Drought effect on isoprene production and consumption in Biosphere 2 tropical rainforest

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Abstract

Isoprene is the most abundant of the hydrocarbon compounds emitted from vegetation and plays a major role in tropospheric chemistry. Models predict that future climate change scenarios may lead to an increase in global isoprene emissions as a consequence of higher temperatures and extended drought periods. Tropical rainforests are responsible for more than 80% of global isoprene emissions, so it is important to obtain experimental data on isoprene production and consumption in these ecosystems under control of environmental variables. We explored isoprene emission and consumption in the tropical rainforest model ecosystem of Biosphere 2 laboratory during a mild water stress, and the relationship with light and temperature. Gross isoprene production (GIP) was not significantly affected by mild water stress in this experiment because the isoprene emitters were mainly distributed among the large, canopy layer trees with deep roots in the lower soil profile where water content decreased much less than the top 30 cm. However, as found in previous leaf level and whole canopy studies, the ecosystem gross primary production was reduced by (32%) during drought, and as a consequence the percentage of fixed C lost as isoprene tended to increase during drought, from ca. 1% in wet conditions to ca. 2% when soil water content reached its minimum. GIP correlated very well with both light and temperature. Notably, soil isoprene uptake decreased dramatically during the drought, leading to a large increase in daytime net isoprene fluxes.

Keywords: Clitoria racemosa, CO2, isoprene emission, microbial, PTR-MS, soil sink, water stress

Received 7 March 2005; revised version received 12 July 2005; accepted 25 October 2005

Introduction

Tropical rain forests are known to play a major role in the global carbon cycle (Malhi & Grace, 2000; Monson, 2002; Loescher et al., 2003). Much recent research has focused on the influence of tropical forests on atmospheric [CO2], but forests emit a number of other carbon compounds, particularly nonmethane hydrocarbons, of which isoprene (C5H8) is the most important (estimated by Guenther et al. (1995) to comprise ca. 44% of global emissions). Recent estimates suggest that tropical regions account for the bulk (>80%) of global isoprene emissions (Jacob & Wofsy, 1988; Zimmerman et al., 1988; Guenther et al., 1995). The total amount of carbon per unit area released through tropical isoprene emissions is believed to be in the range of 0.1–0.3 tC ha⁻¹ yr⁻¹ (Harley et al., 2004). Physiological models (Lloyd, 1999), global carbon budget considerations (Malhi & Grace, 2000) and biomass studies (Phillips et al., 1998) all suggest that the tropical forest carbon sink is on the order of 1 tC ha⁻¹ yr⁻¹. Hence, in order to obtain a more realistic relationship between measurements of net CO2 uptake and the net carbon sink, it is important to understand and quantify the amount of carbon ‘lost’ from the forest through isoprene and the role played by the climatic driving forces of this carbon loss.
Isoprene has a major role in regulating the oxidation potential of the troposphere because of its high reactivity with the hydroxyl radical (OH), the principal tropospheric oxidizing agent (Wofsy, 1976; Crutzen & Fishman, 1977; Greenberg et al., 1985; Fehsenfeld et al., 1992). This has several consequences for the habitability of the biosphere, including production of atmospheric pollutants such as ozone (O3) and peroxyacetyl nitrates (PANs), and formation of organic acids. It also has a potential role in climate warming, reducing the effectiveness of methane removal from the troposphere (Zimmerman et al., 1988).

The exact physiological role of isoprene production in plants is still unknown, but several hypotheses have been proposed. The one that has received most attention is that isoprene serves as a thermal protectant, protecting the leaves against high temperature episodes (Sharkey & Singsaas, 1995; Singsaas et al., 1997; Sharkey et al., 2001; Peñuelas et al., 2005; Velikova & Loreto, 2005). This temperature dependency may be linked to the effects of water stress on isoprene emission by plants. It has been observed that in the long-term, withholding water can reduce isoprene emission to 40% of the emission of normally watered plants (Lerdau et al., 1997). In the short-term, whereas photosynthesis is clearly suppressed, isoprene emission can be stimulated by water stress (Tingey et al., 1981; Sharkey & Loreto, 1993; Fang et al., 1996; Pegoraro et al., 2004b). However, although some studies have focused on the effect of water stress on isoprene emission, the link between isoprene emission, water stress, and leaf temperature is still unclear and requires further investigation.

As isoprene emission is highly temperature sensitive, there is an obvious concern that future increases in global temperature and drought, as predicted in climate models (Cox et al., 2000), could result in enhanced isoprene fluxes. This, in turn, could result in a reduced potential for removing tropospheric methane, resulting in even further global warming. Ultimately, these effects are further enhanced indirectly by other consequences of a global rise in temperature: a regional shift in precipitation, biomass/plant species redistribution and increase in the length of the growing season (Turner et al., 1991; Lerdau et al., 1997; White et al., 1999).

Because tropical forest ecosystems are physiologically active year-round, and experience wet–dry season regimes, they are probably the largest single sources of isoprene. Nevertheless, because of high species diversity and difficulty in access, there is still a substantial lack of information on the isoprene source strength from tropical systems and we still depend largely on model extrapolation based on leaf level measurements (Guenther et al., 1995). Here, we report ecosystem level measurements from a tropical rainforest mesocosm. Taking advantage of a unique opportunity at the Biosphere 2 Laboratory (B2L)-controlled environment facilities we established an experiment to quantify the effect of environmental variables on isoprene emission and its contribution to the carbon balance at the ecosystem level.

The specific objectives were: (1) to screen a range of tropical species in the canopy and understorey layer for their capacity to emit isoprene; (2) to understand the relationship between the isoprene flux and environmental variables such as light and temperature; (3) to investigate the effect of water stress on ecosystem scale isoprene flux; (4) to study the changes in the carbon balance of the model system during drought; (5) to explore the potential magnitude of isoprene uptake by soil and its sensitivity to water stress. We tested the hypotheses that isoprene emission is less sensitive to water stress than photosynthesis and that water stress strongly affects isoprene uptake by soil.

Materials and methods

Plant material

The tropical rainforest mesocosm (TRF) of B2L was constructed in 1991 (Dempster, 1999), and although it was never designed to represent any particular natural tropical forest ecosystem it is now structurally and functionally representative of disturbed humid tropical rainforests in South America, but with floristically diverse pan-tropical vegetation (Leigh et al., 1999; Lin et al., 1999). The top canopy in this mesocosm has reached ca. 15 m above ground and has covered major open spaces after a decade of development, reaching a leaf area index (LAI) of 4–5 in 2001. Secondary canopy and understorey plants have also established themselves under or between large trees (Leigh et al., 1999).

The dominant canopy species include *Clitoria racemosa* Sesse & Moc., *Ceiba pentandra* (L.) Gaertn., *Cecropia schreberiana* Miq., *Arena pinnata* (Wurmb) Merr., *Phytolacca dioica* L., *Pterocarpus indicus* Willd., *Hura crepitans* L., *Inga feuillei* DC. and *Hibiscus elatus* SW. Most of these species are generally shade intolerant species, typical of disturbed secondary tropical forests. The most common understorey plants are *Costus* spp., *Epipteripium* spp., *Dieffenbochia* sp., *Ficus pumila* L., *Hedychium* spp., *Piper* spp., and *Coffeea* spp. To reduce light penetration into the forest floor, many edge plants were grown along the four sides of the mesocosm, including *Alpinia* spp., *Musa* spp., *Bambusa* spp., and *Zinger spectabile* Griff.

The soil profile in the tropical rainforest of B2L was assembled with a subsoil layer (up to 5 m deep) and a topsoil layer of variable depth (0.3–3.2 m in depth).
(Leigh et al., 1999). Although soil bulk density, soil organic matter content and major nutrient concentrations in B2L tropical rainforest were very similar to those of several Puerto Rican rainforests (Silver & Fall, 1991), they were more alkaline (pH ca. 7.5 compared with pH of 5.1 found in Puerto Rican rainforests by Silver and Fall, 1991) and contain slightly higher P, K and other nutritional elements (Leigh et al., 1999; Lin et al., 1999). The soil fauna is, however, very limited (ants, cockroaches). No census of soil macrofauna was undertaken (Karl Bil, B2L, personal communication).

**Growth conditions**

The TRF is encased in a glass and metal shell and was operated as a semi-closed system with control of temperature, atmospheric gas composition and precipitation. Arrays of sensors allowed the continuous monitoring of atmospheric composition, climatic conditions (light, temperature, leaf temperature, soil moisture and humidity) and energy and trace gas fluxes throughout the canopy. The concentration of carbon dioxide [CO$_2$] in the tropical rainforest and outside atmosphere were measured continuously using a LI-6262 gas analyser (Li-Cor Inc., Lincoln, NE, USA), which was calibrated once a day with five standard CO$_2$ cylinders. The CO$_2$ injection rate was monitored using Sierra 840M-3-OV1-SV1-E-V4-S4-MP mass flow controllers (Sierra Instruments Inc., Monterey, CA, USA) and the fan speeds were measured using Panametrics GM868 Ultrasonic gas flowmeters (GE Panametrics, Waltham, MA, USA) (for more details see Lin et al., 1999).

The environmental conditions inside B2L were artificially controlled except for light. Photosynthetically active radiation reaching the top of the canopy inside the mesocosm (PAR$_{\text{m}}$) was ca. 75% of the outside PAR (PAR$_{\text{o}}$). However, because of the shadow from the mesocosm structure and light reflection and scattering, light sensors inside the mesocosm were not sufficiently reliable and for the data analysis presented here, we used light data from a weather station (HT205W Rotronics Hydrometer, La Roche sur Foron, Haute-Savoie, France) located just outside the B2L mesocosms. The daily mean air temperature for this tropical rainforest was set at 27° C with an air temperature range of 35° C/20° C day/night, and relative humidity (RH) of ca. 85%. Temperature stratification in the upper canopy, identified by Arain et al. (2000) as the principal artefact of enclosure, was minimized by high speed fans mounted in the structure to ensure adequate mixing of the mesocosm atmosphere. The TRF was regularly watered with an average daily precipitation of 3.6 mm.

**Experimental setup**

Before the experiment was started, the TRF was heavily wetted for 2 weeks (7.7 mm day$^{-1}$), to reach field capacity. From September 23 (Day 1) to October 28 (Day 36), 2002 no water was added and the soil was left to dry naturally. At the beginning of the drought recovery, the TRF was rewatered for 14 days using the predrought regime. This drought treatment was calibrated to result in a mild water stress permitting a rapid and reversible recovery of the tropical rainforest. On Day 32 an isolated soil compartment (approximately 20% of the whole soil surface) was watered with 30,000 L (36 mm) to test the effect of a singular, isolated watering event.

In order to monitor water stress, soil volumetric water content ($\theta$) was continuously monitored over the duration of the entire experiment by Time Domain Reflectometry probes (CS165, Campbell Scientific Instruments, Logan, UT, USA) inserted at five different locations (N, NW, S, SE and centre) in the soil at two depths: 0–30 and 30–60 cm. As a parameter for plant sensitivity to water stress, predawn and midday leaf water potential ($\Psi_{\text{leaf}}$) measurements were performed on four of the large tree species: *Ceiba pentandra*, *Clitoria racemosa*, *Hibiscus elatus* and *Hura crepitans*, using a PMS 1003 digital pressure-bomb (PMS Instruments, Corvallis, OR, USA). For each tree species, four leaves of the outer canopy were sampled at the time of each $\Psi_{\text{leaf}}$ measurement. Leaf temperature of sun leaves was also monitored with three infrared sensors (Apogee Instruments, Logan, UT, USA) pointed at sun leaves located in the midsection of the canopy of the three main isoprene emitter species. The instrument has a field of view of 6.45 cm$^2$ m$^{-1}$ and was mounted ca. 2 m from the canopy, thus monitoring a leaf area of ca. 12 cm$^2$. All data were collected as 15 min averages with a CR10 datalogger (Campbell Scientific Instruments).

Because the glass-enclosed TRF is free of ultra-violet (UV) light, inside the mesocosm atmosphere O$_3$ production and OH radical generation is minimized (Cockell et al., 2000). Therefore, the lack of oxidative destruction of isoprene inside the TRF atmosphere (more details are given in Pegoraro et al., 2005) permitted measurement of isoprene consumption by the soil at night.

**Leaf gas exchange measurements**

Photosynthetic rate, stomatal conductance and intercellular [CO$_2$] were measured using a LI 6400 open path gas exchange measurement system (Li-Cor). All measurements were made under the same conditions imposed by the environment inside the TRF mesocosm: leaf temperature of 32° C, PAR of 1200 µmol m$^{-2}$ s$^{-1}$ and air flow of 400 µmol s$^{-1}$. After a leaf was placed in the cuvette, a minimum of 10 min was allowed for...
equilibration, and all measurements were made after steady-state conditions had been reached, as indicated by continuous monitoring of CO$_2$ and H$_2$O fluxes. To avoid interference of isoprene in the atmosphere outside the cuvette, cylinder air (Praxair Technology, San Ramon, CA, USA), measured and confirmed to be isoprene-free, was delivered to the Li-Cor measurement system. The cylinder was connected to the air inlet of the LI 6400 by a ‘T’ connection allowing exhaust of excess air. In 2002, changes in isoprene concentration inside the LI 6400 cuvette were monitored by collecting an air sample by attaching a Teflon$^\text{®}$ bag (SKC West Inc., CA, USA) of 2.5 dm$^3$ volume to the cuvette exhaust. The bag was then brought to the laboratory and isoprene concentration determined by gas chromatography (for more details, see Pegoraro et al., 2004a,b). In 2003, changes in isoprene concentrations were measured online via a 5 m length of Teflon$^\text{®}$ PFA tubing (1.6 mm inside diameter), inserted through a ‘T’ connection to the leaf cuvette exhaust and connected to a proton transfer reaction mass spectrometer (PTR-MS, Ionicon GmbH, Innsbruck, Austria). Operational details of PTR-MS are described elsewhere (Hansel et al., 1995; Lindinger et al., 1998; Hayward et al., 2002). The air sample was pulled by the PTR-MS at a constant flow rate of ca. 20 mL min$^{-1}$ and isoprene concentration was determined using a dwell time of 2 s, with a high temporal resolution between successive measurements of the same mass (ca. 7 s). The instrument was calibrated before and after the experiment (Pegoraro et al., 2005).

**Ecosystem level gas exchange measurements**

**Isoprene fluxes.** One-minute averages of ecosystem isoprene atmospheric concentration from the mesocosm were measured and recorded with a Fast Isoprene Sensor (FIS-02-AUTO, Hills Scientific, Boulder, CO, USA) mounted in an adjacent Laboratory. The mesocosm was sampled continuously for 15 min each hour. The FIS was calibrated before, during and after the experiments. A more detailed description of the measurement system and flux calculation is given in Pegoraro et al. (2005). All flux calculations where performed exclusively for a closed system, excluding the periods when the pull/push fans where exchanging air with the outside. The net isoprene exchange (NIE) (isoprene emission minus isoprene consumption), was calculated continuously at 15 min intervals as average nanomoles (nmol) of isoprene exchange per metre square of ground area per second for the entire experimental period as

$$\text{NIE} = \frac{AC}{At} = \frac{(C_{t+1} + (C_{t+1} \times L) - (C_{t-1} + (C_{t-1} \times L))}{2 \times At},$$

where $C_{t+1}$ is the concentration in the mesocosm for the following 15 min period with respect to time ‘$t$’, $C_{t-1}$ is the same for the previous 15 min period, $L$ is the leak rate and $At$ is the time period (15 min in this case). Soil isoprene uptake was measured both at the whole mesocosm level and in the small soil chambers (Pegoraro et al., 2005) and was used to calculate the soil isoprene uptake flux ($IF_{\text{soil}}$): $IF_{\text{soil}} = -k \times C$ (nmol m$^{-2}$ s$^{-1}$), where $k$ (m$^{-1}$) is the isoprene deposition velocity. Gross isoprene production (GIP, nmol m$^{-2}$ s$^{-1}$) was then calculated every 15 min as

$$\text{GIP} = \text{NIE} - IF_{\text{soil}}.$$

**Ecosystem CO$_2$ uptake.** Net ecosystem exchange (NEE) was calculated as average of micromoles of CO$_2$ exchange per metre square of ground area per second at 15 min intervals continuously for the entire experimental period according to:

$$\text{NEE} = F_{\text{in}} + F_{\text{tank}} - F_{\text{out}} - F_{\text{leak}} - F_{\text{atm}},$$

where $F_{\text{atm}}$ is the flux because of change in CO$_2$ storage in the mesocosm, $F_{\text{leak}}$ is the flux of CO$_2$ leakage across the mesocosm isolation curtain, $F_{\text{tank}}$ is CO$_2$ injected from the CO$_2$ tank, $F_{\text{in}}$ and $F_{\text{out}}$ are the rates of CO$_2$ exchange by the pull/push fans (for more details, see Lin et al., 1999). Following the tradition of ecophysiological research, we denote positive NEE values for the net CO$_2$ uptake by the whole ecosystem.

Leakage through the curtain separating the rainforest from the adjacent mesocosm (B2L Savanna) was estimated to be ca. 1.6% of total air volume per hour based on sulphur hexafluoride (SF$_6$) tracer experiments (Pegoraro et al., 2005). The soil air volume is small (<1%) compared with the total volume of the bay, therefore only ca. 0.2% of the total leak rate could be the result of diffusion into the soil.

Daytime respiration was estimated by establishing night time NEE and soil temperature relationships and extrapolating thus for daylight hours, and gross primary productivity (GPP) was then calculated by subtracting daytime respiration from NEE (Lin et al., 1999).

**Soil gas exchange**

The absence of atmospheric isoprene oxidation and the minimization of isoprene leaked to the outside by regulation of the mesocosm exhaust system resulted in elevated isoprene concentrations inside the TRF (between 200 and 1200 nmol mol$^{-1}$ (ppb)). In tropical ecosystems they are much lower ranging typically between 3 and 7 ppb (Rasmussen & Khalil, 1988; Zimmerman et al., 1988; Rinne et al., 2002; Greenberg et al., 2004; Trostdorf et al., 2004). As a result of the high diffusion gradient, these high ambient isoprene concentrations resulted in high isoprene consumption fluxes.
by the soil. While extrapolation to real natural conditions is thus limited, they nonetheless allowed exploration of the potential of soil isoprene consumption under a range of environmental conditions. Observed isoprene consumption in the dark at whole ecosystem level and in small soil chambers always followed an exponential decay function. It was possible to calculate the deposition velocity as: \[ k = \frac{\ln(C_2/C_1)}{(t_2 - t_1)} \]
where \( C_2 \) is isoprene concentration at time \( t_2 \) and \( C_1 \) is isoprene concentration at time \( t_1 \). Although the proportionality constant \( k \) of the exponential decay is commonly called *deposition velocity* (Cleveland & Yavitt, 1997), here it has been called *soil activity factor* to include all physical and biological capabilities of soil to uptake isoprene (Pegoraro et al., 2005).

For *in situ* soil isoprene uptake measurements, five soil collars were inserted ca. 3 cm deep into the soil in five different locations (mainly along a North to South transect), 2 weeks before the start of the experiment to give the soil time to recover from disturbance. Soil isoprene exchange measurements were made with small (30 × 30 × 40 cm³) aluminium static soil chambers (a more detailed description of the system is given in Pegoraro et al., 2005). At the start of each measurement period the chamber was fitted onto the collar, thus preventing any gas exchange with the outside. Soil isoprene concentrations attained in the tropical rainforest as a whole at the time of measurements. To insure inertness the chambers were leak tested as follows: an exact replicate of the soil collar-chamber device, without soil and containing a plastic floor sealed to the bottom of the collar, was set up inside the TRF. No appreciable variation in isoprene concentration could be observed in the empty chambers by repeating the experimental protocol.

Measurements of isoprene concentration in the soil profile were made by taking air samples from three different soil depths. The PTR-MS inlet was connected by a 9 m Teflon® tube (1.6 mm inside diameter) to stainless steel soil probes, sampling at 5, 10 and 15 cm depth, installed at one location in the centre of the TRF. To prevent pulling in air from above the soil surface during soil profile sampling, the PTR-MS air flow was regulated at its minimum: ca. 15 mL min⁻¹, and the sampling time was minimized (ca. 2 min) to flush the tubing and collect a significant sample of air.

**Results**

Screening for the production and emission of isoprene by the large canopy trees and the most common understory and edge plants was relatively easy because of canopy access inside the tropical rainforest of B2L. None of the screened understory and edge plants was found to be isoprene emitters. Table 1 shows emitters of the canopy layer together with their average isoprene emission rate in nonstressed conditions.

**Growth conditions**

Before and after the drought experiment of 2002, in nonstressed conditions, soil water content (θ) in the top 30 cm of soil was maintained at ca. 0.27 m³ m⁻³ (Fig. 1a). After the last rain, θ decreased, rapidly reaching 0.13 m³ m⁻³ on the last day of the drought. However, the decrease in θ was particularly strong in the top 30 cm of soil; below 60 cm soil depth the decrease in θ was much less pronounced (for more details see Rascher et al., 2004). Over the 3-month period comprising the drought experiment and the recovery period, average daytime outside PAR decreased steadily from 1300 μmol m⁻² s⁻¹ at the end of September to ca. 750 μmol m⁻² s⁻¹ (40% less) in mid December (Fig. 1b). Both air temperatures inside the TRF and leaf temperature of one of the major emitting species, closely followed variations in the outside light also showing a decreasing trend (Fig. 1c). Leaf temperature was always a few degrees Celsius lower than air temperature, although this difference was reduced to a minimum in the last days of the drought when it was ca. 1 °C. GIP also decreased steadily over the experimental period (Fig. 1d) tracking changes in light and temperature. However, GIP did not show any response to water stress. As expected GPP also decreased following the decrease in light and temperature (Fig. 1d). Furthermore, it decreased more rapidly during the water stress period and showed a rapid recovery at the end of the drought period on the day after the first rain.

A detailed analysis of drought effects on CO₂ exchange in B2L TRF can be found in Rascher et al. (2004).

**Table 1** Average leaf isoprene emission rate ± SE (measured at leaf temperature of 32 °C and PAR of 1200 μmol m⁻² s⁻¹) in nonstressed conditions for five canopy dominant species of the tropical rainforest of Biosphere 2 Laboratory (n = number of leaves tested)

<table>
<thead>
<tr>
<th>Species</th>
<th>Isoprene emission rate (nmol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arenga pinnata</td>
<td>38.8 ± 3.3 (n = 12)</td>
</tr>
<tr>
<td>Chrysalidocarpus lutescens</td>
<td>19.2 ± 8.4 (n = 9)</td>
</tr>
<tr>
<td>Clitoria racemosa</td>
<td>58.3 ± 2.6 (n = 58)</td>
</tr>
<tr>
<td>Inga sapindoides</td>
<td>20.1 ± 2.0 (n = 13)</td>
</tr>
<tr>
<td>Pterocarpus indicus</td>
<td>23.0 ± 3.4 (n = 12)</td>
</tr>
</tbody>
</table>

All measurements were performed between 12:00 and 14:00 hours.
Drought effect on gas exchange

The strong decrease in $\theta$ observed in the top 30 cm of soil did not affect isoprene emission from the large tree canopy species (Fig. 2a). However, the decrease in soil moisture had a strong effect on GPP, which started to decline noticeably when $\theta$ was lower than ca. 0.22 m$^3$ m$^{-3}$ (Fig. 2b).
GIP variations were essentially explained by variations in outside light and temperature (Figs. 3a and b). However, most of the GIP variation was mainly a result of changes in light. When compared with results obtained from Cottonwood trees grown at 430 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) CO\(_2\) in the intensive forestry mesocosm (IFM) and that had a LAI of ca. 2.04 (Pegoraro et al., 2005), the relationship with both outside light and air temperature was very similar to the TRF (relationships from 15 min flux data are shown in Fig. 4). Although light intensity was below normal saturation levels (reaching a maximum of ca. 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) inside the TRF biome, GIP measured in the TRF appeared to have higher light and temperature saturation levels (Figs. 4a and b). Temperature inside the TRF is largely controlled, and the two variables showed a strong correlation with each other as strong variations in the outside solar radiation always resulted in a variation in temperature. GPP also showed a strong relationship with light, but a weaker relationship with temperature (Figs. 5a and b). GPP increased very quickly with increasing outside light until light levels of ca. 600 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Then GPP continued to increase, but at a much lower rate and remained practically constant between outside light levels of 1400 and 1800 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). GPP clearly showed a saturation level in its relationship with temperature, rapidly increasing until reaching its optimum at ca. 35 °C; it then began to decrease quickly with further increasing temperature.

The isoprene carbon loss as a fraction of total photosynthesis varied remarkably over the drought period (Fig. 1d). Before the drought GIP represented a carbon loss at 0.94% of the carbon assimilation. Carbon loss increased rapidly at the beginning of the drought and on Day 12 it was 100% higher. Carbon loss then decreased slowly until the last day of the drought, when GPP dropped to its minimum and consequently the carbon loss increased again reaching its peak (2.00%). Because of the difference in the response to light be-

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**Fig. 2** Relationship between daytime average gross isoprene production (GIP) (nmol m\(^{-2}\) s\(^{-1}\)) (a) and gross primary production (GPP) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)) (b), and soil moisture (\( \theta \)) (m\(^3\) m\(^{-3}\)) measured in the top 30 cm of the tropical rainforest soil of Biosphere 2 Laboratory. Days with low light levels (cloudy days) were not considered.

**Fig. 3** Relationship between daytime average outside photosynthetic active radiation (PAR\(_{\text{out}}\)) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)) (a) and air temperature (°C) (b), and daytime average gross isoprene production (GIP) (nmol m\(^{-2}\) s\(^{-1}\)) for the tropical rainforest of Biosphere 2 Laboratory during the drought experiment in 2002.

between GPP and GIP (the first clearly showing saturation at medium–high light intensities and the second showing no saturation even at high light intensities), the lower light intensity in December caused a larger decrease in isoprene production than in CO2 fixation and as a result the carbon loss stabilized over the recovery period at a lower level (0.66%) than the predrought period.

To demonstrate the broad effect of water stress on leaf physiology amongst the major canopy trees, variable responses of predawn and midday leaf water potential ($\Psi_{\text{leaf}}$) to drought, and recovery after drought, are given in Fig. 6 for the four investigated tree species: C. pentandra, C. racemosa, H. elatus and H. crepitants. In general, both predawn and midday $\Psi_{\text{leaf}}$ tended not to decrease significantly until the very end of the drought. Of the four species, C. racemosa is the only isoprene emitter and it is the species that displayed the largest decrease in $\Psi_{\text{leaf}}$ during the drought with predawn $\Psi_{\text{leaf}}$ decreasing below −1.2 MPa and midday $\Psi_{\text{leaf}}$ dropping below −1.5 MPa (Fig. 6), with no appreciable recovery in either predawn or midday $\Psi_{\text{leaf}}$ after rainfall commenced. Leaves of C. racemosa strongly responded to the singular, isolated watering experiment at Day 32, with midday $\Psi_{\text{leaf}}$ equalling predawn values. For a more detailed description of the effect of the drought on $\Psi_{\text{leaf}}$ of B2L TRF trees, see Rascher et al. (2004).

Although drought did not affect GIP, it had a large effect on the soil activity of the top-soil layer. When soil was wet ($\theta > 0.24 \text{ m}^3\text{ m}^{-3}$) isoprene soil uptake fluxes rapidly increased with increasing atmospheric isoprene concentration, whereas when measured in dryer conditions ($\theta < 0.21 \text{ m}^3\text{ m}^{-3}$) soil fluxes showed a slower response to increases in concentration (Fig. 7; Pegoraro et al., 2005). This was reflected by the drop of 91.40%
in $k$ values over the drought period (Fig. 8). The relationship between soil activity factor $k$ (m min$^{-1}$) in the TRF and soil moisture (Figs 9a and b; Pegoraro et al., 2005) also shows that this soil system was evidently very sensitive to soil water content. Soil activity factor $k$ was estimated for each day of the period 17 September, 2002–20 June, 2003 by using an exponential function of the regression that fit the relationship between $k$ observed and soil moisture. This relationship was found by using $k$ measured in the whole system ($k_{observed}$) during days when the system was closed. Observed and estimated $k$ for the 2002 drought period are presented in Fig. 8 that shows how the model nicely predicts the soil activity factor during the drought period, although it overestimates $k$ when soil moisture reaches its maximum level after the drought.

The assumption that most isoprene consumption occurs in the top cm of soil was also confirmed by soil profile measurements showing that only ca. 2% of the atmospheric isoprene concentration reached 5 cm depth during the wet period. During the dry period, the decrease in $k$ slowed down isoprene uptake and ca. 23% of the atmospheric isoprene concentration reached 5 cm depth.

Discussion

Because of difficulties in access and the high species diversity of tropical rainforest ecosystems, studies on isoprene emission both at leaf and whole canopy level have mainly focused on temperate ecosystems. Models estimating isoprene emissions for tropical regions were often developed before any leaf-level data on tropical
emissions had been collected (Guenther et al., 1995) and, therefore, rely mostly on data from temperate plants for their leaf-level controls and constraints. This may lead to important biases. For example, previous work performed on several tropical tree species (Lerdau & Keller, 1997; Keller & Lerdau, 1999; Lerdau & Throop, 2000) has shown that isoprene emissions tend to saturate at a much higher light intensity than in temperate tree species. Current estimates of tropical isoprene emissions may be confirmed or adjusted with information from leaf-level and whole tropical ecosystems measurements. The ability for different variables to intervene in both isoprene production and consumption processes at the whole ecosystem level has been explored here.

Recently, new technology (such as eddy covariance both by proton transfer reaction mass spectrometry [Karl et al., 2003] or by fast isoprene sensor [Guenther & Hills, 1998], and disjunct eddy accumulation [Karl et al., 2004]) has become available to facilitate above canopy measurements of trace gases such as isoprene, and the number of studies on isoprene emission from tropical ecosystems has rapidly increased in the last few years. However, it is often difficult in the field to control all variables, and therefore data often represent a simple observation more than a targeted experiment. The large-scale facility of B2L gave us the unique opportunity to measure the instantaneous response of isoprene fluxes from tropical species during a mild drought, something that is difficult in natural situations because of practical constraints. A major restraint of the B2L facility is the limitation to replicate experiments. However, repetition of experiments is possible and has been performed, for example, to assess the B2L system variability (Lin et al., 1998; Rosenthal, 1998, 1999; Tubiello et al., 1999). Replication in time (in series) is routine for experimental research in the laboratory and is well appreciated in site-specific measurement systems such as flux towers. Although serial replication runs the risk of memory effects, especially in long-term experiments, these effects can, and have been, tested in successive years in controlled facilities such as B2L (Osmond et al., 2004).

Variations in GIP in the TRF were mostly driven by variation in light and their response to both light and temperature was similar to that observed from Cottonwood trees by Pegoraro et al. (2005). The intensity of the water stress used for this experiment was not sufficiently severe to significantly affect isoprene production, probably because the larger change in $\theta$ affected mainly the top 30 cm of soil. As indicated by our screening of a large number of species within both the top canopy and the understory vegetation layers, the isoprene emitters where mainly distributed among the large trees of the canopy layer which are likely to have a developed root system extending well below 60 cm depth, as is observed in natural Amazonian forests, where big trees utilize deep water resources during drought (Nepstad et al., 1994; Meinzer et al., 1999). The small variation in predawn $\psi_{leaf}$ values is a clear indication that the top canopy trees were not strongly affected by the soil water deficit. The sampled species are shade-intolerant trees that mostly occur in open sites or in forest gaps. As an adaptation strategy to dry soil environments caused by strong evaporation as a consequence of soil surface exposure to direct light, these species may quickly close their stomata as soon as they sense a decrease in soil moisture in order to keep water losses to a minimum. As a consequence, even when exposed to a mild water stress their CO$_2$ assimilation may decrease substantially although the physio-
logical apparatus is not critically affected. The midday $\Psi_{\text{leaf}}$ data reported in this study compare very well with field data reported by Huc et al. (1994) for three early stage and pioneer species in French Guiana during a natural dry season. The midday $\Psi_{\text{leaf}}$ was not markedly altered and reached daily lowest values between $-1.5$ and $-1.8 \text{ MPa}$. However, it is likely that the trees sampled by Huc et al. (1994) experienced an even milder water stress than the trees used in our study as the dry season of French Guiana was characterized by (maximum) 20-day periods without significant thunderstorm rainfall (up to 30 mm day$^{-1}$), but with small, frequent precipitation events.

Although isoprene production was not affected by water stress, the mild water limitation had several other important consequences on the system. As found in previous leaf level and whole canopy studies (Tingey et al., 1981; Sharkey & Loreto, 1993; Fang et al., 1996; Guenther et al., 1999; Pegoraro et al., 2004a, b), opposite of isoprene production, $CO_2$ uptake was very sensitive to water stress. As reported in detail by Rascher et al. (2004), even the mild water stress imposed in this study had a significant effect on the deep-rooted canopy trees. Moreover, it strongly affected the shallow-rooted understorey. As a consequence, over the drought period GPP was strongly reduced and the isoprene carbon loss as a fraction of total photosynthesis doubled from 1% to 2%.

Furthermore, this study confirmed the sink capacity of soils for atmospheric isoprene (Cleveland & Yavitt, 1997) and suggests that it is mainly the result of soil bacterial metabolism which is not limited to recycling of soil-derived substrate (Fall & Copley, 2000). Previous experiments on soil sink properties for isoprene (Cleveland & Yavitt, 1997) used concentrations ca. two orders of magnitude higher than in natural ecosystems. Ambient isoprene concentrations in our experiment were even higher as a result of the experimental constraints described before which poses considerable uncertainty when extrapolating observed uptake rates and values of $k$ to atmospheric levels of isoprene observed under natural conditions. However, the tremendous advantage of B2L was to allow experiments in a complex TRF ecosystem instead of a simple incubation chamber, and therefore consider more realistic interplay between environmental variables. In well-watered conditions, the values for $k$ measured in the TRF in this study agree remarkably well with results found by Karl et al. (2004) in Costa Rica and both estimates are much higher than what was previously estimated for tropical soils (Cleveland & Yavitt, 1997).

The presence of isoprene-degrading bacteria was not determined using microbiological techniques. Nonetheless, all of the evidence presented (Pegoraro et al., 2005) indicates the existence of a biological sink and its predominance over physical processes such as dissolution into water or diffusion into the soil air space. Such processes are possible, but are very small (Henry’s law constant for isoprene solubility in water is very low, ca. $2.8 \times 10^{-2} \text{ M atm}^{-1}$), and previous studies (Cleveland & Yavitt, 1997) have used sterilized soil maintained at the same soil moisture level to verify that biological rather than physical processes consumed isoprene and that leakage into the soil was not responsible for declines in isoprene headspace concentrations. Measurements of soil diffusion coefficients using SF$_6$, during wet conditions were close to free-air diffusion coefficients (J. van Haren, unpublished data), suggesting that the soil contains a well-developed macropore system. Therefore, isoprene not consumed in the superficial layer of the soil would have diffused rapidly into the soil. We observed little isoprene below 10 cm suggesting it is actually consumed in the superficial layer. Moreover, isoprene concentration measured in the soil air at 5 cm depth dramatically increased during the dry period. Although consistent with increasing diffusion owing to increase in soil air fraction, the magnitude difference cannot be explained by diffusion increase, especially considering that wet diffusion rates are already high. We, therefore, contend that the change in isoprene uptake between wet and dry periods likely depends on a change in microbial activity.

Our results also suggest that when substrate availability is the only limiting factor to a microbial population’s growth, soil contribution to isoprene removal from the atmosphere increases very rapidly with increasing atmospheric isoprene concentration and shows an extremely high-saturation point (well above 2 ppm). However, even during the mild drought imposed in the present experiment, soil isoprene uptake decreased dramatically leading to a large increase in daytime net isoprene fluxes and to accumulation of isoprene in the TRF atmosphere. Drastic changes in soil sink capacity may therefore play an important role in the observed increase in isoprene fluxes during dry seasons in Amazonia (Frostdorf et al., 2004). Dry deposition of isoprene might currently be substantially underestimated (Karl et al., 2004) and tropical ecosystems, therefore, may not only play an important role as a source but can have the capability to effectively consume part of the emitted isoprene (Pegoraro et al., 2005).

While the drought intensity used in this experiment was not severe enough to significantly affect isoprene production, it may well represent an extended dry season in the Amazon. Similar dry periods are observed in Amazonia in El Nin˜o years (Trenberth & Hoar, 1997) and may be more likely if deforestation continues (Nepstad et al., 2004). Our results suggest it is possible that warming and drying in the Amazon basin pre-
dicted by some global climate models (Cox et al., 2000) may have a large effect on both sources and sinks for isoprene, leading to a major increase in isoprene emission and above canopy concentrations, with a significant impact on regional air quality.

Tropical rainforests play an important role in global carbon and water cycles, representing ca. 35% of the global net primary production (Loescher et al., 2003). In order to reliably predict the long-term responses of tropical rainforests to a changing environment it is important to better understand the processes that underlie whole ecosystem response to local climate and feedbacks expected on the regional climate (Field et al., 1995; Cox et al., 2000). The effects of drought on tropical rainforests are potentially large and complex, but so far only a few studies have addressed the mechanistic effects of drought in a rainforest ecosystem (Nepstad et al., 2004), and implications from effects on trace gas emissions have not been considered. Experimentally the tropical rainforest inside B2L provided an ideal model system, as the easy canopy access enabled leaf-level measurements in tight correlation with whole ecosystem carbon budgeting (Lin et al., 1999, 2001), and NEE values measured inside the TRF were comparable with those reported for field sites in the wet tropics (Andreade et al., 2002; Osmond et al., 2004).

Acknowledgements

E. P. was supported by a graduate student stipend from a program enhancement grant provided by the Office of the Executive Vice Provost, Columbia University (Michael Crow) and by Edward P. Bass, with equipment support from the Packard Foundation. PTR-MS instrumentation and support for LA came from NSF (CHE-0216226) and a Chemistry Biosphere 2 Program grant from Office of the Executive Vice Provost, Columbia University (Michael Crow). The authors thank Russ Monson for advice in the course of the project and for comments on the manuscript. We thank anonymous reviewers for their helpful comments.

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