1980)). Using a model allows us to dynamically link stomatal conductance, leaf water potential and energy availability to account for the multiple factors that determine stomatal conductance. The model we have used is the soil–plant–atmosphere (SPA) model (Williams *et al*. 1996). The SPA model is a multilayer soil–vegetation–atmosphere transfer (SVAT) model, the stomatal conductance algorithm of which is based on the isohydric hypothesis. We parameterized the SPA model, using measured estimates of hydraulic parameters, and compared the diurnal predictions of the model with the tree physiology data to test the isohydric hypothesis embedded in the model.

The second area of uncertainty investigated was whether soil water potential or soil-to-leaf hydraulic resistance was the factor that limits soil-to-leaf water supply under water-stressed conditions. Soil-to-leaf water transport, along with atmospheric demand, determines leaf water status, but it is not clear whether soil water potential or soil-to-leaf hydraulic resistance is the major factor that constrains water uptake during the dry season. Some previous modelling studies on tropical and seasonal temperate forests (Williams *et al*. 1996, 2001a) suggest that changes in ecosystem water use during the dry season could be explained only through changes in soil-to-leaf hydraulic resistance, as only small changes in soil water potential have been observed. However, other studies have found very large seasonal changes in soil water potential between seasons (Misson, Panek & Goldstein 2004). In this paper, we use measured estimates of soil water potential and soil-to-leaf hydraulic resistance, as well as the description of soil-leaf water transport in the SPA model, to isolate the relative importance of both transport factors in constraining evapotranspiration during the dry season.

The third area of uncertainty investigated in this paper is the distribution of hydraulic resistance within the soil-to-leaf continuum. Soil-to-leaf hydraulic resistance consists of several hydraulic resistances in series in the leaves, branches, trunks, roots, root–soil interface and soil matrix. Much information exists describing the resistance of excised branch segments under different water potentials (Mencuccini 2002), but little information exists on the relative magnitudes of branch, trunk and below-ground resistance components (Sperry *et al*. 1998, 2002). It is impractical to obtain all information to model the dynamics of every resistance in the SPA continuum, it is more efficient to deduce the location of the major resistance to water transport. In this paper, we split the resistance of the soil-to-leaf pathway into above- and below-ground components using stem psychrometry measurements, and thus determine the location of the greatest resistance to water transport.

The following key questions are addressed in this paper:

1. Are the leaf water potential, sap flow and stomatal conductance data consistent with the hypothesis that stomata function to prevent leaf water potential declining below a minimum critical value under water stressed circumstances?
2. Are changes in soil-to-leaf water supply dominated by changes in soil water potential or by soil-to-leaf hydraulic resistance?
3. If there is a major change in soil-to-leaf hydraulic resistance between seasons, is the change in resistance located above or below ground?

We first address how the data alone may be used to answer these questions, then we investigate what additional conclusions we may draw, by comparing the data with the predictions of the SPA model.

METHODS

Site

The experimental site is located in the Caxiuanã National Forest, Pará, Brazil (1°43′3.5″S, 5°27′36″W). The forest is a lowland *terre firme* rain forest. The mean annual rainfall is 2272 mm (± 193 mm), but with a pronounced dry season between July and December, when on average only 555 mm (± 116 mm) of rainfall is recorded (data from 1999 to 2003). The soil is a yellow oxisol (Brazilian classification latosol), with a 0.3–0.4 m thick stony/laterite layer at 3–4 m depth. The soil texture (0.0–0.5 m) is 75–83% sand, 12–19% clay and 6–10% silt (Ruivo & Cunha 2003). The soil consists of mainly kaolin in the clay fraction and quartz in the sand fraction (Ruivo & Cunha 2003). The site elevation is 15 m above river level, and the water table has been observed at a depth of 10 m during the wet season.

TFE experiment

To investigate the limitation of soil water on forest gas exchange in drier conditions than those normally experienced, an artificial soil drought was created by using TFE. This work was carried out as part of the LBA (Large-Scale Biosphere Atmosphere Experiment in Amazonia) Ecology program (Avissar & Nobre 2002). Two 100 × 100 m plots, a control and a treatment TFE plot, were established, and the borders trenched to a depth of 1 m to reduce the lateral flow of water. In the TFE plot, a roof of transparent plastic sheeting and wooden guttering was installed at a height of approximately 2 m height in November 2001, to keep the soil free from rainfall.

A 30-m-tall canopy access tower was installed in each plot. Nine trees were accessible from each tower. Of these trees, five in the TFE plot and four in the control plot were equipped with sap flow monitors. The species, canopy heights and diameter at breast height (DBH) were recorded (Table 1). The sap-flow-equipped trees were the tallest trees accessible from the towers, and their leaves ranged from 11 to 28 m in height in the control plot and 16–28 m in the TFE plot. Trees were measured up to the top of the canopy. A meteorological station (Campbell Scientific, Loughborough, UK), installed on a 55-m-tall tower located 700 m from the experimental site, recorded climatic conditions [wet and dry bulb temperatures, rainfall, wind speed and direction, incoming and outgoing photosynthetically active radiation (PAR), short-wave and long-wave radiation] every 15 min.

Tree physiology measurements

Canopy leaf area index (LAI) was measured with an LAI-2000 Plant Canopy Analyser (Li-Cor, Inc., Lincoln, NE, USA) on a 10 × 10 m grid in both plots, in May 2003 and November 2003. The grid covered the full extent (100 × 100 m) of each plot. Diurnal courses of leaf water potential were monitored by using a digital pressure bomb (Skye Instruments, Llandrindnod Wells, UK) on 17 and 19 May 2003 (late wet season) and on 19 and 20 November 2003 (late dry season). On each of these days, four to five leaves were sampled from each of the intensively studied trees at 0600, 0900, 1100, 1300, 1500 and 1630 h, and leaf water potential was determined for each leaf by using the pressure bomb. Sap flow rates were measured for each of the intensively studied trees by using the trunk segment heat balance method (Environmental Measuring Systems, Brno, Czech Republic) (Cermak, Deml & Penka 1973; Cermak, Kucera & Nadezhdina 2004). The heat balance sensors measure sap flow over an entire sector of circumference, therefore they do not require calibration for xylem depth if the sensors (which are 30–50 mm long) penetrate through all of the active xylem tissue. Xylem depth was estimated in wood cores both visually and using dye previously injected below the point of measurement, to confirm that water was not transported beyond 30 mm depth. Xylem depth measurements of 47 trees, which ranged from 0.1 to 1.3 m in diameter, indicated that the xylem rarely extended beneath 20 mm depth, irrespective of tree size (data not shown); therefore, the 30-mm-long sap flow sensors cut through all of the conductive tissue. Water flux was logged every 15 min throughout each day.

The heat balance sap flow measurement method suffers from calibration errors around zero, such that when there is zero flow, a slight positive flow is recorded and a calibration is necessary. This is typically achieved by taking the minimum point over a period of several days and subtracting it from the raw data, so that the minimum becomes the zero point. This method is problematic if sap flow data are used to establish that the trees and soil have reached equilibrium, based on the achievement of zero sap flow during the night. However, if flow continues through the night as the leaves refill, then the flow will constantly decline as the soil–leaf water potential gradient becomes smaller. If flow were to stop altogether, then the apparent flow would be constant. For all of the trees studied the refilling period appeared to last only until between 2200 h and midnight. Thereafter, sap flow values remained constant to within 0.002 kg s^{-1} cm^{-1}. This constant value was used as the zero

Table 1. Details of the intensively studied trees equipped with sap flow monitors and accessible from the canopy tower (C trees are the trees in the control plot, T trees are the ones in the TFE plot)

Tree code	Species	DBH (m)	Ψ_{crit} MPa	Measurement height (m)	Canopy height (m)
C1	*Mezilaurus mahuba*	0.156	–1.9	11	6–21
C2	*Licania heteromorpha*	0.187	–0.9	19	18–26
C3	*Manilkara bidentata*	0.515	–4.3	28	21–30
C4	*Manilkara bidentata*	0.439	–2.7	27	23–31
T1	*Licaria ameniaca*	0.159	–2.2	16	10–22
T2	*Hirtela bicornis*	0.295	–2.1	20	15–30
T3	*Lecythis confertiflora*	0.366	–2.9	25	21–32
T4	*Swartzia racemosa*	0.485	–3.2	27	22–32

TFE, through-fall exclusion; DBH, diameter at breast height; $\Psi_{crit,}$ critical leaf water potential.

point in all cases, and the existence of unchanging sap flow for several hours was used as evidence of the existence of zero flow.

Stomatal conductance was measured using an LI-1600 leaf porometer (Li-Cor, Inc.). Diurnal measurements of the ambient transpiration rates, stomatal conductance and other associated meteorological variables (humidity, photon flux density, leaf and cuvette temperature) were made on the control plot on 27 May 2003 and on 31 October 2003, and in the TFE plot on 23 May 2003 and on 2 November 2003. (The May dates represent the late wet season while the October and November dates represent the late dry season.) Four to five leaves were sampled from each of the intensively studied trees. Measurement times were 0900, 1030, 1200, 1330, 1500 and 1630 h. Prior to 0900 h, very high (> 90%) humidity prevented accurate readings from being obtained from the porometer, because of low transpiration rates. Leaves were not divided into shade and sun leaves, because the sun/shade definition of a mid-canopy leaf changes very frequently as the position of the sun shifts throughout the day. It was assumed that at a given canopy level, all leaves experienced a similar proportion of sun and shade conditions.

Stem psychrometers (Plant Water Systems, Guelph, Ontario, Canada), in conjunction with a manual microvoltmeter (Wescor, Logan, UT, USA), were used to measure the water potential of the xylem at the base of each of the intensively studied trees. We collected these measurements at the same time as the leaf water potential measurements, in order to compare the water potentials of the leaves and stem. Prior to installation, the psychrometer sensors were calibrated against the pressure bomb measurements of leaf water potential. Nine leaves were collected from trees at different levels in the canopy at midday. From each leaf, a piece of the lamina was removed and measured with the psychrometers according to the Wescor protocol, while the water potential of the remaining leaf was measured by using the pressure bomb. After the calibration, we installed the sensors between the height of 0.2 and 0.3 m at the base of the intensively measured trees. The sensors were insulated with a foam with depth of 0.1 m and an aluminium foil radiation shield. This insulation was highly effective at removing temperature gradients between the two thermocouple junctions – the main source of error in psychrometer measurements – and the voltage gradient was never higher than 0.1 μV, which was within the range recommended by the manufacturers.

We measured the ambient hydraulic resistance of the excised segments of terminal branches during November 2002, May 2003 and November 2003 as another means of observing changes in above-ground hydraulic resistance. Four branches were collected from each intensively measured tree over several days. Branches were collected between 1400 and 1500 h to ensure that embolism risk was maximal. The leaves and petioles were removed immediately to prevent further water loss, and measurements were made within 3 h of collection to minimize the effects of cavitation recovery (Zwieniecki & Holbrook 2000). A low-pressure hydraulic resistance measurement system similar to that described by Sperry & Tyree (1988) was used to measure hydraulic resistance. Branch segments were between 0.09 and 0.15 m in length and 10–14 mm in diameter. Leaf area distal to each measured segment was found by measuring the area/mass ratio of a subset of leaves from each branch, using digital photography and Scion Image software (Scion Corporation, Frederick, MD, USA).

RESULTS

Meteorology

The average meteorology changed between the measured wet and dry season days (Fig. 1). The average air vapour pressure deficit (VPD) between saturation and the atmosphere over a 24 h period was higher in the dry season (0.5 kPa) than in the wet season (0.38 kPa). The average short-wave radiation increased from 183 W m^{-2} in the wet season to 216 W m^{-2} in the dry season. The average temperature was higher in the dry season (25.3 °C) than in the wet season (24.6 °C).

Sap flow

In the control plot, sap flow was 44% higher in the dry season than in the wet season. However, in the TFE plot, sap flow was 15% lower in the dry season than in the wet season (Fig. 2 and Table 2). The majority of the changes between seasons occurred in the upper canopy trees (C3, C4, T3 and T4). In particular, tree T3 showed a very large decline from 1134 to 16 kg d^{-1}. The sap flow data in Fig. 2 are normalized for tree leaf area (see modelling section), so differences between the trees are due to factors other than size.

Stomatal conductance

In control and TFE plots, the stomatal conductance of six out of the eight trees measured remained high (> 100 mmol m^{-2} s^{-1}) for the majority of the day during the

Table 2. Average daily sap flow value for the control plot (C) and TFE plot (T) trees during the wet and dry seasons

		Daily sap flow (kg d^{-1})	
Plot	Tree code	Wet season	Dry season
Control	C1	27	40
	C2	13	13
	C3	184	285
	C4	438	777
TFE	T1	40	45
	T2	34	45
	T3	134	16
	T4	157	134

TFE, through-fall exclusion.

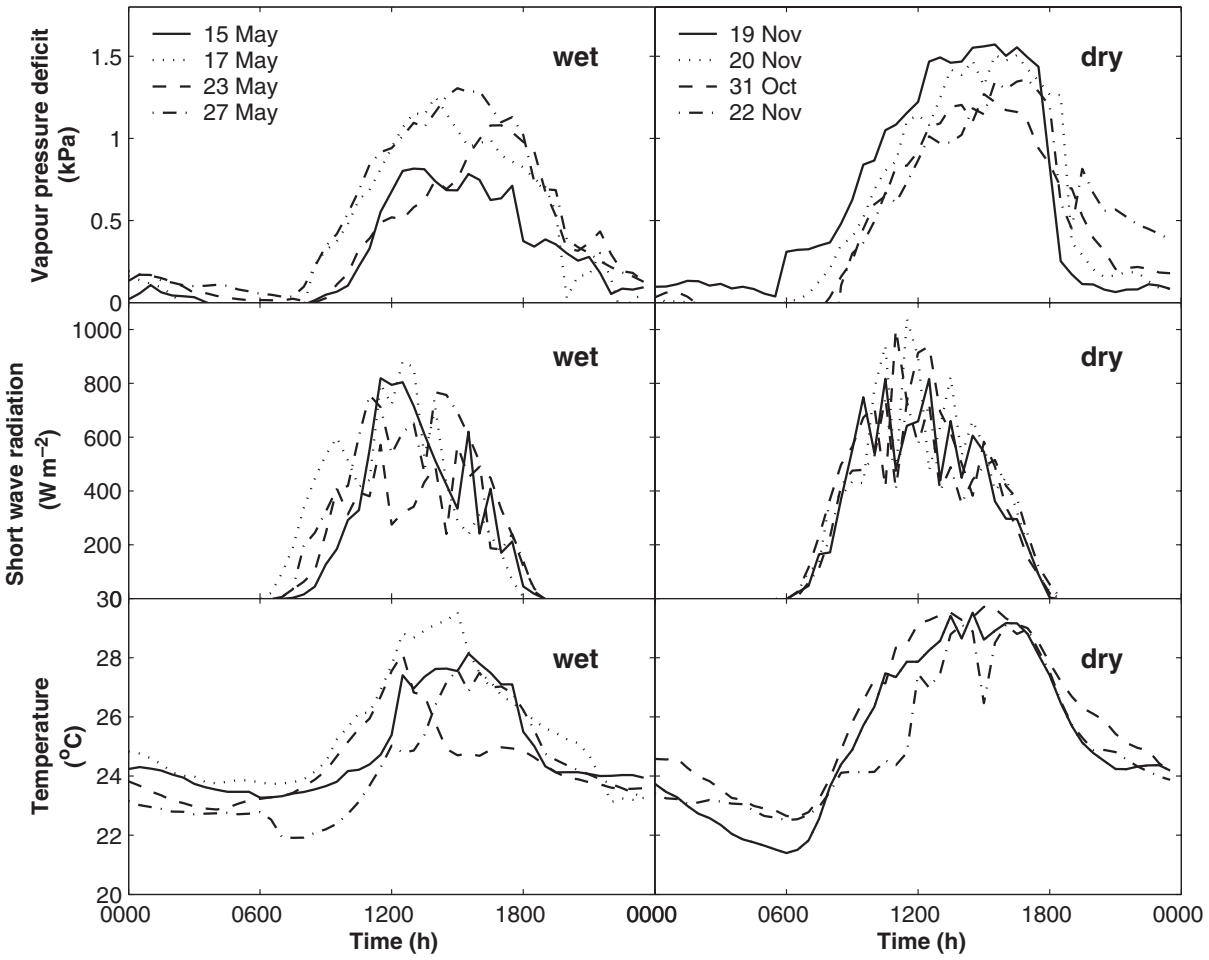

Figure 1. Diurnal courses of vapour pressure deficit, short wave radiation and air temperature at 30 m height on wet season days (15, 17, 23 and 27 May 2003) (left-hand panels) and dry season days (19 November, 20 November, 31 October, 2 November 2003) (right-hand panels).

wet season (Fig. 3). Average maximum stomatal conductance was 184 mmol m^{-2} s^{-1} and the only period with low (< 100 mmol m^{-2} s^{-1}) stomatal conductance occurred at 1630 h in the TFE plot, which corresponds to a period of low radiation and temperature (Fig. 1). In the dry season, g_s was 35 and 39% lower than in the wet season in the control and TFE plots, respectively. In the dry season, g_s declined gradually between 0900 and 1530 h in the control plot, but no diurnal pattern was observed in the TFE plot, and g_s remained between 45 and 85 mmol m^{-2} s^{-1} for the whole day.

Leaf water potential

We found that the daytime leaf water potentials were lower in the dry season than in the wet season for both plots (Fig. 4). The average minimum leaf water potential reached was –1.71 MPa in the wet season, and –2.47 MPa in the dry season. For each measurement period, there was no significant difference in daytime leaf water potential values between plots ($P > 0.05$). During the dry season, leaf water potential declined quickly each morning in all trees, reaching a plateau around a minimum value by 0900 or 1100 h and remaining within 0.5 MPa of the minimum value for the majority of the day (from 0900 or 1100 h until at least 1500 h). The exception to this was tree C1, which showed gradual recovery in leaf water potential throughout the afternoon of 20 November. However, this tree did display a minimum plateau around –1.7 MPa on the 19th November, below which it did not decline at any other time. Between 1500 and 1630 h, leaf water potential recovered slightly in six out of the eight trees studied. We found large differences in the minimum leaf water potential values reached by the different trees (Table 1). The minimum leaf water potential reached was negatively correlated with height ($r^2 = 0.74$). In the wet season, none of the trees reached the same minimum level observed in the dry season (with the exception of tree T2).

Stem water potential

The calibration procedure showed that the psychrometers provided an unbiased estimate of stem water potential. Regression analysis of the data indicated that very little

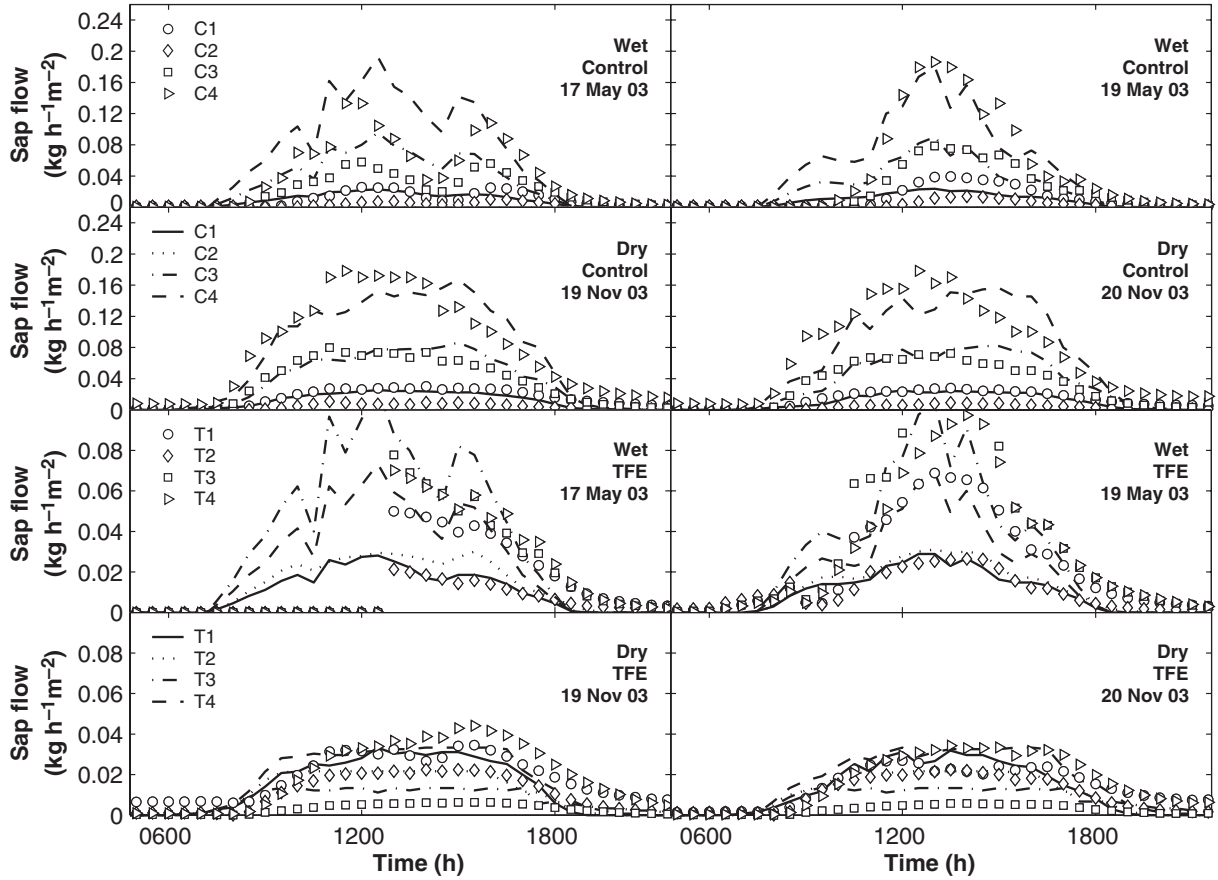

Figure 2. Measured (symbols) and modelled (lines) sap flow per m^2 leaf area for individual trees in the control (top four panels) and through-fall exclusion (TFE) (bottom four panels) plots for wet and dry seasons. C1–C4 are trees in the control plot and T1–T4 are trees in the TFE plot. Note the change in scale between control and TFE plot figures.

calibration was necessary, as the slope of the psychrometer/pressure bomb relationship was 1.03 and the intercept was 0.092 MPa with an r^2 value of 0.94, thus indicating that the psychrometers provided a reliable estimate of leaf water potential compared with that of the pressure bomb. In other investigations, stem psychrometers have been calibrated against sections of wood that are subject to drying (Irvine & Grace 1997). In this instance, the variability of tree species and the logistics of felling large rain forest trees meant that this approach was not possible. We were therefore limited to calibration against leaf laminar measurements. In this case, however, virtually no correction was made to the psychrometer outputs following calibration. It is feasible that an error in the psychrometer measurements could have been introduced as a result of measurements being made on woody stems instead of leaf laminae. In the wet season, the values of stem water potential were consistently higher than –0.5 MPa, close to the values of soil water potential estimated from pre-dawn leaf water potential (–0.08 to –0.09 MPa). In the dry season, the data on stem water potential show similar plateaux to the leaf water potential measurements (Fig. 5), with the levels of the plateaux being slightly (0.7 MPa average difference) wetter than the values of leaf water potential. The average dry season stem water potential was –1.69 MPa in the control and –1.53 MPa in the TFE plot.

Branch resistivity

We found no difference in the ambient resistivity of the excised terminal branch segments between seasons (Fig. 6), but there were large differences between the resistivity values for the different trees. The highest resistivity (0.37 m^{-2} s MPa $mmol^{-1}$), which was recorded for tree C1, was 9.4 times higher than the least resistivity (0.04 m^{-2} s MPa $mmol^{-1}$), which was recorded for tree C3.

Modelling

Justification of modelling exercise

The data presented here indicate, from an initial analysis, that they are broadly consistent with the isohydric hypothesis that the stomata respond to leaf water potential in order to prevent leaf water potential from decreasing to a

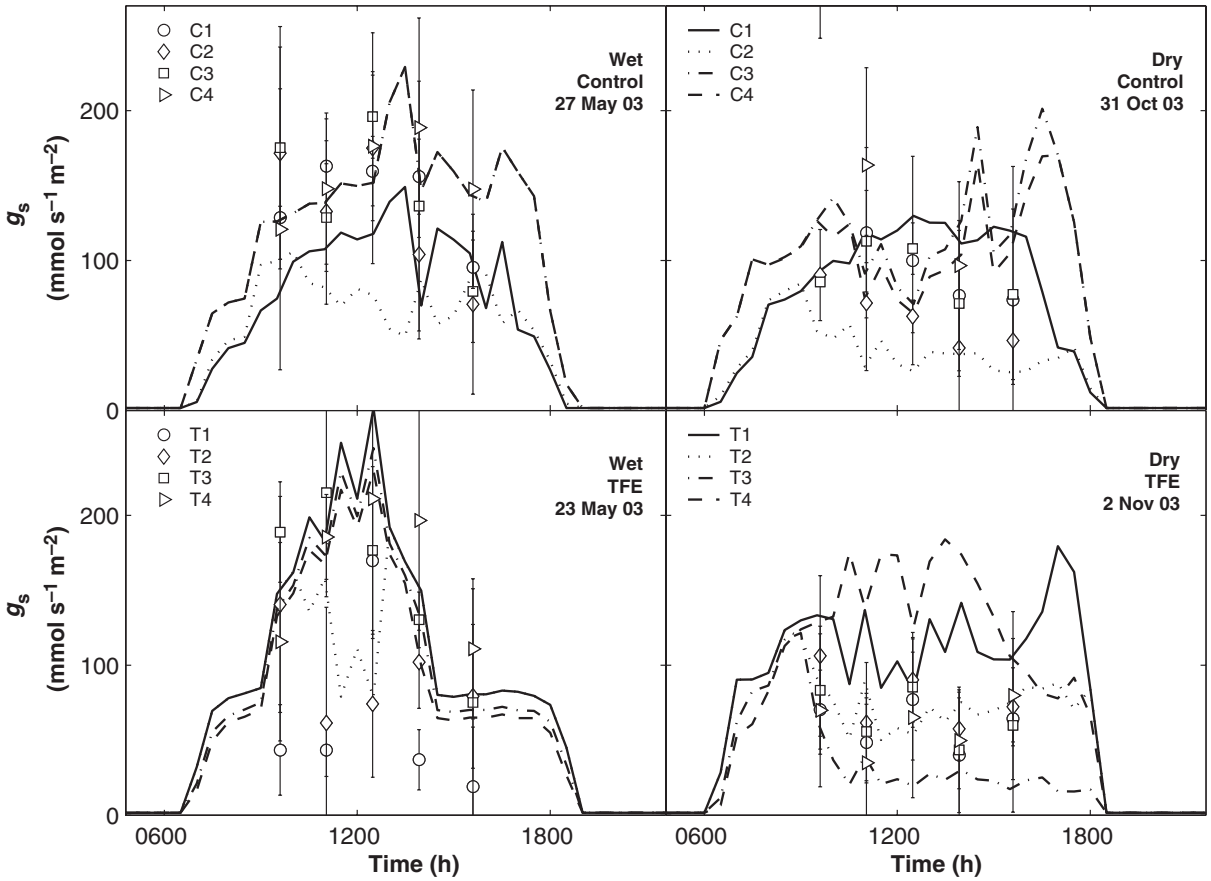

Figure 3. Measured (symbols) and modelled (lines) stomatal conductance for individual trees in the control (top two panels) and throughfall exclusion (TFE) (bottom two panels) plots for wet and dry seasons. C1–C4 are trees in the control plot and T1–T4 are trees in the TFE plot.

level below a critical threshold value under water-limited conditions. A persistent minimum leaf water potential plateau was observed during the dry season in all trees. When the leaves were at their minimum leaf water potential during the dry season, the stomatal conductance was consistently low. During the wet season, high stomatal conductances were observed and the leaf water potential was not near the minimum value.

However, the leaf water potential data alone do not actually confirm the existence of an isohydric mechanism. The plateaux in leaf water potential could possibly have been due to the meteorological conditions, for example, low atmospheric demand for moisture may have caused the fortuitous maintenance of stable leaf water potentials. To exclude this possibility, we must establish that the atmospheric demand at all the different canopy levels was high enough to reduce leaf water potentials to a level below the measured values, in the absence of stomatal control. Therefore, construction of the predictions of the isohydric model necessitated the use of a dynamic simulation model. We chose to use the SPA model (Williams *et al*. 1996) to simulate the predictions of the isohydric hypothesis for each data set for the following reasons:

1 The isohydric hypothesis proposes that leaf water potential is the dominant control over stomatal conductance and water use in water-limited conditions. Leaf water potential is the balance of soil-to-leaf water supply and atmospheric loss. To generate 'expected' leaf water potential values, we must simultaneously model both atmospheric demand and soil-to-leaf water supply. Both these processes are explicitly simulated by the SPA model.

2 Stomatal conductance may be limited either by hydraulic stress or by low light energy levels. Therefore, to predict the model expectations of stomatal conductance, we need simulations of both the leaf water potential and the availability of light energy to the leaves. The SPA model includes a radiative transfer scheme and a model of leaf water potential to allow both the factors that determine stomatal conductance to be simulated.

3 Tree-level predictions of sap flow must be constructed from leaf level estimates of evapotranspiration. Leaves at different heights in the canopy of a single tree have different rates of sap flow depending on their respective energy supply and hydraulic limitation. The SPA model includes a multilayer model of the forest canopy and a radiative transfer model, so it is possible to scale from leaf-level evapotranspiration predictions to tree-scale

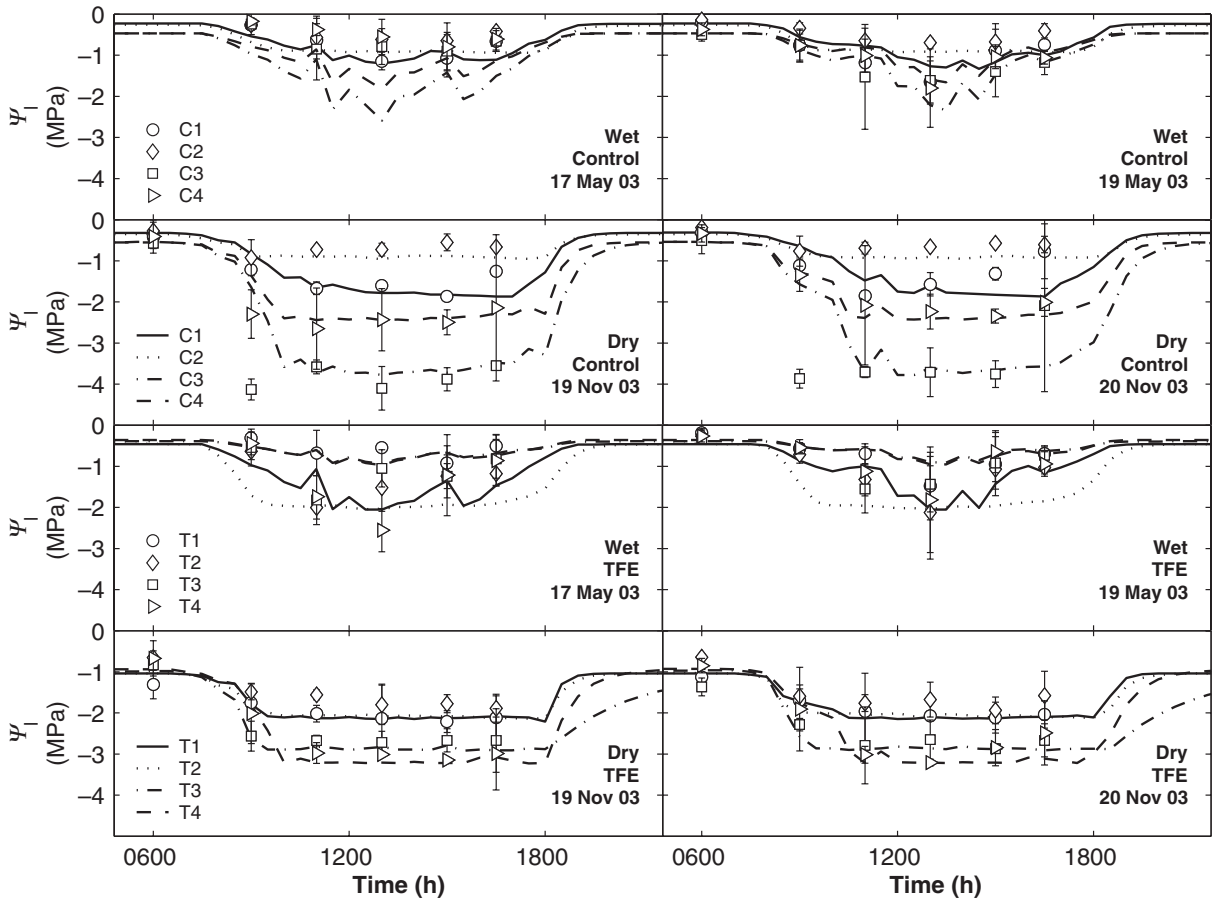

Figure 4. Measured (symbols) and modelled (lines) leaf water potential for individual trees in the control (top four panels) and throughfall exclusion (TFE) (bottom four panels) plots for wet and dry seasons. C1–C4 are trees in the control plot and T1–T4 are trees in the TFE plot.

sap flow predictions, therefore producing patterns of sap flow that are consistent with the isohydric hypothesis.

4 Prediction of stem water potential from leaf water potential is difficult, because water storage, or capacitance, in the tree leads to a lag between the leaf water potential and the stem water potential measured at the base of the tree in the morning, as water is supplied from above the ground and not drawn from the soil. Estimates of stem water potential dynamics consistent with the hypothesis can be made by using a dynamic model of the interaction between capacitance and resistance that has been incorporated into the SPA model.

The SPA model

The SPA model is a multilayered SVAT model that is designed to represent processes that are common to vascular plants, so that ecosystem–atmosphere exchange may be understood in terms of similar processes in different locations. The model has previously been tested in temperate deciduous and evergreen forests, arctic tundra and tropical rain forest ecosystems (Williams *et al*. 1996, 1998, 2000, 2001a; Williams, Bond & Ryan 2001b).

SPA is ideally suited to investigating the impact of drought on forest ecosystems because of its explicit modelling of water transport to leaves. In the model, stomatal conductance is controlled such that photosynthesis is maximized while not allowing leaf water potential to drop below a critical minimum value. If leaf water potential (Ψ_l) reaches the critical minimum leaf water potential (Ψ_{crit}), stomatal conductance (g_s) decreases and further water loss is prevented, therefore causing isohydric model behaviour under water-stressed conditions. Leaf water potential is determined from the balance of atmospheric demand, simulated by using the Penman–Monteith equation (Jones 1992), and leaf water supply, as shown in Eqn 1.

$$\frac{\delta\Psi_l}{\delta t} = \frac{\Psi_s - \rho g h - ER - \Psi_l}{CR}, \quad (1)$$

where Ψ_s is the soil water potential, ρ is the density of liquid water (kg m^3), g is gravitational acceleration (9.8 m s^{-2}) and h is the height (m) of the canopy layer. Strictly, this should be the vertical distance between the point of water uptake and the leaves. However, we are, at this stage, unsure of the height of the water uptake and therefore are not able to correct for these changes. However, a 10 m difference in

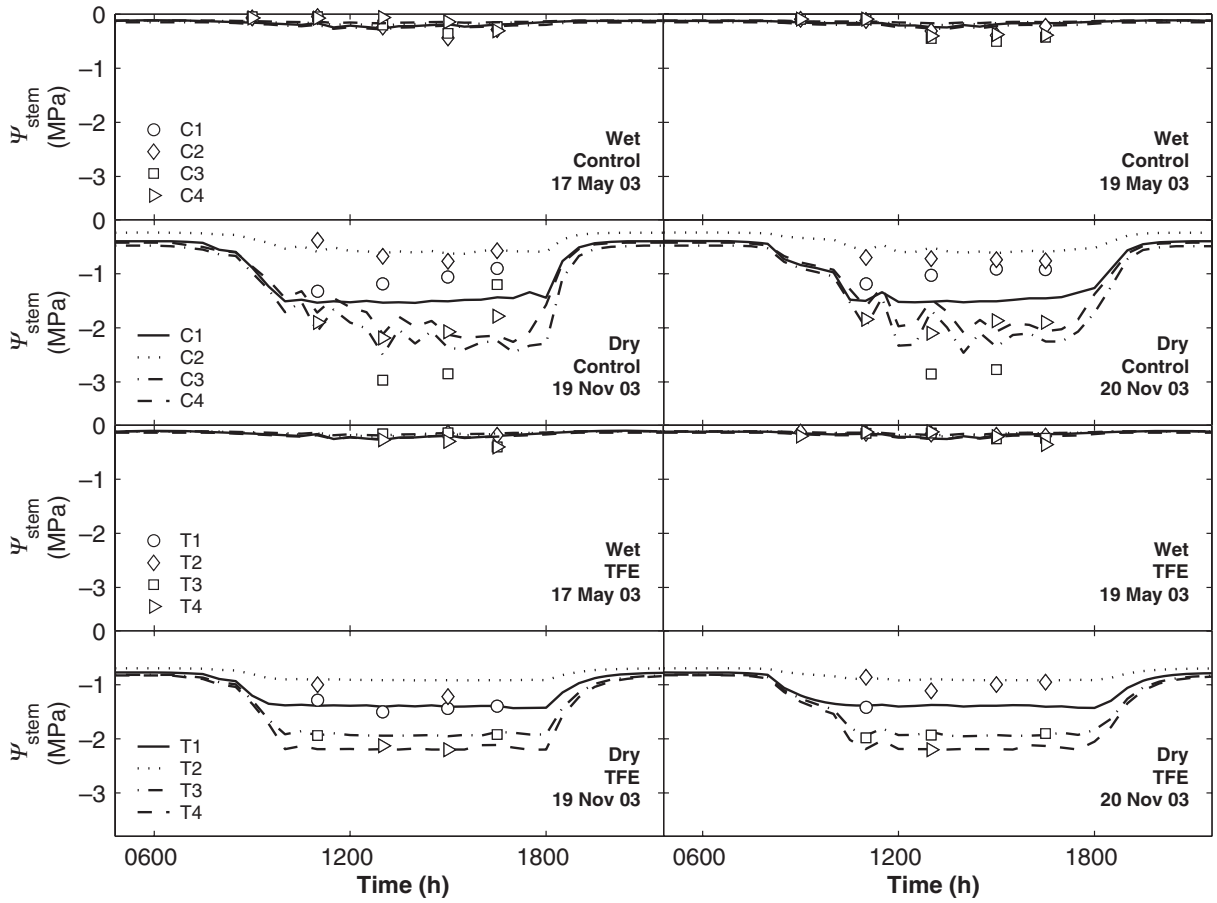

Figure 5. Measured (symbols) and modelled (lines) stem water potential for individual trees in the control (top four panels) and throughfall exclusion (TFE) (bottom four panels) plots for wet and dry seasons. C1–C4 are trees in the control plot and T1–T4 are trees in the TFE plot.

height causes only a 0.1 MPa difference in gravitational leaf water potential, so this is thought to be of little consequence in relation to the observed changes in leaf water water potential in all trees. E is the rate of evapotranspiration (mmol m^{-2} s^{-1}), C is the capacitance (mmol m^{-2} MPa^{-1}) and R is the soil–leaf hydraulic resistance (m^{-2} s MPa $mmol^{-1}$). To solve this equation, we must first define the hydraulic properties that determine water supply to the leaves, R, C and Ψ_s. All these parameters are physical properties of trees or ecosystems and can be estimated either independently or, in the case of R, from a subset of the data. The hypothesis underlying the SPA stomatal conductance algorithm has not been previously tested against high-resolution diurnal time series tree physiology data as presented here.

Models inputs

We ran the SPA model for each tree for each of the intensively measured days. All parameters in SPA remain as given by Williams *et al.* (1996) unless stated otherwise. Of those inputs that were changed to reflect local observations, some were common to all trees, plots and seasons, and others were varied according to available data (Table 3).

C was estimated as 2300 mmol MPa^{-1} m^{-2} from measurements made by Goldstein *et al.* (1998) for a seasonal tropical forest in Panama, the only published estimate of C for tropical forest trees (see Appendix) and was assumed to be constant between seasons. Ψ_s was determined from the averaged pre-dawn Ψ_l measurements (Fig. 4), for each plot and season. Sap flow decreased to zero during the night (Fig. 2), indicating that equilibrium between the soil and tree had been reached, so pre-dawn Ψ_l should be a reasonable estimate of Ψ_s (Donovan, Linton & Richards 2001). LAI was determined from the LAI-2000 measurements (Table 3). Ψ_{crit} was determined for each tree from the minimum observed leaf water potential (Table 1) and was kept the same between seasons.

Soil-to-leaf hydraulic resistance (m^2 s MPa $mmol^{-1}$) varies between trees and seasons, and was calculated as shown in Eqn 2.

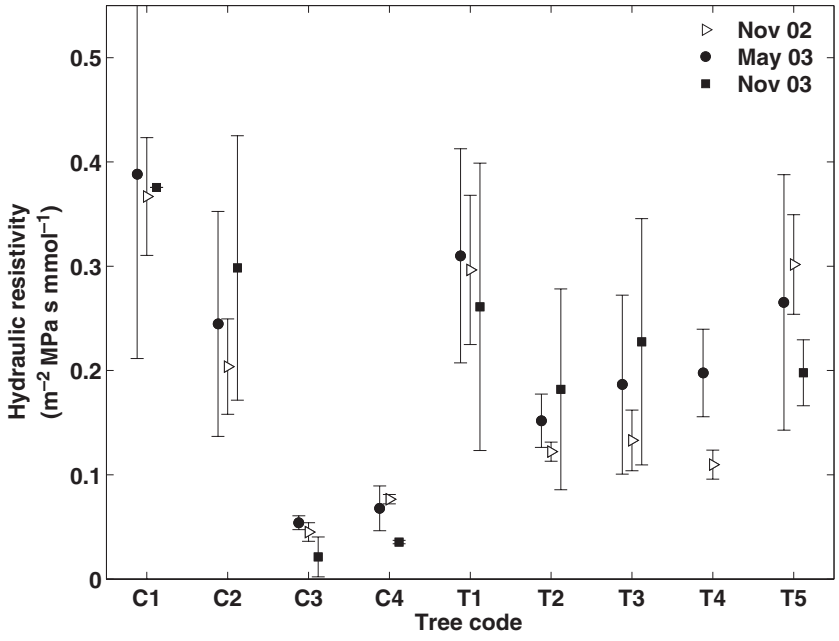

Figure 6. Resistivity of excised branch segments in the November (dry season) of 2002 and 2003 and May (wet season) of 2003. Measured using the method of Sperry (1988) for branch segments 0.09–0.15 m long and 10–14 mm in diameter. Error bars are standard deviation of four measurements.

$$R = \frac{A(\Psi_l - \Psi_s)}{S} \quad (2)$$

where S is the tree-level sap flow (mmol s^{-1}) and A is the tree leaf area (m^2) calculated from the tree diameter. The diameter was assumed to be proportional to leaf area since we found no relationship between xylem depth and tree diameter. The ratio between diameter and leaf area was derived from the tree survey diameter data and canopy LAI. We estimated R from each measurement of Ψ_l. The estimates of R at 0900 h were very high, since the release of stored water from plant tissues above the sap flow sensor allowed Ψ_l to drop without causing a corresponding increase in sap flow rate and thus creating a high apparent resistance. Thereafter, R reached a plateau that is stable to within 1 m^2 s MPa mmol^{-1}. We took the average value of this post-0900 h plateau and used it as the R parameter in the model (Table 4).

The SPA model inputs were independent of the verification data, with the exception of three parameters, Ψ_{crit}, Ψ_s and R. However, all three of these parameters were found deterministically, by using the methods previously described. Critically, these parameters were determined without reference to the fit between the model and the data; therefore, they are not 'fitted' or 'optimized' parameters but representations of real physical or biological properties.

Processing of model output for comparison with data

The SPA model provided predictions of gas exchange and physiology for layers of leaves at different heights in the canopy. Leaf water potential and stomatal conductance data were compared directly with the model output at the height at which they were measured. To allow comparison of the tree-level sap flow data with the model output, the leaf-level model predictions were scaled to the tree level. We again allocated a total leaf area to each tree (A) and distributed it evenly between the lowest and highest canopy layers occupied by each tree (Table 1). Sap flow was estimated for each layer from the SPA model predictions, and

Table 3. Characteristics of the control and TFE plot used as input to the SPA model.

		Control		TFE		
Parameter	Units	Wet	Dry	Wet	Dry	Source
Canopy height	m		30			Measurement from tower
Capacitance	mmol m^{-2} MPa^{-1}		2300			Goldstein *et al.* (1998)
Soil water potential	MPa	–0.09	–0.17	–0.08	–0.66	Pre-dawn Ψ_l
LAI	m^3 m^{-3}	5.0	5.8	4.3	4.6	LAI 2000

Some parameters were constant between seasons (canopy height and capacitance) and some were varied between seasons according to measured values (LAI, soil water potential).
TFE, through-fall execution; SPA, soil–plant–atmosphere; Ψ_l, leaf water potential; LAI, leaf area index.

Table 4. Values of R (soil-leaf hydraulic resistance) calculated using Eqn 2, b (the proportion of resistance located below ground) fitted to the Ψ_{stem} data, and the R_{ag} and R_{bg} (above- and below-ground resistance, respectively) calculated from the values of R and b

			Control plot				TFE plot			
Parameter	Units	Season	C1	C2	C3	C4	T1	T2	T3	T4
R	$m^{-2}s$ MPa $mmol^{-1}$	Wet	2.0	3.0	1.1	0.6	1.1	3.7	0.5	0.8
		Dry	2.8	3.0	4.1	2.5	2.1	3.5	19.7	6.1
b	–	Wet	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2
		Dry	0.6	0.2	0.9	0.8	0.3	0.1	0.7	0.6
R_{ag}	$m^{-2}s$ MPa $mmol^{-1}$	Wet	1.8	2.7	0.9	0.5	1.0	3.3	0.5	0.6
		Dry	1.1	2.4	0.4	0.5	1.5	3.1	5.9	2.4
R_{bg}	$m^{-2}s$ MPa $mmol^{-1}$	Wet	0.2	0.3	0.2	0.06	0.1	0.4	0.05	0.2
		Dry	1.7	0.6	3.7	2.0	0.6	0.3	13.8	3.7

TFE, through-fall exclusion.

the tree-level sap flow was the sum of all the sap flow from the individual layers (see Appendix for full details of the scaling approach).

To compare the predictions of the isohydric hypothesis, as embedded in the SPA model, with the data on stem water potential, we used the SPA model output of Ψ_l to create estimates of modelled stem water potential for each tree. However, stem water potential depends not only on Ψ_l and Ψ_s but also on the distribution of hydraulic resistance above and below ground, b, which can be obtained through Eqn 3.

$$b = \frac{R_{bg}}{R} \tag{3}$$

where R_{bg} is the leaf-specific hydraulic resistance located below ground, in the roots and soil, and R is the total soil-to-leaf hydraulic resistance (both m^2 s MPa $mmol^{-1}$). To find the value of b, which best explained the stem water potential data, we created several different scenarios of the modelled stem water potential, with values of b ranging from 0 and 1, in increments of 0.1. We calculated the modelled stem water potential, as shown in Eqn 4.

$$\Psi_{stem} = [b(\Psi_l - \Psi_s)] + \Psi_s \tag{4}$$

where Ψ_l and Ψ_{stem} are the modelled leaf and stem water potential, respectively, and Ψ_s is the soil water potential estimated from pre-dawn leaf water potential. We then determined which value of b gave rise to the smallest root mean square error (RMSE) between the modelled and measured Ψ_{stem} values. The value of b, fitted in this manner, is therefore an estimate of the proportion of the soil-to-leaf resistance which was located below ground in each season. We then estimated the absolute values of the above- and below-ground resistance (R_{ag} and R_{bg}, respectively) by multiplying the total soil–leaf resistance (R) by the proportion located below ground (b) or above ground ($1 - b$).

Model sensitivity to wet–dry season input changes

Water supply to leaves, at a given leaf water potential, depends on soil-to-leaf hydraulic resistance and soil water potential. It is unclear which of these two factors change the most between seasons and which has the greatest impact on water supply within the range experienced. In addition, sap flow rates are influenced by meteorology and LAI changes. We used the SPA model to investigate which of these factors was the dominant cause of altered sap flow between seasons, by generating SPA output for each plot six times. For the 'baseline' run, we used wet season input values of LAI, Ψ_s, R and meteorology data (Table 4). For the second run, we altered the meteorology data to its dry season value to test the impact of increasing temperature, VPD and solar radiation. The remaining four model runs tested the impact of altering the LAI, Ψ_s and R to their dry season values (Table 5, column 1). In each instance, we estimated the canopy sap flow, averaged for the two modelled days in each season and for the four tree-specific parameterizations. This allowed us to create a stepwise estimate of the effect of the different variables on the total sap flow.

Model verification

The isohydric hypothesis, as incorporated into the SPA model, showed good agreement with the data in the

Table 5. Modelled average daily sap flow in mm d^{-1}

Variables included	Control plot	TFE plot
Baseline	3.4	3.7
Met	4.2	4.6
Met + LAI	4.3	4.7
Met + LAI + Ψ_s	4.2	4.4
Met + LAI + R	4.0	3.6
Met + LAI + Ψ_s + R	3.9	3.1

Baseline refers to the predictions made using wet season meteorology data and parameters. The model inputs were then sequentially changed to their dry season values to identify which factor was the main cause of interseason variation in water use. Differences between plots in the baseline values are due to differences in wet season LAI.
TFE, through-fall exclusion; Met, meteorology data; LAI, leaf area index; Ψ_s, soil water potential; R, soil-leaf hydraulic resistance.

majority of cases. The model accounted for an average of 85% of the variation of Ψ_l, 83% of the variation of sap flow, 57% of the variation in g_s and 98% of the variation in Ψ_{stem} (Table 6). In the wet season, stomata were fully open, leaf water potential did not drop down to the minimum value and sap flow values were high (Fig. 4). In the dry season, as a result of changes in model inputs (R, Ψ_s and LAI) and meteorological drivers, the SPA model captured the reduction in leaf water potential to the minimum value in all trees (Fig. 4), and the resulting reduction in stomatal conductance (Fig. 3). The reduction in g_s in the TFE plot was strong enough to cause a large reduction in the sap flow of the TFE plot trees, in agreement with the data (Fig. 2). In the control plot, the stomatal closure necessary to maintain the minimum leaf water potential was not sufficient to cause a major decline in sap flow rates, and in fact, the rising VPD meant that sap flow increased in the control plot in the dry season, again in agreement with the data. Other features of the data that were well described by the model include the reduction in the rate of sap flow during the evening (this is codependent on the resistance and capacitance values) and the difference in leaf-area-specific sap flow between trees, which occurs as a result of their different illuminations at different heights in the canopy. Predictions of the magnitude of reduction in leaf water potential in the wet season, when no hydraulic limitation is present, agrees with the data.

One inconsistency between the model hypothesis and the data is that a modelled increase in stomatal conductance was found on several of the trees at 1630 h during the dry season (Fig. 3), when no increase in stomatal conductance has been observed in the data. This coincides with a recovery in leaf water potential in both the model and the data (Fig. 4). This indicates that in the model the energy available was sufficient to justify some stomatal opening in the late afternoon. However, the data show that the trees did not respond to the removal of hydraulic limitation in the same manner. The data therefore indicate that some further stomatal closure mechanism may be necessary to explain the observed stomatal behaviour.

Table 6. r^2 and slope values of the relationship between data and the SPA model predictions of leaf water potential, sap flow, stomatal conductance and stem water potential for individual trees Data for different seasons are combined

Parameter	Tree	Ψ_l	Sap flow	g_s	Ψ_{stem}
r^2	C1	0.78	0.90	0.39	0.98
	C2	0.83	0.90	0.74	0.92
	C3	0.80	0.92	0.29	0.93
	C4	0.89	0.90	0.90	0.97
	T1	0.85	0.72	0.70	0.99
	T2	0.87	0.81	0.38	0.99
	T3	0.94	0.72	0.87	0.99
	T4	0.91	0.75	0.30	0.99
Slope	C1	0.73	1.06	1.34	0.71
	C2	0.73	0.91	1.24	1.11
	C3	0.92	0.79	0.49	1.11
	C4	0.99	0.85	1.05	0.98
	T1	1.00	1.02	0.53	1.01
	T2	0.89	0.63	0.26	1.12
	T3	0.81	0.71	0.71	1.00
	T4	0.81	0.88	0.42	0.99

Data for different seasons are combined.
SPA, soil–plant–atmosphere; ψ_l, leaf water potential; g_s, stomatal conductance; ψ_{stem}, stem water potential.

DISCUSSION

Are the leaf water potential, sap flow and stomatal conductance data consistent with the hypothesis that the stomata function to maintain isohydric conditions within the plant under water-stressed circumstances?

The isohydric hypothesis, as embedded in the SPA model, produced results that were broadly consistent with the data. Importantly, the SPA model contains no 'optimized parameters', or parameters whose value is adjusted to minimize the model-data error. A minimum plateau of leaf water potential was observed during the dry season in the majority of cases, at the same time as reduced stomatal conductance was observed. The SPA model analysis indicates that these patterns are consistent with a hypothesis of hydraulic limitation and not of reduced atmospheric demand or light availability. Because the model is based on underlying physical factors, we have been able to use it to test the physiological hypothesis underlying stomatal function, with encouraging results.

The main consistent exception to the isohydric hypothesis was the afternoon stomatal opening which the SPA model predicted for some trees in the dry season but was not observed in the data. There are at least two possible explanations for the observed lack of afternoon stomatal reopening in the absence of hydraulic stress. Firstly, there is a direct response of stomata to VPD, such that the VPD observed at 1600 h during the dry season (1.2–1.5 kPa) causes stomatal closure even in the absence of actual hydraulic stress. This sort of response is implicit in Jarvis-type stomatal conductance models, and the implied mechanism is a direct response of the guard cells to VPD. A second possible explanation, invoked by Tardieu (1993), involves the chemical signalling of soil water status, whereby abscisic acid is produced in root tissue in dry conditions and transported to the stomata, where it invokes stomatal closure (Zhang & Davies 1989). The sap flow velocities measured in this study were sufficient to allow the transport of abscisic acid at a rate of 8–15 m d^{-1}, around the height of the smallest trees. This is not sufficient to allow abscisic acid to be the cause of afternoon stomatal closure or any sort of subdiurnal pattern. It is possible that the afternoon stomatal closure may be explained by an interaction between chemical and hydraulic signals, as described by Tardieu (1993). However, since we did not measure abscisic acid concentrations, and because the dis-

crepancy is only a minor one, the integration of additional hypotheses into the model is not a research priority at this stage.

We have shown here that it is possible to model leaf water potential dynamics, stomatal conductance and sap flow, given a knowledge of the soil water potential and soil-leaf hydraulic resistance. However, modelling the dynamics of the soil-to-leaf hydraulic resistance is a further challenge (Sperry *et al*. 1998; Williams *et al*. 2001a; Tuzet, Perrier & Leuning 2003; Misson *et al*. 2004). To reduce uncertainty in the modelling of leaf water potential, we recommend that efforts should be concentrated on the estimation of soil and plant hydraulic resistance dynamics.

Are changes in soil-to-leaf water supply dominated by changes in soil water potential or by soil hydraulic resistance?

We tested the impact of changing the model input variables from their wet to their dry season values to investigate which factor had the greatest impact on gas exchange. Water supply to leaves is proportional to both the Ψ_s to Ψ_l gradient and to $1/R$ (Eqn 1). R appears to be more sensitive to changes in ecosystem water status and therefore was the dominant cause of reduced water use during the dry season. There is a small change in the Ψ_s to Ψ_l gradient (an average factor of 2.2). while a large change in $1/R$ between seasons has been recorded (an average factor of 7.6) (Table 3). Altering the model input meteorology and LAI from wet to dry season caused an increase in the SPA-simulated sap flow (Table 5). Subsequently decreasing Ψ_s to dry season values caused only a very small reduction of 0.07 mm d^{-1} in the control plot and a larger decrease of 0.26 mm d^{-1} in the TFE plot. However, setting R to its dry season values caused the simulated sap flow to decrease by 0.23 and 1.07 mm d^{-1} in the control and TFE plots, respectively. Some authors (Donovan *et al*. 2001) have criticized the use of pre-dawn leaf water potential as a proxy for soil water potential, using plants from desert ecosystems which are prone to night-time transpiration. In this instance, we believe that sap flow reaches zero during the night and that night-time transpiration is unlikely. In addition, if errors were introduced by the disequilibrium between soil and leaf water potential, correction of this would have the effect of reducing the estimated soil water potential. In this case, this would decrease the impact of soil water potential on model predictions even further and would reinforce the conclusions already drawn.

In addition, this analysis indicates that very little restriction on sap flow occurred in the control plot even in the height of the dry season. The maximum unstressed sap flow in the control plot was 4.2 mm d^{-1}, compared with 3.9 mm d^{-1} when resistance and soil water potential were changed to their dry season values. This finding mirrors that of Carswell *et al*. (2002), who found no seasonality in the eddy covariance gas exchange measurements performed at a flux tower 1 km from the TFE experiment at Caxiuanõ.

If there is a major change in soil-to-leaf hydraulic resistance, is the change in resistance located above or below ground?

We have shown that ecosystem sap flow is sensitive to soil-to-leaf hydraulic resistance and that soil-to-leaf hydraulic resistance is highly variable both between trees and through time. If simulation of the supply of water to leaves is a realistic and accurate means of simulating tree and forest sap flow as demonstrated here, then the next goal of the development of hydraulic limitation simulation must be to develop a process-based model for the a priori prediction of soil-to-leaf hydraulic resistance from other ecosystem level data. To achieve this goal, we must first deduce which part of the SPA continuum provides the greatest resistance to water movements under hydraulically stressed conditions.

We found that during the wet season, the stem water potential measurements were very close to the soil water potential measurements, indicating that most of the soil-to-leaf hydraulic resistance was located above ground. However, the main change in resistance between seasons was located below ground. The optimization of the parameter b indicated that an average of only 13% of the resistance was located below ground during the wet season, increasing to 45% during the dry season. Between seasons, the total soil-leaf resistance increase in all trees, except in trees C2 and T2. The total above-ground resistance did not change by more than 0.5 s MPa m^2 $mmol^{-1}$ between seasons for all trees, except for trees T3 and T4. These results suggest that under control or ambient conditions, changes in R_{bg} are the dominating factor in the dry season response of the trees. Under some more extreme dry conditions in the TFE plot, some increase in R_{ag} was triggered by the low system water potential. Branch resistivity values indicate no change in above-ground resistance between seasons.

We have therefore found that changes in below-ground resistance are the likely cause of stomatal limitation in the dry season. It remains to be determined whether this is due to increases in root xylem or soil-to-leaf hydraulic resistance.

CONCLUSIONS

We tested the hypothesis that stomata function to maintain isohydric conditions in rain forest trees under hydraulically stressed situations. We tested this hypothesis against diurnal time series in leaf water potential, stomatal conductance, sap flow and stem water potential from a tropical rain forest. The hypothesis, as embedded in the SPA model and including no optimized parameters, is in broad agreement with the data. Further model analyses indicate that variations in soil-to-leaf resistance are the major factor that limits water use during the dry season and that very little hydraulic limitation of sap flow occurred in the control plot during the dry season. Stem psychrometer measurements indicate that the major change in resistance between seasons is located below ground, and we suggest that advances in understanding the response of tropical forests to gas

exchange will result from intensification of research on the dynamics of soil-to-leaf hydraulic resistance.

We expect that increasing confidence in process-based representations of drought stress will eventually allow identification of the critical factors that controls forest vulnerability to drought stress. This will allow integration of our increasing understanding of forest hydrology and gas exchange processes with our concern about the effect of drier climates on biosphere–atmosphere interactions, both within Amazonia and in other drought-threatened ecosystems.

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APPENDIX

We used an estimate of capacitance (C) derived using the data of Goldstein *et al*. (1998) for a seasonal tropical forest in Panama, the only published estimate of C for tropical forest trees. Goldstein *et al*. (1998) found a relationship between tree basal area and tree capacitance by measuring the lag between sap flow in terminal branches and at the base of the trunk. We converted these estimates of C to the units required for SPA, using Eqn 5:

$$C = \frac{1}{n}\sum \frac{c_i}{r\Psi_{min,i}} \quad (5)$$

where C is the new value of capacitance [mmol MPa^{-1} m^{-2} (leaf area)], c_i is the value of capacitance [mmol m^{-2} (basal area)] calculated by Goldstein *et al*. (1998) for the ith tree, Ψ_{min} is the minimum value of Ψ_l reported by Goldstein *et al*. for the ith tree (MPa), n is the number of trees sampled and R is the ratio between basal area and leaf area calculated for our site (5.5 m^2 m^{-2}). The sensitivity of soil–plant–atmosphere (SPA) to C is low, except at the extremes of the ranges (Williams *et al*. 1998), so this method of calculation, using data from elsewhere in the tropics, is a tolerable level of uncertainty, given that there are no other data sets available for tropical species. Using this method, we find a mean value of C of 2300 mmol m^{-2} MPa^{-1}.

The simulated sap flow (S_j) in mm h^{-1} was calculated as shown in Eqn 6.

$$S_j = \sum_{i=l_j}^{h_j} \frac{s_i L_{j,i}}{l_i} \quad (6)$$

where j is a given tree and i is one of the 10 modelled canopy layers. s_i is the SPA-simulated sap flow (mm h^{-1} m^{-2} – ground area) of layer i. l_i is the modelled leaf area (m^{-2}) in layer i per m^{-2} of ground area. $L_{i,j}$ is the leaf area estimated for layer i of tree j (m^{-2}).

Chapter 6

Shifts in plant respiration and carbon use efficiency at a large-scale drought experiment in the eastern Amazon

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Summary

- The effects of drought on the Amazon rainforest are potentially large but remain poorly understood. Here, carbon (C) cycling after 5 yr of a large-scale through-fall exclusion (TFE) experiment excluding about 50% of incident rainfall from an eastern Amazon rainforest was compared with a nearby control plot.
- Principal C stocks and fluxes were intensively measured in 2005. Additional minor components were either quantified in later site measurements or derived from the available literature.
- Total ecosystem respiration (R_{eco}) and total plant C expenditure (PCE, the sum of net primary productivity (NPP) and autotrophic respiration (R_{auto})), were elevated on the TFE plot relative to the control. The increase in PCE and R_{eco} was mainly caused by a rise in R_{auto} from foliage and roots. Heterotrophic respiration did not differ substantially between plots. NPP was 2.4 ± 1.4 t C ha^{-1} yr^{-1} lower on the TFE than the control. Ecosystem carbon use efficiency, the proportion of PCE invested in NPP, was lower in the TFE plot (0.24 ± 0.04) than in the control (0.32 ± 0.04).
- Drought caused by the TFE treatment appeared to drive fundamental shifts in ecosystem C cycling with potentially important consequences for long-term forest C storage.

Introduction

Tropical forests play a key role in global biogeochemical cycles and climate. The Amazon rainforest alone contains 70–120 billion tonnes of carbon (C) in vegetation, an amount of C equivalent to over a decade of global anthropogenic emissions (Houghton *et al.*, 2001; Malhi *et al.*, 2006; Saatchi *et al.*, 2007). Recent analyses predict an increased probability of greater drought frequency and severity across the Amazon over the next 100 yr because of climate change, regional deforestation and fire (Werth & Avissar, 2002; Christensen *et al.*, 2007; Cox *et al.*, 2008; Harris *et al.*, 2008; Malhi *et al.*, 2008). The effects of drought upon ecosystem structure and function in the Amazon are potentially large but remain poorly defined. Relatively little information from field studies is available to test whether the modelled representation of drought effects in the region – decreased forest photosynthesis and increased soil CO_2 efflux (Tian *et al.*, 1998; Peylin *et al.*, 2005; Zeng *et al.*, 2005) – is realistic. Model projections are constrained particularly by a lack of detailed knowledge about the physical controls upon ecosystem C partitioning and soil CO_2 efflux. A range of studies from drought experiments in the Amazon have examined numerous C cycle components in isolation (Nepstad *et al.*, 2002; Davidson *et al.*, 2004, 2008; Sotta *et al.*, 2007; Metcalfe *et al.*, 2007a, 2008, 2010; Brando *et al.*, 2008; da Silva *et al.*, 2009; Meir *et al.*, 2009; da Costa *et al.*, 2010), but none have yet synthesized these

individual components to construct a full C budget of a droughted Amazon forest.

The overall purpose of this study, therefore, was to examine the impacts of a large-scale through-fall exclusion (TFE) treatment in an eastern Amazon primary rainforest on ecosystem C cycling and partitioning. Our analysis here is centred on measurements made across one full seasonal cycle, 4 yr after imposition of the TFE treatment, in 2005, comparing data from the TFE and a nearby control plot. While the TFE treatment was not replicated (Hurlbert, 1984, 2004), it provides insights into ecosystem processes that would otherwise have been impossible to capture in smaller-scale experiments (Carpenter, 1996; Sullivan, 1997; Osmond *et al.*, 2004; Stokstad, 2005). On both plots, for the focal period of 2005, we estimated and integrated all key ecosystem C fluxes to measure forest net primary productivity (NPP) and ecosystem respiration (R_{eco}):

$$R_{eco} = R_{hetero} + R_{auto} \quad \text{Eqn 1}$$

where R_{hetero} and R_{auto} represent R from heterotrophic and autotrophic sources, respectively. The total amount of C expended by trees at a stand scale (plant C expenditure, PCE) was estimated as:

$$\text{PCE} = \text{NPP} + R_{auto} \quad \text{Eqn 2}$$

Under steady-state conditions, where C inputs equal outputs, the following should hold true:

$$\text{GPP} \approx \text{PCE} \approx R_{eco} \quad \text{Eqn 3}$$

where GPP (gross primary productivity) is the total quantity of C entering the forest via photosynthesis. We assessed whether this assumption was valid for both plots by comparing GPP estimated from a previous study, which applied a site-parameterized ecophysiological model to both plots (Fisher *et al.*, 2007), with our estimates of plot-level PCE and R_{eco}. In the case of a substantial imbalance between tree GPP and PCE

$$\text{NTP} = \text{PCE} - \text{GPP} \quad \text{Eqn 4}$$

We examined the implications for the net change in tree C balance (net tree production, NTP).

Materials and Methods

Field site and experimental design

The study site is located in the Caxiuanã National Forest, Pará State, northeastern Brazil (1°43′3.5″S, 51°27′36″W). The forest is a lowland *terra firme* rainforest with high annual rainfall (2000–2500 mm) and a pronounced dry season (Table 1). Across the entire year, mean soil surface temperature is approximately 25°C, with little seasonal and diurnal variation. The soil type is a highly weathered yellow Oxisol (Quesada *et al.*, 2009). In January 2002, a 1 ha plot (TFE plot) was modified by the installation of plastic panels placed at 1–2 m above the ground, excluding approximately 50% of incident rainfall, and causing a shift in soil water availability, plant water relations, leaf physiology and, ultimately, tree growth and survival (Fig. 1). The change in annual rainfall magnitude and dry season length imposed by the TFE treatment simulated some key aspects of a precipitation regime more commonly encountered in some savannas and deciduous forests in the region (Betts *et al.*, 2004; Malhi *et al.*, 2009a), which is consistent with long-term climate predictions for the region from at least one major global climate model- HadCM3 (Collins *et al.*, 2001).

Air temperature beneath the TFE panels was *c.* 2°C warmer than ambient air during the dry season, although soil temperature remained similar to ambient values throughout. During the wet season, air temperatures above and below the TFE panels were similar (da Costa *et al.*, 2006). The boundary of the TFE plot was trenched to a depth of 1–2 m and lined with plastic to minimize lateral ingress of water from adjacent, wetter soil. The control plot perimeter was also trenched to avoid confounding treatment effects. All measurements were taken at least 10 m inside the perimeter of each plot to minimize edge effects.

Above-ground carbon stocks and solid fluxes

Canopy leaf area density and leaf morphology data were derived from Metcalfe *et al.* (2010). To calculate leaf area

Table 1 Key vegetation and soil features for each plot surveyed

Plot characteristics	Control	TFE
Vegetation		
Tree number ha^{-1}	532	501
Tree species number	118	113
Surface litter mass (t C ha^{-1})	2.7 ± 0.2	3.4 ± 0.5
Soil 0–10 cm		
Bulk density (t m^3)	1.4	1.2
Clay content (%)	18	13
Silt content (%)	5	4
Sand content (%)	77	83
pH	4	4
Carbon concentration (mg g^{-1})	9	12
Nitrogen concentration (mg g^{-1})	0.4	0.3
Phosphorus concentration (mg g^{-1})	0.1	0.2
Carbon : nitrogen ratio	23	35
Soil cation exchange (cmol dm^{-3})	0.8	0.7

Values indicate mean ± 95% confidence intervals (where available and appropriate). Surface litter means are derived from 25 replicates. TFE, through-fall exclusion. Tree number and basal area represent all individuals over 10 cm diameter at breast height, measured in January 2005. Soil values are collated from data in Sotta *et al.* (2007).

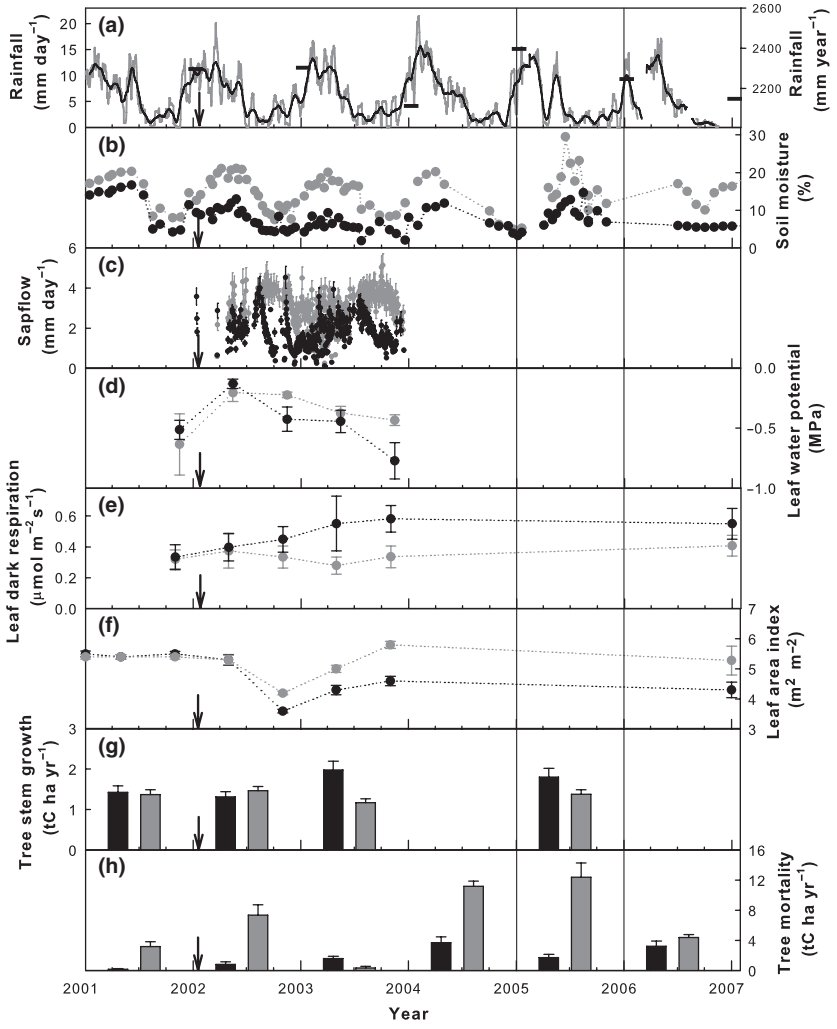

Fig. 1 Through-fall exclusion (TFE) effects on key forest processes before, during and after the study period of 2005 (highlighted). Grey circles and bars, control; closed circles and bars, through-fall exclusion. The arrow at the base of each panel indicates the beginning of the TFE treatment. (a) Rainfall is presented as daily totals (grey line), 30 d moving average (black line) and annual totals for the preceding year (black bars). (b) Soil volumetric moisture values before and after 2005 represent the mean of hourly measurements from time domain reflectometer probes (TDR) installed at 5,100 and 250 cm soil depths in a single soil pit on each plot. During 2005, soil moisture values are the mean of 25 TDR soil surface (30 cm soil depth) monthly measurements along a regularly spaced grid within each plot. (c) Sapflow and leaf water potential (d) data are derived from Fisher *et al.* (2006). Leaf dark respiration (*R*) (e) and leaf area index (f) data are reproduced from Metcalfe *et al.* (2010). (g) Tree stem growth and mortality (h) estimates include only stems > 10 cm diameter at breast height (DBH); these data are reproduced from da Costa *et al.* (2010). Error bars indicate 95% confidence intervals around plot means. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape scale heterogeneity.

index (LAI, m^2 leaf m^{-2} ground), images of the canopy were recorded at 25 locations within each plot in late 2004 and early 2007 with a digital camera and hemispherical lens; total LAI was apportioned into canopy height categories with LAI height profile data collected at a tower in the centre of each plot. Mean leaf mass per unit area (LMA) for each plot was calculated for the same periods by harvesting leaves from different canopy layers, determining area and dry mass of each leaf, then dividing dry mass by one-sided area. Values intermediate to those calculated on the two sampling dates were used to estimate LAI and LMA in 2005. To derive estimates of total plot foliar biomass, LAI and LMA from each canopy layer were multiplied and then the estimated foliar biomass for each layer was summed.

The monthly flux of litter falling from the canopy in 2005 was recorded in 20 mesh traps (area = 1 m^2 per trap) placed at 1 m above the ground surface on the control plot, and above the plastic panels on the TFE plot (height 2–2.5 m). Litter retrieved from the traps was dried at 70°C to constant mass, separated into leaves, flowers, fruits and seeds, woody material < 2 cm diameter and weighed. Previous studies have shown that 36–40% of litterfall is intercepted before it reaches litter traps and decomposed within the canopy (Edwards, 1977; Frangi & Lugo, 1985), so we multiplied recorded litterfall by 1.3 to provide a conservative correction for this 'canopy storage' term. In addition, litterfall collection in mesh traps does not account for material lost via herbivory (12–30% of canopy leaf mass, Clark *et al.*, 2001). Therefore, we conservatively estimated mean herbivory on both plots as 10% of leaf litterfall.

Branches > 2 cm diameter falling from live trees were not adequately sampled by mesh traps and so this flux was separately monitored between December 2008 and August 2009 by collecting, drying at 70°C to constant mass and weighing all woody material > 2 cm diameter which appeared along four 1 × 80 m transects per plot that had previously been cleared of all woody material (see the Ground carbon stocks and solid fluxes section).

Plot values for live and dead stem standing biomass, growth, recruitment and mortality were obtained from da

Costa *et al.* (2010). Annual stem growth increment was recorded for all live tree stems > 10 cm diameter at breast height (DBH, 1.35 m) between 2001 and 2008. Recruitment of new trees into the > 10 cm DBH category was recorded in August 2005. Tree diameter was converted to mean (± 95% confidence intervals) above-ground stem mass using eight previously published allometric equations (da Costa *et al.*, 2010). Mortality was assessed as death or disappearance of previously permanently marked stems.

In addition to stem mass loss via mortality, we included a term for mass loss via live tree damage (heartwood rot, crown and partial trunk loss) of 0.44 ± 0.10 t C ha^{-1} yr^{-1} from Chambers *et al.* (2001). The biomass of smaller stems was estimated once in March 2005 by recording diameter of all stems between 2 and 10 cm DBH in a 20 × 20 m area on both plots, and using the same DBH–biomass conversion equations as for the larger stems using mean wood density for trees > 10 cm DBH on each plot (0.7 g cm^{-3}), and extrapolating this value to the rest of the plot area. The growth of stems between 2 and 10 cm DBH was estimated by quantifying the proportion of growth to biomass for stems > 10 cm DBH, and multiplying this value by estimated plot biomass of stems between 2 and 10 cm DBH. This method assumed that tree growth was similar across size classes, which was unlikely, but in the absence of direct measurements of the growth of stems 2–10 cm DBH it yielded an approximation of this relatively minor component (< 1% of total NPP in our analysis).

Ground carbon stocks and solid fluxes

Coarse woody debris (CWD) necromass was calculated in December 2009 by removing and weighing *in situ* all woody material > 2 cm diameter along four 1 × 80 m transects within each plot. A subset of this material was then dried at 70°C to constant mass and reweighed to derive a correction factor for the wet mass values from the rest of the material. Then each piece of the subset was measured with callipers to estimate surface area. The correlation between piece surface area (cm^2) and dry weight (g) was used to estimate surface area of all pieces collected on the plots (r^2 = 0.81, mass = 16.49 × $area^{0.63}$). Finally, each piece from the subset was placed into a water-filled cylinder to measure piece volume, and hence tissue density (dry mass/volume). Density was estimated separately for five classes of wood decomposition following Harmon *et al.* (1995). In cases where material within the transect was too large to remove and weigh manually, the diameter at three points was recorded to estimate surface area and volume, and wood density associated with the decomposition class was used to convert the volume of each piece into mass. Total plot CWD mass and surface area were calculated as the sum of the smaller pieces removed from the transects and the larger pieces remaining on the transects. To back-calculate CWD biomass for 2005, our study period, we assumed that the rate of CWD accumulation necessary to achieve the observed 2009 plot difference was proportional to stem mortality, quantified annually by da Costa *et al.* (2010).

Ground surface fine litter mass (including woody material < 2 cm diameter) was collected from 25 areas (0.25 m^2) in each plot in December 2009. Litter samples were cleaned of inorganic detritus, dried at 70°C to constant mass and weighed.

Fine root biomass and production data were derived from Metcalfe *et al.* (2008). Briefly, 27 soil cores down to 30 cm depth were removed from each plot in 2005, fine roots (< 2 mm diameter) were removed following the method of Metcalfe *et al.* (2007b), dried at 70°C to constant mass and then weighed. Fine roots below 30 cm and coarse roots (> 2 mm diameter) were not sampled with these cores. To correct for this, four 1.5 × 1.5 m holes were excavated to 3 m soil depth in each plot in June 2008. All roots retrieved were collected, dried at 70°C to constant mass and weighed. Dry root mass was apportioned into soil depth and diameter categories. From these data (D Galbraith, unpublished), the proportions of fine root mass through the entire soil column down to 3 m soil depth located within the surface (control = 0.63, TFE = 0.65), and of total root mass represented by roots < 2 mm diameter (control = 0.08, TFE = 0.49), were calculated and applied to the measured surface fine root values to estimate total root mass and production down to 3 m soil depth and for all root diameters. Because of the low sample size in this study, we applied error estimates of 13% around coarse root standing biomass and growth values from a more extensive sampling programme in a similar forest (Silver *et al.*, 2000). To back-calculate coarse root biomass for 2005, our main period of interest, we assumed that the rate of coarse root mortality necessary to achieve the observed difference between plots in 2008 was proportional to the measured rate of stem mortality (da Costa *et al.*, 2010). To quantify coarse root growth, the proportion of growth to biomass of stems > 10 cm DBH was quantified, and this value was multiplied by the estimated plot biomass of coarse roots, down to 3 m soil depth. This method assumed that coarse root growth and stem growth were similar and that coarse root growth was constant down the soil profile. This was a source of uncertainty but in the absence of direct measurements of coarse root growth anywhere in the soil profile and fine root growth below 30 cm soil depth it yielded an approximation of this relatively minor component (< 11% of total NPP in our analysis).

Net dissolved organic carbon (DOC) export (DOC runoff – DOC deposition) was taken as 0.19 ± 0.07 t C ha^{-1} yr^{-1} from an intensive study of DOC dynamics in a central Amazon catchment (Waterloo *et al.*, 2006).

Carbon dioxide efflux

Leaf area index and leaf dark *R* data were derived from Metcalfe *et al.* (2010). Leaf dark *R* was recorded from leaves throughout the canopy in 2003 and 2007. All measurement campaigns sampled fully expanded, nonsenescent, undiseased leaves. Leaf dark *R* was recorded after CO_2 gas exchange in dark conditions had stabilized (usually after *c.* 10 min), at ambient air CO_2 concentration (360–380 ppm) and humidity (60–80%). Estimates of leaf dark *R* per unit leaf area in each canopy layer was multiplied by mean plot LAI located within the same canopy layer, and then all layers were summed to derive plot-level estimates of night-time leaf *R* assuming 12 h of darkness each day throughout the year and a constant temperature of 25°C (Metcalfe *et al.*, 2010). Values for 2005 were estimated as the mean of the measurements in November 2003 and January 2007. Leaf light *R* on both plots was estimated as 67% of leaf dark *R* from Lloyd *et al.* (2009), who used light response curves from another lowland Amazon forest (Domingues *et al.*, 2005) and applied light-inhibition equations from eucalyptus seedlings (Atkin *et al.*, 2000).

Emissions of volatile organic compounds (VOCs) constitute another minor source of C from leaves. We used a value of 0.13 ± 0.05 t C ha^{-1} yr^{-1} for this component (Malhi *et al.*, 2009b) which sums published estimates of VOC, including isoprene and terpene (Kuhn *et al.*, 2007), and methane (do Carmo *et al.*, 2006) emissions from tropical forests.

No site measurements of live tree stem *R* at 1.3 m were available, so a value of 0.6 ± 0.08 μmol m^{-2} stem surface s^{-1} was taken from the existing literature (Nepstad *et al.*, 2002; Meir & Grace, 2002) and applied to both plots. This may underestimate total stem *R* rates since portions of stem higher up (Yoda, 1983) and branches (Cavaleri *et al.*, 2006) tend to have higher CO_2 effluxes than the main bole near the ground. Tree stem area was estimated using a taper function to estimate stem basal diameter for all trees > 2 cm DBH on both plots (Chambers *et al.*, 2000) and then applying an equation relating basal diameter to total stem surface area (Yoneda, 1993) from 315 *terra firme* Amazon trees (Chambers *et al.*, 2004). Plot-level scale stem C efflux was estimated by multiplying R_{stem} per unit stem area by total plot live stem area.

Soil CO_2 efflux data were derived from Metcalfe *et al.* (2007a). Total soil CO_2 efflux (R_{soil}) was recorded each month through 2005 at 25 points in each plot. R_{soil} was partitioned into contributions from surface organic litter, roots and soil organic matter at nine points on each plot in the dry (November 2004) and wet (June 2005) seasons. Monthly contributions from each of the R_{soil} components were linearly interpolated between these two periods.

R from coarse woody debris was recorded once in August 2009 from 12 to 16 pieces of dead wood on the ground for each of the five decomposition classes (see the Ground carbon stocks and solid fluxes section for details of the CWD necromass survey) randomly selected on the control and TFE plots. We included the *R* contribution from standing dead trees using the same surface area estimation methodology as live stems, and assuming they had similar CO_2 efflux rates to ground CWD, which was a source of uncertainty but in the absence of detailed information about wood decomposition patterns in the tropics it yielded an approximation of this relatively minor component (< 11% of total *R* in our analysis). Plot-level dead wood *R* was estimated by multiplying stem *R* per unit dead wood area by total plot dead wood surface area.

Data analysis and presentation

The lack of treatment replication precluded fully comparative statistical analysis (Hurlbert, 1984, 2004), but 95% confidence intervals were calculated around means as an indication of the reliability of the observed mean differences at a plot scale. Throughout the manuscript, therefore, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. Errors were propagated by quadrature of absolute errors for addition and subtraction, and quadrature of relative errors for division and multiplication (Aragão *et al.*, 2009). This assumes that the errors are independent and normally distributed. All *R* terms were summed into R_{auto} and R_{hetero} contributions, which together make up R_{eco}. Total NPP was calculated as the sum of all plant growth components, and PCE was then estimated as the sum of total NPP and R_{auto}. Carbon use efficiency (CUE) at an ecosystem level and individually for different plant components (canopy, stems, roots) was calculated as:

$$\mathrm{CUE} = \frac{\mathrm{NPP}}{\mathrm{NPP} + R_{auto}} \qquad \text{Eqn 5}$$

Turnover time (1/turnover rate) estimates for specific components on the control plot were derived by dividing C fluxes by stocks. Independent checks on C flux estimates were derived from eddy covariance (Carswell *et al.*, 2002) and detailed modelling studies (Fisher *et al.*, 2007) at the site, albeit for different time periods from the current study. In addition, data were compared to published equations, which use a mass balance approach assuming steady-state conditions, to estimate total below-ground allocation (TBCA; equations from Raich & Nadelhoffer (1989) were modified to include contributions from coarse wood, root litter and DOC) and soil CO_2 efflux (Malhi *et al.*, 2009b)

Results

Ecosystem C balance, partitioning and CUE

Estimated PCE during the period of measurement was slightly greater in the TFE (33.9 ± 3.6 t C ha^{-1} yr^{-1}) than in the

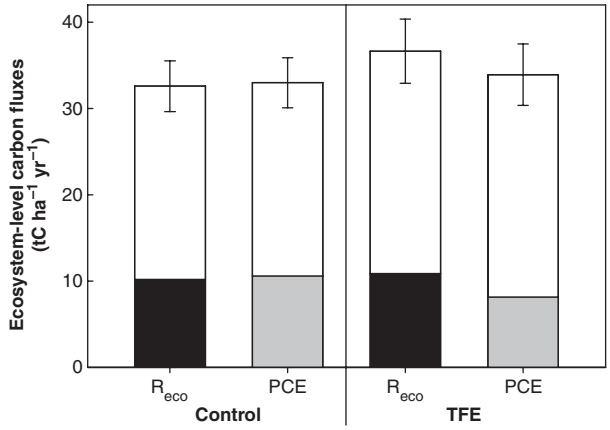

Fig. 2 Total ecosystem-level carbon fluxes on the plots. Grey bars, net primary productivity (NPP); open bars, autotrophic respiration (R_{auto}); closed bars, heterotrophic respiration (R_{hetero}). All data and their sources are specified in Table 2. Error bars indicate 95% confidence intervals around the total flux values. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. R_{eco}, ecosystem respiration; TFE, through-fall exclusion; PCE, plant carbon expenditure.

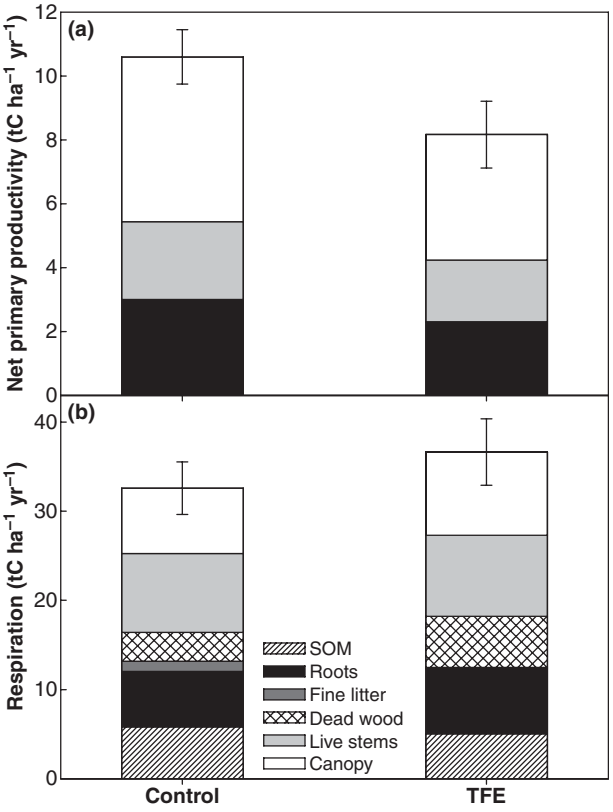

Fig. 3 Net primary productivity (NPP) (a) and respiration (*R*) (b) from different above- and below-ground ecosystem components on both plots. All data and their sources are specified in Table 2. Error bars indicate 95% confidence intervals around the total flux values. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. Foliage *R* incorporates measured leaf dark *R* (Metcalfe *et al.*, 2010) and modelled leaf light *R* (Lloyd *et al.*, 2009); stem *R* is from all stems > 2 cm diameter at breast height assuming the same value of *R* per unit stem surface area on both plots derived from the existing literature (Meir & Grace, 2002; Nepstad *et al.*, 2002), while dead wood *R* includes contributions from coarse woody debris (CWD) on the ground and standing dead stems. Dead wood *R* was measured in 2009 and back-calculated to 2005 assuming dead stem and CWD stock accumulation was proportional to measured tree mortality. Canopy production incorporates measured litterfall and literature-based estimates for herbivory, canopy storage of litter and volatile organic carbon emissions. Stem NPP values are derived from da Costa *et al.* (2010) and include growth of all stems > 2 cm diameter at breast height, branch fall and recruitment. Root growth includes both fine and coarse root growth down to 3 m soil depth using root profile data from D Galbraith (unpublished). TFE, through-fall exclusion; SOM, soil organic matter.

control plot (33.0 ± 2.9 t C ha^{-1} yr^{-1}) (Table 2, Fig. 2). R_{eco} was elevated on the TFE plot compared with the control plot, although there was substantial uncertainty around the plot means (Table 2, Fig. 2; 36.6 ± 3.7 and 32.6 ± 2.9 t C ha^{-1} yr^{-1}, respectively). Greater R_{eco} in the TFE plot was mainly attributable to the higher R_{auto} flux of 25.8 ± 3.4 t C ha^{-1} yr^{-1} compared with 22.4 ± 2.8 t C ha^{-1} yr^{-1} in the control (Table 2, Fig. 2), which in turn was driven by a rise in canopy and root *R* (Table 2, Fig. 3b). By contrast, R_{hetero} was similar between plots (*c.* 10.5 t C ha^{-1} yr^{-1}) because greater estimated TFE dead wood *R* was offset by lower soil heterotrophic CO_2 efflux (Table 2, Fig. 3b).

Total estimated NPP was 2.4 ± 1.4 t C ha^{-1} yr^{-1} lower on the TFE plot relative to the control (Table 2, Fig. 3a). On both plots, approximately half of NPP was derived from the canopy, with the remainder split evenly between roots and stems (Table 2, Fig. 3a). The trees on both plots allocated slightly more total assimilated C (NPP and R_{auto}) to the canopy (*c.* 38%) than stems (*c.* 33%) or roots (*c.* 28%), of which 70–80% was comprised of R_{auto} for every component (Table 2). On both plots, canopy CUE was higher than either stem or root CUE (Fig. 4). The TFE treatment was associated with a lower CUE in all plant organs, but particularly canopy CUE (control, 0.41 ± 0.07; TFE, 0.30 ± 0.07). The overall effect was a lower ecosystem CUE in the TFE plot of 0.24 ± 0.04 compared with 0.32 ± 0.04 in the control plot (Table 2, Fig. 4).

Quantities and dynamics of C stocks

The ratio of below to above-ground live plant C stocks was 0.64 ± 0.21 in the control plot compared with 0.15 ± 0.05 in the TFE plot (Fig. 5). In the control plot, estimated mean turnover time of live canopy foliage, fine litter on the ground, CWD, fine roots and live stems were 0.5, 0.7, 4.4, 3.4 and 88.0 yr, respectively (Fig. 6). Stocks of C in ground fine litter were slightly elevated in the TFE plot (Table 1, *c.* 0.7 t C ha^{-1} yr^{-1}), despite lower influx from canopy

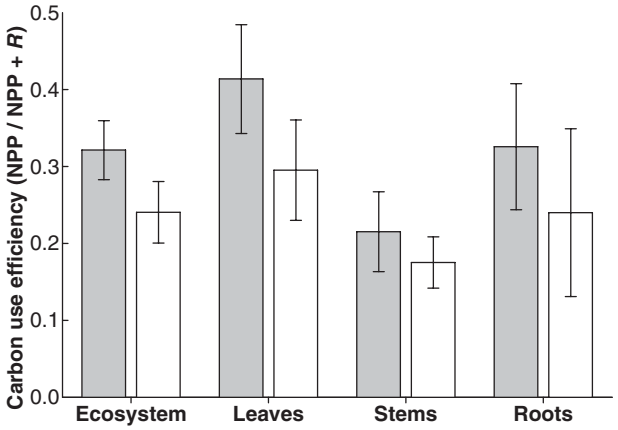

Fig. 4 Carbon use efficiency (CUE) at the ecosystem level and for different above- and below-ground plant components on both plots. Grey bars, control; open bars, through-fall exclusion. All data and their sources are specified in Table 2. Error bars indicate 95% confidence intervals around the mean CUE values. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. NPP, net primary productivity; *R*, respiration.

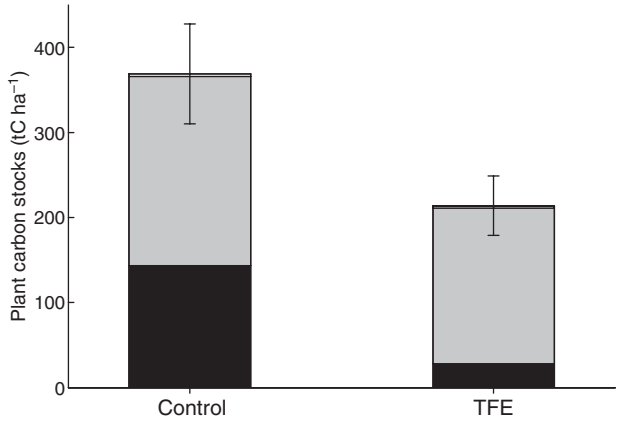

Fig. 5 Stocks of carbon in different above- and below-ground plant components on both plots. Open bars, canopy; grey bars, stems; closed bars, roots. Error bars indicate 95% confidence intervals around the total stocks. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. Root stocks are presented down to 3 m soil depth using root profile data from D Galbraith (unpublished). TFE, through-fall exclusion.

litterfall (Table 2, Fig. 3a; 0.9 ± 0.2 t C ha^{-1} yr^{-1}) compared with the control. In the TFE plot, microbial fine litter *R* removed only around 4% of ground fine litter C stock each year (Tables 1, 2), which means that to balance C inputs from litterfall whilst accounting for the observed increase in ground litter C stock relative to the control, 75 ± 6% of the TFE fine litter stock must annually been removed by processes other than microbial *R* (Fig. 7), such as physical disintegration and/or consumption by detritivores. Using the same approach, a greater percentage of the control plot standing surface litter C stock was removed each year via both microbial *R* (43 ± 4%) and other processes (95 ± 11%; Fig. 7). In the control plot, the annual decomposition metrics for ground litter exceeded 100% because turnover time was < 1 yr (Fig. 6).

Quantities and dynamics of CO_2 fluxes

Plot differences in CO_2 efflux were the net product of shifts in both *R* per unit area of plant material (e.g. leaves, CWD), and the total amount of plant material area. These two properties often responded in different ways to the TFE treatment. For example, across all measurement periods, mean dark leaf *R* per unit leaf area was greater in the TFE plot (0.51 ± 0.05 μmol m^{-2} s^{-1}) than in the control plot (0.34 ± 0.03 μmol m^{-2} s^{-1}), while LAI declined over 7 yr following the imposition of the TFE treatment by *c.* 1 m^2 m^{-2} relative to the control (Metcalfe *et al.*, 2010). The net product of these interacting factors was a marked increase in plot-level dark leaf *R* (Table 2, Fig. 3b; Metcalfe *et al.*, 2010) in the TFE plot (5.6 ± 2.4 t C ha^{-1} yr^{-1}) relative to the control (4.4 ± 1.7 t C ha^{-1} yr^{-1}).

Total dead wood surface areas were 0.19 ± 0.05 and 0.27 ± 0.06 m^2 m^{-2} in the control and TFE plots, respectively. In both plots, *c.* 70% of dead wood area was in the form of standing stems with the remainder comprising ground CWD. The mean CWD *R* values per unit wood area were 4.6 ± 0.9 and 5.5 ± 1.6 μmol m^{-2} s^{-1} in the control and TFE plots, respectively. The weak trend towards higher mean CWD *R* on the TFE plot was specifically attributable to the greater quantity of relatively undecomposed CWD in the TFE plot (Fig. 8b), possibly from a greater recent input of new wood via increased tree mortality and branch fall, which appeared to have a higher rate of *R* (Fig. 8a). The combination of a greater quantity of fresh, undecomposed CWD in the TFE plot and elevated rates of *R* per unit area of fresh CWD translated into total CWD *R* of 5.7 ± 1.5 t C ha^{-1} yr^{-1} in the TFE plot compared with 3.3 ± 0.9 t C ha^{-1} yr^{-1} in the control (Table 2, Fig. 3b).

Estimated plot-level stem *R* emissions (Table 2, Fig. 3b) were similar in the TFE (9.1 ± 1.8 t C ha^{-1} yr^{-1}) and control plots (8.8 ± 1.8 t C ha^{-1} yr^{-1}) because the lower stem area of trees > 10 cm DBH in the TFE plot was outweighed by the higher stem area of trees between 2 and 10 cm DBH.

R_{soil}, the sum of fine litter, root and soil organic matter respiration, was only slightly diminished by 0.69 ± 0.14 t C ha^{-1} yr^{-1} in the TFE plot relative to the control during the measurement year of 2005 (Table 2, Fig. 3b; Metcalfe *et al.*, 2007a). The relative contribution of autotrophic and heterotrophic sources to R_{soil} differed between plots. Thus, in the control plot, R_{soil} was divided almost equally between heterotrophic (53%, 6.9 ± 0.3 t C ha^{-1} yr^{-1}) and autotrophic (47%, 6.2 ± 0.3 t C ha^{-1} yr^{-1}) contributions,

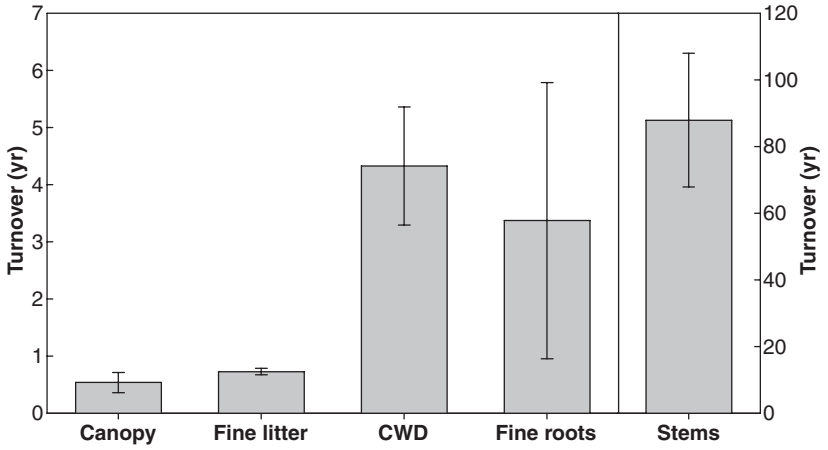

Fig. 6 Turnover time of different ecosystem components on the control plot. Turnover is calculated assuming steady-state conditions as input or output/stock. Error bars indicate 95% confidence intervals around the mean turnover time values. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. CWD, coarse woody debris.

whereas R_{soil} in the TFE plot was dominated to a greater extent by autotrophic sources (59%, 7.3 ± 0.3 t C ha^{-1} yr^{-1}) and heterotrophic R (41%, 5.1 ± 0.2 t C ha^{-1} yr^{-1}) contributed relatively less (Table 2, Fig. 3b). Expected R_{soil} and TBCA, calculated for the control plot from C inputs and assuming steady-state conditions, were both lower than our measurements (Table 2).

Discussion

Drought effects on net carbon fluxes: patterns and processes

A key assumption of the multi-component integration 'bottom-up' approach employed here to examine C cycling at an eastern Amazon rainforest, is that steady state conditions exist at the site, and therefore that PCE is approximately equal to GPP. In the control plot, where steady-state conditions are plausible (at least over the timescale of the experiment), PCE estimated for the year of 2005 for this study (33.0 ± 2.9 t C ha^{-1} yr^{-1}) was quite similar to estimates of GPP over 2002 and 2003 made using an ecophysiological model parameterized at the two plots (*c.* 30 t C ha^{-1} yr^{-1}; Table 2; Fisher *et al.*, 2007). By contrast, PCE in the TFE plot (33.9 ± 3.6 t C ha^{-1} yr^{-1}) was higher than the modelled GPP in the TFE plot of 26.9 and 27.1 t C ha^{-1} yr^{-1} in 2002 and 2003, respectively (Table 2; Fisher *et al.*, 2007). As a preliminary exploration of the possible consequences of this mismatch between PCE and GPP in the TFE plot, we conducted the following analysis. We calculated the mean of the two annual modelled GPP estimates from the TFE plot and, assuming these estimates were representative of the forest in 2005 and had an uncertainty of 10% (Fox *et al.*, 2009), we then subtracted this GPP value from measured PCE for 2005. The result implies that the TFE forest was expending 7.0 ± 4.5 t C ha^{-1} yr^{-1} more than it was assimilating (net tree production; Table 2). Clearly, a major uncertainty with this analysis is the assumption that modelled 2002 and 2003 GPP values are representative of conditions in 2005 – although, if anything, stand-level GPP values would be expected to decline further after 2003, and preliminary runs of the site-parameterized ecophysiological model beyond 2003 support this view (R Fisher, pers. comm.), which would suggest that the discrepancy between 2005 GPP and PCE/R_{eco} was likely to be even larger. Useful future lines of enquiry would be to quantify the sources of uncertainty that could not be incorporated into this analysis (e.g. TFE effects on stem allometry, stem R, leaf light R, herbivory, canopy litter storage, leaf temperature) to test this hypothesis further.

Notwithstanding the uncertainty surrounding this analysis, the substantial apparent 'overspend' of C by the forest could feasibly be sourced from nonstructural carbohydrate (NSC) stores and reductions in NPP. From the available literature, we estimate that the TFE forest may have had *c.* 20 t C ha^{-1} of available NSC (*c.* 8% of live biomass; Graham *et al.*, 2003; Würth *et al.*, 2005; Poorter & Kitajima, 2007) to draw upon at the beginning of the TFE treatment. In addition, during the treatment, the TFE forest would be making annual savings from lower NPP construction (25% of biomass; Penning de Vries, 1975) and maintenance R costs on the order of 3 t C ha^{-1} yr^{-1} (data not shown).

Previous work at the study site has indicated that the larger canopy trees responded isohydrically to drought, by maintaining leaf water potential above a minimum critical value to avoid xylem embolism, but thereby also reducing C assimilation rates (Fisher *et al.*, 2006). This 'C starvation' hypothesis (McDowell *et al.*, 2008; McDowell & Sevanto, 2010) could provide one potential mechanistic explanation for the observed increase in tree mortality on the TFE plot (da Costa *et al.*, 2010) and decline in reproduction (DB Metcalfe, unpublished). Our estimate of a large possible C overspend relative to likely NSC reserves is consistent with a scenario whereby trees may reach critically low amounts of NSC under extended drought conditions, and contrary to previous suggestions that large NSC pool sizes in forest trees render C starvation-induced mortality unlikely (Sala *et al.*, 2010). An obvious next step is to verify whether trees

Table 2 Summary of plot carbon fluxes

No.	Component	Control plot Mean	Control plot 95% CI	TFE plot Mean	TFE plot 95% CI	Source
Net primary productivity (NPP, t C ha^{-1} yr^{-1})						
1	Leaves	2.5	0.1	2.1	0.1	This study
2	Twigs	0.6	0.1	0.5	0.1	This study
3	Reproduction	0.5	0.1	0.2	0.1	This study
4	Herbivory	0.3	0.01	0.2	0.01	Clark *et al.* (2001)
5	Canopy storage	1.1	0.1	0.8	0.04	Edwards (1977)
6	Branch	0.5	0.4	0.4	0.1	This study
7	Stem > 10 cm DBH	1.8	0.2	1.4	0.1	da Costa *et al.* (2010)
8	Stem 2–10 cm DBH	0.1	0.02	0.1	0.02	This study
9	Recruitment	0.1	0.02	0.1	0.02	This study
10	Fine root	1.7	0.6	2.0	1.0	Metcalfe *et al.* (2008)
11	Coarse root	1.1	0.4	0.2	0.1	This study
12	Dissolved organic carbon	0.2	0.07	0.2	0.07	Waterloo *et al.* (2006)
13	Volatile organic compounds	0.1	0.05	0.1	0.05	Malhi *et al.* (2009b)
Respiration (R, t C ha^{-1} yr^{-1})						
14	Dark leaf	4.4	1.7	5.6	2.4	Metcalfe *et al.* (2010)
15	Light leaf	2.9	1.1	3.8	1.6	Lloyd *et al.* (2009)
16	Live stems > 10 cm DBH	7.7	1.8	7.4	1.7	da Costa *et al.* (2010)
17	Live stems 2–10 cm DBH	1.2	0.3	1.7	0.4	This study
18	Dead stems	2.5	0.7	3.9	1.3	This study
19	Coarse woody debris	0.8	0.6	1.9	0.7	This study
20	Fine litter	1.1	0.1	0.1	0.005	Metcalfe *et al.* (2007a)
21	Roots	6.2	0.3	7.3	0.3	Metcalfe *et al.* (2007a)
22	Soil organic matter	5.8	0.3	5.0	0.2	Metcalfe *et al.* (2007a)
Measured ecosystem level fluxes (t C ha^{-1} yr^{-1})						
23	Total NPP	10.6	0.9	8.2	1.0	$\sum$ 1–13
24	R_{eco}	32.6	2.9	36.6	3.7	$\sum$ 14–22
25	R_{auto}	22.4	2.8	25.8	3.4	$\sum$ 14–17, 21
26	R_{hetero}	10.2	1.0	10.9	1.5	$\sum$ 18–20, 22
27	PCE	33.0	2.9	33.9	3.6	23 + 25
28	NTP[a]	1.8	4.3	7.0	4.5	27 – mean of (31 + 32)
29	TBCA	9.2	0.8	9.6	1.1	$\sum$ 10–12, 21
30	Ecosystem CUE	0.32	0.04	0.24	0.04	23/(23 + 25)
Alternative ecosystem level fluxes (t C ha^{-1} yr^{-1})						
31	Model GPP	30.9	3.1[b]	26.9	2.7[b]	2002, Fisher *et al.* (2007)
32	Model GPP	31.4	3.1[b]	27.1	2.7[b]	2003, Fisher *et al.* (2007)
33	Eddy covariance GPP	36.3	—	—	—	Carswell *et al.* (2002)
34	Component R_{soil}	16.3	—	12.1	—	Sotta *et al.* (2007)
35	Model R_{soil}	11.7	—	9.5	—	Malhi *et al.* (2009b)
36	Model TBCA	6.0	0.9	7.0	1.1	Raich & Nadelhoffer (1989)

Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. Values in source calculations refer to the parameter identity numbers (first column).
TFE, through-fall exclusion; DBH, diameter at breast height; PCE, plant carbon expenditure; TBCA, total below-ground carbon (C) allocation from the method of Raich & Nadelhoffer (1989) modified to include the contributions of coarse branches, root litter and dissolved organic carbon (DOC); CUE, carbon use efficiency; R_{eco}, ecosystem respiration; R_{auto}, autotrophic respiration; R_{hetero}, heterotrophic respiration; R_{soil}, soil CO_2 efflux.
[a]NTP, net tree production, is the balance between C entering and exiting live plant biomass.
[b]10% of gross primary productivity (GPP), derived from Fox *et al.* (2009). Alternative ecosystem level C fluxes are separated into three broad sources: modelled data, eddy covariance data and 'component' data from component-scale field measurements.

in the TFE plot do indeed have lower NSC contents relative to trees in the control.

Drought effects on carbon partitioning and turnover

In the TFE plot there was a large decrease in plant C stocks, particularly below ground, attributable to an elevated rate of tree mortality relative to the control (Fig. 5; da Costa *et al.*, 2010). Estimated plant C stocks situated below ground constituted 24 ± 8 and 8 ± 3% of the total in the control and TFE plots, respectively (Fig. 5), compared with global and regional syntheses suggesting a value of around 21% for tropical forests (Jackson *et al.*, 1996; Cairns *et al.*, 1997).

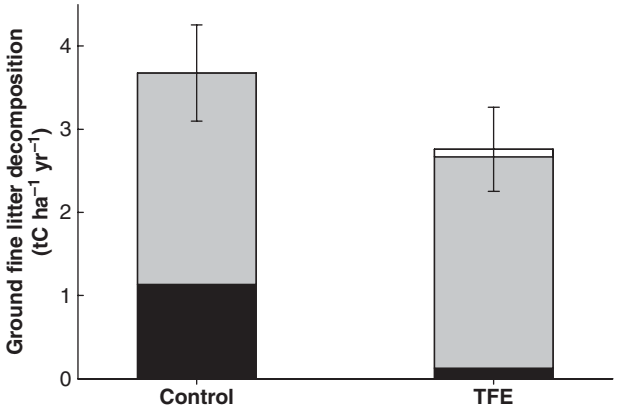

Fig. 7 Contribution of microbial respiration (closed bars) and other processes (grey bars) to decomposition of ground fine litter. Other processes could include physical disintegration and/or consumption by detritivores. Shortfall (*S*, open bars) represents the imbalance between litter inputs (*L*) and decomposition required to account for the observed increases in fine litter stock in the through-fall exclusion (TFE) plot relative to the control, assuming that there was no difference in stocks between plots before the TFE treatment. Microbial respiration (*M*) was estimated by measurement of soil CO_2 efflux (R_{soil}) at the same point with and without surface litter (Metcalfe *et al.*, 2007a); physical/macrofaunal removal in the plots was calculated as $L - M - S$. Error bars indicate 95% confidence intervals around the total values. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity.

The relatively modest NPP reduction on the TFE plot is surprising, particularly given the substantial decline in live plant biomass. The two largest components of NPP – stem and canopy production – are also the most reliably measured, and bear out the general conclusion that NPP declined, though not by much, in the TFE plot compared with the control (da Silva *et al.*, 2009; da Costa *et al.*, 2010). While large trees (> 20 cm DBH) showed substantial reductions in NPP, it was clear, from studies both at this site (da Costa *et al.*, 2010) and another TFE experiment in the Amazon (Brando *et al.*, 2008), that smaller trees (< 20 cm DBH) appeared to be relatively resilient to drought. This could indicate that understorey trees benefited from increased light availability as the canopy thinned, and/or subcanopy conditions (less wind, higher air humidity and CO_2 concentrations) which promoted water-use efficiency.

Plant *R* responses reported in this study are surprising because drought almost always inhibits *R* in actively growing plant tissues (Atkin & Macherel, 2009 and references therein). However, perhaps of significance for this study, responses from slow-growing, mature plants appear to be more variable. This study therefore adds to the minority of documented occurrences of drought-induced increases in plant *R* (Zagdanska, 1995; Ghashghaie *et al.*, 2001; Bartoli *et al.*, 2005). Other studies in the Amazon have reported dry-season increases in leaf dark *R* at a standardized temperature (Domingues *et al.*, 2005; Miranda *et al.*, 2005), and a survey of 208 woody plant species from 20, mainly temperate, sites indicated that mean leaf dark *R* increased as site annual rainfall declined (Wright *et al.*, 2006). Possible physiological mechanisms for drought-induced leaf dark *R* increase include greater energy demand for maintenance of vacuolar solute gradients, repair of water-stress-induced cell damage and/or increased wastage respiration via futile cycles (Hue, 1982; Lambers, 1997; Lambers *et al.*, 1998; Cannell & Thornley, 2000; Flexas *et al.*, 2005; Würth *et al.*, 2005; Wright *et al.*, 2006; Atkin & Macherel, 2009). Relatively less work has been conducted on the underlying mechanisms controlling *R* from other plant tissues but at least some of the same processes could be operating. Further work is required to explore how consistent these plant *R* responses to drought are across the Amazon and other mature tropical forests.

The considerable additional respiratory cost imposed on plants by the TFE treatment meant the estimated proportion of PCE used to construct plant tissue (CUE) was 0.24 ± 0.04, compared with 0.32 ± 0.04 in the control (Table 2, Fig. 4). Estimated CUE of the control plot adds to a growing body of evidence indicating that tropical forests generally have higher respiratory costs relative to tissue growth and therefore a lower CUE, of *c.* 0.3 (Chambers *et al.*, 2004; Malhi *et al.*, 2009b) compared with values usually between 0.4 and 0.6 for boreal and temperate forests (DeLucia *et al.*, 2007). To accurately simulate current and future forest C cycling, it may be important to incorporate these physiological responses into current models, many of which assume constant CUE over space and time (e.g. Hyland, Levy *et al.*, 2004; 3PG, Landsberg & Waring, 1997; CASA, Potter *et al.*, 1993; Forest-BGC, Running & Coughlan, 1988). In addition, further work is required to collect similar data at more tropical forest sites to explore the generality of this pattern.

The 'functional balance' theory suggests that plants might respond to the TFE treatment by shifting partitioning of C towards roots, at the expense of other tissues, where photosynthate can be used to increase water uptake (Thornley, 1972; Cannell & Dewar, 1994). Our data provide no clear support for this theory: the proportions of total NPP invested in roots and foliage slightly declined in the TFE plot compared with the control (although these mean plot differences were well within 95% confidence intervals) (Table 2, Fig. 3a). The lack of a clear NPP allocation response could indicate that the forest is adapting to drought in other ways, such as increasing water uptake per unit root mass by increasing specific root length and specific root area (Metcalfe *et al.*, 2008), or that other processes are dominant, such as drought-associated shifts in root turgor pressure and/or soil density, which impede the development of root systems irrespective of plant allocation patterns (Whalley *et al.*, 1998; Bingham & Bengough, 2003; Bengough *et al.*, 2006). Quantifying partitioning as total forest C expenditure (NPP + R_{auto}) provides another test of the functional

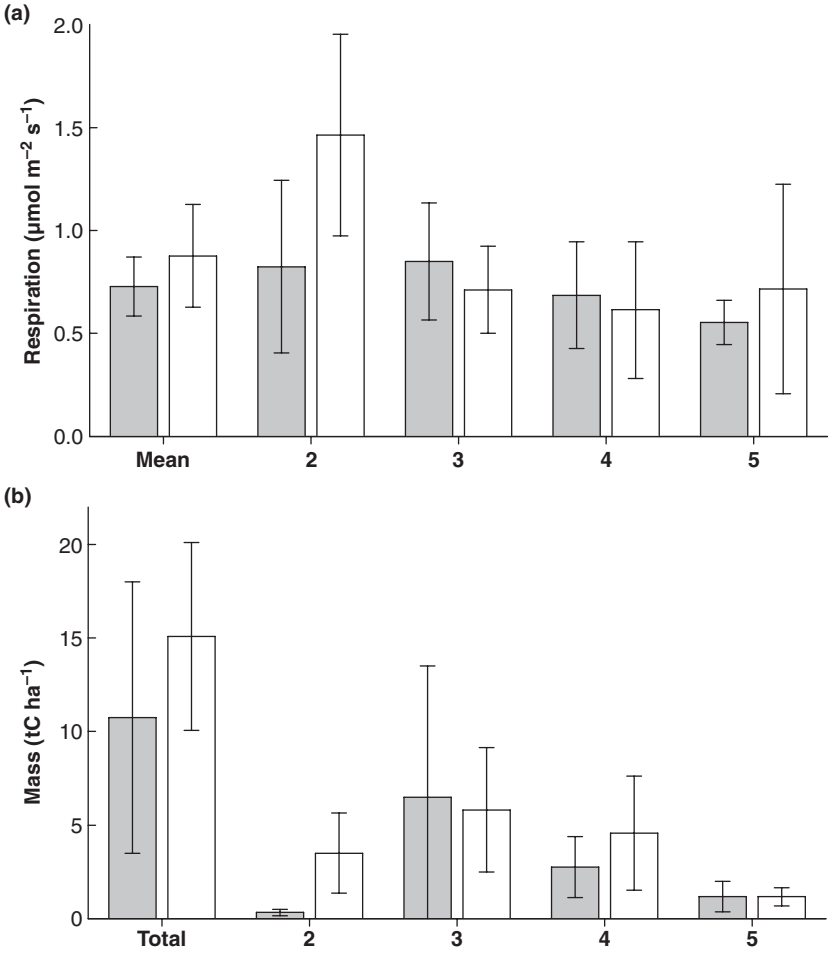

Fig. 8 Coarse woody debris (CWD) respiration (a) and carbon stocks (b) in both plots. Grey bars, control; open bars, through-fall exclusion (TFE). Error bars indicate 95% confidence intervals around the values. CWD stocks were measured in 2009 and back-calculated to 2005 assuming dead stem and CWD stock accumulation was proportional to measured tree mortality. Given the unreplicated nature of the plots, error bars represent only within-plot spatial variation and measurement error rather than landscape-scale heterogeneity. Values indicate means/totals for the entire plot and are divided into the following four decomposition categories following Harmon *et al.* (1995): 2, firm wood with bark intact but no leaves or fine twigs; 3, firm wood with rotten/sloughing bark; 4, partially rotten wood which can be broken when kicked; 5, completely rotten wood which can be broken apart by hand.

balance theory, and reveals a different pattern to that of NPP alone. The decline in NPP in the TFE plot was offset by a substantial estimated rise in canopy *R*, with the net effect that total C partitioning to both roots and canopy apparently increased in the TFE plot relative to the control, although, again, this trend should be interpreted with caution given the substantial uncertainties around the means (Table 2).

da Costa *et al.* (2010) estimate that increased stem mortality in the TFE plot produced *c.* 33 t C ha^{-1} more dead woody material than in the control over the 7 yr from 2002 to 2008. It might be expected that this necromass would decompose relatively slowly under the drier conditions in the TFE plot, but in this study we observed no clear inhibition of CWD respiration on the TFE treatment (Fig. 8a). Thus, if there was any difference in amounts of CWD moisture between plots, this appeared to have little effect on microbial activity on the CWD surface. *R* per unit wood surface appears to have remained similar between plots, whilst the total quantity of dead wood dramatically increased in the TFE plot (Fig. 1; da Costa *et al.*, 2010), with the result that stand-level dead wood *R* was greater in the TFE plot than in the control (Table 2). There was also a weak trend towards higher *R* from fresh wood material in both plots (Fig. 8a), perhaps because more labile C was accessible to microbes on the surface of the wood, and there were greater quantities of fresh wood in the TFE plot (Fig. 8b), which further contributed to increased CWD emissions in the TFE plot relative to the control (Table 2, Fig. 3b). By contrast, fine litter *R* was approximately 10 times lower in the TFE plot than in the control (Table 2), but the measured increase in fine litter C stocks in the TFE plot was relatively minor (Table 1). Taken together, this suggests that other decomposition processes, such as physical disintegration and consumption by detritivores, became more important in the TFE plot (Fig. 7). The shifts in R_{hetero} observed in this study may have important consequences for turnover of C stocks which, together with TFE-induced changes in C stocks, could translate into important changes in ecosystem CO_2 emissions.

Method validation and intercomparison

This study has compiled all available site data and, where necessary, taken values from other Amazon forests to

construct a detailed snapshot of estimated C fluxes in the fourth year of a drought experiment in eastern Amazonia. Analyses of longer-term change of some components and more detailed work focused on individual components may be found elsewhere (Nepstad *et al.*, 2002; Davidson *et al.*, 2004, 2008; Sotta *et al.*, 2007; Metcalfe *et al.*, 2007a, 2008, 2010; Brando *et al.*, 2008; Meir *et al.*, 2008; da Silva *et al.*, 2009; Meir *et al.*, 2009; da Costa *et al.*, 2010). The 'bottom-up' approach used in this study to estimate PCE and R_{eco} in the control plot showed a reasonable degree of consistency with earlier estimates of stand-level C fluxes at this site made using both eddy covariance and modelling methods (Table 2). However, from the equations which estimate R_{soil} (Malhi *et al.*, 2009b) and TBCA (Raich & Nadelhoffer, 1989) from above- and below-ground C inputs, there is an indication that there is higher measured R_{soil} and TBCA than expected (Table 2), which could indicate insufficient field sampling or that further work is required to parameterize the mass balance models. Eddy covariance studies in central/eastern Amazon *terra firme* old-growth forest have recorded very different rates of annual ecosystem C uptake and release, with distinct seasonal patterns (Saleska *et al.*, 2003; Ometto *et al.*, 2005). Bottom-up measurements could help to specify which components of R_{eco} (e.g. R_{auto} vs R_{hetero}) and GPP (e.g. R vs NPP) contribute to explaining the observed differences. This study provides some of the first insights into ecosystem-level shifts in Amazon forest C metabolism associated with drought, which, although constrained by numerous uncertainties, provide a foundation for future modelling and experimental work testing questions and patterns arising from the data presented.

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Chapter 7

Conclusions

The Amazon basin appears to be at risk from a drying climate that could compromise the viability of the rain forest ecosystem. The loss of the Amazon rain forest would have dramatic consequences for both the climate and humanity, not only in terms of the global carbon balance but also in terms of biodiversity loss, landscape changes and even culture.

In this thesis, we have found that the tropical rain forest trees would be able to withstand short-term drought periods (2 years) without major impact in carbon assimilation. Stomata closed to prevent water loss with the imposition of drought stress but the maximal photosynthetic rate (A_{max}) was restored close to its original values by the end of the study period, associated to increased instantaneous water use efficiency. Morphological changes also took place, by means of a reduction of the specific leaf area in the TFE plot trees, compared to the Control plot. Furthermore, leaf optical properties changed, indicating structural changes as well. Finally, no metabolic limitations were observed during the course of the study.

The forest therefore potentially has much greater resilience to dry periods than initially supposed. So it may be the case that the forest may be able to withstand a modest reduction in rainfall with no direct effect on growth or gas exchange.

Leaf dark respiration increased significantly in the TFE plot trees. Increased tree mortality was also observed, mainly in large trees. This will probably lead to shifts in the forest, both as a carbon sink, because the carbon dioxide emissions to the atmosphere will increase, and in species composition, as top canopy species will be more vulnerable to death.

A vertical canopy gradient was observed on most of the parameters measured, the top canopy species being the ones showing the highest photosynthetic capacity, but also being the species that suffered the most with imposed drought.

In any case, the effects of the observed inter-annual variation and imposed seasonal variation on carbon assimilation were reversed in the following wet season. During the dry season, forest canopy foliage is maintained via deep rooting access to soil water reserves allowing a fast recovery when water becomes fully available again. Water table has been found to be at 10 m depth during the wet season, in this forest. Rooting depths in the field can be much greater than those initially supposed vegetation models. In fact,

soil hydraulics and rooting depth, which are extremely poorly documented for the whole Amazon region, were here identified as important forest properties controlling the resilience to drought.

The low values of A_{max} observed in all studied trees suggest that phosphorus, and eventually potassium, may be limiting the photosynthetic potential of this forest. This limitation is likely to persist because soil drying may increase phosphorus deficiency and eventually constrain even more the carbon assimilation of these trees.

This thesis reinforces the importance, by presenting new data, of considering several important factors in future climate change models, in order to reduce the current uncertainty about the future of Amazonia. Models should be able to include physiological acclimation to drought or be capable of simulating P limitations or even take into account different functional groups, of which canopy vertical profile can be regarded as the simplest one.

Reflectance based indices proved to be useful in detecting drought stress in tropical rain forests and support the idea that imaging spectroscopy sensing can contribute to increase the accuracy of ecological studies in tropic rain forests. Because a canopy vertical gradient was observed in the reflectance, the extrapolation of remote sensing to whole canopy should be carefully done, to avoid overestimation of the drought impact on the forest.