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The interacting effects of elevated atmospheric CO_2 concentration, drought and leaf-to-air vapour pressure deficit on ecosystem isoprene fluxes

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Abstract Isoprene is the most abundant biogenic hydrocarbon released from vegetation and it plays a major role in tropospheric chemistry. Because of its link to climate change, there is interest in understanding the relationship between CO_2, water availability and isoprene emission. We explored the effect of atmospheric elevated CO_2 concentration and its interaction with vapour pressure deficit (VPD) and water stress, on gross isoprene production (GIP) and net ecosystem exchange of CO_2 (NEE) in two *Populus deltoides* plantations grown at ambient and elevated atmospheric CO_2 concentration in the Biosphere 2 Laboratory facility. Although GIP and NEE showed a similar response to light and temperature, their responses to CO_2 and VPD were opposite; NEE was stimulated by elevated CO_2 and depressed by high VPD, while GIP was inhibited by elevated CO_2 and stimulated by high VPD. The difference in response between isoprene production and photosynthesis was also evident during water stress. GIP was stimulated in the short term and declined only when the stress was severe, whereas NEE started to decrease from the beginning of the experiment. This contrasting response led the carbon lost as isoprene in both the ambient and the elevated CO_2 treatments to increase as water stress progressed. Our results suggest that water limitation can override the inhibitory effect of elevated CO_2 leading to increased global isoprene emissions in a climate change scenario with warmer and drier climate.

Keywords Biosphere 2 · Climate change · Cottonwood · Photosynthesis · Water stress

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Introduction

Although the short-term effects of some environmental variables, such as light and temperature, on leaf isoprene emission are well known (Harley et al. 1999), the effects of other environmental variables, such as atmospheric CO_2 concentration and water stress have been less studied. It has been found that the effect of elevated CO_2 (above ambient atmospheric concentrations) is to reduce isoprene production (e.g. Monson and Fall 1989; Sharkey et al. 1991; Guenther et al. 1991; Rosenstiel et al. 2003) and that even at ambient atmospheric CO_2 concentrations, isoprene production is inhibited compared to trees grown at lower atmospheric CO_2 concentrations (Monson and Fall 1989; Sharkey et al. 1991). It has also been observed that isoprene emission, in contrast to photosynthesis, is not inhibited by a mild drought, and starts to decline only when the stress is severe and causes prolonged and large declines in photosynthesis (Tingey et al. 1981; Sharkey and Loreto 1993; Fang et al. 1996; Pegoraro et al. 2004a). However, most of the published studies on iso-

prene emission concern leaf level experiments carried out on potted plants; studies on water stress and elevated CO_2 effects on isoprene fluxes from entire forest tree canopies are rare (e.g. Guenther et al. 1999; Rosenstiel et al. 2003; Rapparini et al. 2004; Centritto et al. 2004; Scholefield et al. 2004; Pegoraro et al. 2004b).

Eastern cottonwood (*Populus deltoides* Bartr.) is a common species grown in commercial agriforest plantations, mostly in temperate climates. The increased establishment of short-rotation agriforests has been promoted as a means of satisfying the increasing demand for wood and paper products (Fenning and Gershenzon 2002), while sequestering atmospheric carbon until more permanent solutions are developed to mitigate the problem of increasing atmospheric CO_2 concentration (Brown et al. 1996). Because almost all agriforest species are strong isoprene emitters, the proliferation of agriforest plantations (estimated to be 10.5 Mha $year^{-1}$, FAO 1995), may have a significant impact on regional atmospheric chemistry (Trainer et al. 1987; Chameides et al. 1988). In particular, we might expect the increased production of atmospheric pollutants, such as ozone, and organic peroxy radicals (Monson and Holland 2001), and an increase in the lifetime of methane (Poisson et al. 2000), an important determinant of global climate. Although the increasing number of agriforest plantations may lead to increased local isoprene emission, it has been argued that future increases in atmospheric CO_2 concentrations may partially compensate this trend by inhibiting isoprene production while stimulating biomass production (Rosenstiel et al. 2003). Rapparini et al. (2004) suggested that environmental stresses such as temperature and drought may counteract the effect of elevated CO_2 and lead to increased global isoprene emission in the context of increases in global mean temperature and extended droughts as suggested by some future climate scenarios (e.g. Cox et al. 2000). In particular, water stress caused by the lack of soil water or increased leaf-to-air vapour pressure deficit (VPD) might be hypothesised to cause a decrease in stomatal conductance, and concomitantly, an increase in leaf temperature and decrease in intercellular CO_2 concentration, both of which may cause an increase in the isoprene emission.

In order to improve our understanding of the control of environmental parameters on isoprene emission from a cottonwood agriforestry plantation, we set up an experiment inside the controlled environment research facility of the intensive forestry mesocosm (IFM) of Columbia University's Biosphere 2 Laboratory (B2L). The overall objective of the study was to improve our understanding of the interacting effects of elevated atmospheric CO_2 concentration, soil water deficit and leaf-to-air water VPD on isoprene emission. Specifically, we aimed to test the hypothesis that high VPD and reduced soil water availability may override the suppression of isoprene emission at high CO_2 concentration. The specific objectives of this study were: (1) to investigate the effect of elevated atmospheric CO_2 concentration on ecosystem isoprene emission; (2) to examine the interaction between elevated atmospheric CO_2 concentration with VPD and drought stress; (3) to study the relationship between isoprene emission and photosynthesis during water stress; and (4) to analyse the fundamental canopy-level relationships between ecosystem isoprene emission and environmental variables such as light, temperature, soil moisture and VPD.

Material and methods

Plant material

Experiments were carried out during the autumn of 2002 in the Intensive Forestry Mesocosm (IFM) facility at the Biosphere 2 Laboratory (B2L) Oracle, AZ, USA (1,130 m elevation, 32°35′ N latitude, 110°51′ W longitude), in three agriforest cottonwood plantations (day neutral clones of *P. deltoides* Bartr.) grown in three separate experimental bays (of approx. 12,000 m^3 volume and 550 m^2 soil surface) with independent daytime control of atmospheric CO_2 concentration: 430, 800 and 1,200 μmol mol^{-1}, air circulation, temperature and precipitation (Rosenstiel et al. 2003; Osmond et al. 2004). The three mesocosms were operated as semi-closed systems with a set of push–pull fans working only for a 2 h period at dawn to facilitate the expulsion of nighttime respired CO_2, exchanging the air inside the mesocosms with outside air (reaching an exchange rate of up to 110 m^3 min^{-1} for each mesocosm, a turnover time of 100 min). Air handlers and three additional fans keep each mesocosm well mixed (SF_6 is well mixed within 12 min after injection).

The cottonwoods were planted from cuttings in 1998, coppiced at the end of each growing season through 2002 and exposed to controlled atmospheric CO_2 concentrations during each growing season in 1999–2003. Because of CO_2 fertilisation effect, in October 2002 (the time of the experiment) the trees grown in the elevated CO_2 concentrations had accumulated more biomass than the trees grown at ambient CO_2 concentration: trees were 6.5 and 6.8 m tall with a leaf area index (LAI) of 1.9 and 3, in the 430 and 1,200 μmol mol^{-1} CO_2 concentration bays, respectively. The constructed silt loam soil (1 m deep) of the agriforest had been evolving *in situ* over 12 years and had developed the physical and nutritional profiles of "natural soils" (Torbert and Johnson 2001), comparable to those used for agriforestry in the SE United States. It now shows metabolic (Murthy et al. 2004) and microbiological properties (Lipson et al., in review) "within a reasonable range for natural soils" (Kudeyarov et al. 2002), with a soil organic carbon content of ca. 2–3% and a carbon:nitrogen ratio of 8.32.

Growth conditions

Although the glass structure of the Biosphere transmits 72% of incoming photosynthetic photon flux density

(PPFD), the low latitude of the site allowed maximum PPFD values to reach 2,000 μmol photons m^{-2} s^{-1}. Average daily total PPFD at the canopy level was 15.62 ± 3.40, 13.98 ± 3.13 and 15.71 ± 3.74 mol photons m^{-2} day^{-1} for the 1,200, 800 and 430 μmol mol^{-1} CO_2 bay, respectively. Day length was 11.5 h at the start of the experiments and 11.0 h at the end, air temperature (T_{air}) was set at 34°C/22°C day/night, and relative humidity (RH) was ca. 75 and 30% for the low (1 kPa) and high (3 kPa) VPD settings, respectively.

Arrays of sensors facilitated continuous monitoring of atmospheric CO_2 concentration, environmental conditions (light, air temperature, leaf temperature, and relative humidity) and trace gas fluxes. PPFD was measured in each bay with 12 sensors (Apogee Instruments, Logan, UT, USA) installed at four evenly distributed locations in each bay (NE, NW, SE and SW) and mounted at three different heights (3, 6 and 9 m above the surface) for each location. VPD was calculated from air temperature and RH data which were measured using a weather station (HT205W Rotronics Hydrometer, La Roche sur Foron, Haute-Savoie, France) mounted at ca. 9 m height in each bay and shielded from solar heating. Soil moisture (SM) was monitored with Time Domain Reflectometry probes (TDR CS165, Campbell Scientific Instruments, Logan, UT, USA) inserted at four location in each bay at two depths: 20 and 80 cm. All data were collected for every 15 s, averaged and stored every for 15 min using dataloggers (Campbell-CR10X, Campbell Scientific Inc., Logan, UT, USA).

Experimental design

As the mesocosms are arranged east to west (430, 800 and 1,200 μmol mol^{-1} CO_2, respectively), this study focuses on results from the 430 and 1,200 μmol mol^{-1} mesocosms, since these two mesocosms received similar integrated light and are most directly comparable. The experiment was carried out towards the end of the 2002 growing season: from October 23 (day 0) to November 29 (day 37) water was withheld from the trees in the bays and the soil was left to dry out naturally. The two CO_2 treatments were combined in temporal sequence with two levels of VPD, imposed in four alternate cycles during the drought. Each cycle consisted of one low VPD period set at a midday value of ca. 1 kPa and one high VPD period set at a midday value of ca. 3 kPa. Each VPD phase was maintained for 3 days, starting on day 3 with the low VPD, until day 23 when the VPD level was left on high for the remainder of the experiment to accelerate drying out of the soil and to accentuate the water stress on the trees. The two phases (low and high VPD) of the first cycle occurred when soil moisture was maximal, whereas the following phases were associated with decreasing soil moisture. However, only the last cycle showed a clear interaction with water stress in the low VPD phase.

Ecosystem CO_2 exchange

Net ecosystem exchange of CO_2 (NEE, μmol m^{-2} s^{-1}) was calculated continuously at 15 min intervals for each bay as μmol of CO_2 exchange per m^2 of ground area per second for the entire experimental period (for a more detailed description see Murthy et al. 2004). Data for NEE calculation for each sampling period were collected at the end of the sampling period.

Ecosystem isoprene flux measurements

For each mesocosm, Dekoron tubing (9.5 mm diameter, 50–90 m length) and three Air Cadet pumps (Cole-Palmer, Vernon Hills, IL, USA) running at more than 6 dm^3 min^{-1} were used to circulate air in three loops drawing air from the three mesocosms into a laboratory and then returning it to the mesocosms. Inside the laboratory, isoprene concentrations were measured with a Fast Isoprene Sensor (FIS) from Hills Scientific (Boulder, CO, USA) (Guenther and Hills 1998). Before the air sample was drawn inside the reaction cell of the FIS, a solenoid array allowed for automatic sampling between the air sampling lines of the three biomes. The mesocosms were sampled at a height of 16 m above the ground, 2 m below the top of the mesocosm frame. The air sample was drawn into the analytical system at a rate of 1.2 dm^3 min^{-1}. Inside the reaction cell of the FIS, the air sample was mixed with 1.0 dm^3 min^{-1} of pure ozone. The FIS was calibrated before and after the experiment by diluting an isoprene standard (5 μmol mol^{-1}, Scott-Marrin, Riverside, CA, USA) over the range of 50 nmol mol^{-1} to 1 μmol mol^{-1} isoprene. System stability throughout the experiment was monitored by running an automated calibration cycle every day at midnight using a standard (100 nmol mol^{-1}) and zero air obtained by passing the sample stream through a scrubber before entering the reaction cell. The system analysed the air coming from a mesocosm during a period of 15 min before changing to the next one. Isoprene concentration was measured every minute at the end of the sampling period discarding the first data automatically to allow complete flushing of the short inlet line from the array of valves to the FIS. The isoprene concentration data were collected every minute on irregular 15 min periods switching between the four biomes, i.e. the biomes were never sampled simultaneously, whereas, the environmental variables and NEE data were collected on a regular 15 min period. For this reason, the 1 min raw isoprene concentration data were averaged within the 15 min sampling period and a spline model was used to fill gaps smaller than 1 h and centre the data on regular consecutive 15 min periods for all biomes to obtain uniform and comparable datasets.

Gross isoprene production (GIP)

The isoprene ecosystem flux, which in our case corresponds to the *net isoprene exchange* (NIE) (isoprene

emission minus isoprene consumption), was calculated continuously for a "closed" system at 15 min intervals as average nanomoles (nmol) of isoprene exchange per m^2 of ground area per second (nnmol m^{-2} s^{-1}) for the entire experimental period with the following equation:

$$\mathrm{NIE} = \frac{\Delta C}{\Delta t} = \frac{C_{t+1} - C_{t-1}}{2 \times \Delta t},$$

where C_{t+1} is the averaged fractional concentration in the mesocosm for the following 15 min period and C_{t-1} is the same for the previous 15 min period. Δt is the length of the time period (15 min). This had the effect of centring the derivative on the current time period, and simultaneously introduced some smoothing.

The B2L was leak-tested by injections of different inert trace gases in the different biomes and leak rates were determined by the tracer decay rate and the rate at which a tracer gas appeared in the contiguous biome or outside. Although the enclosure was found to be 99% air tight, calculated leak rates were taken into account in the isoprene flux calculations by adding the leak flux to the calculated isoprene flux. Diffusion into the soil was also determined by tracer gases injections. After SF_6 addition to the biomes, substantial increases of its concentration in the soil airspace were observed only up to 30 cm in depth. As the soil air volume is small (less then 1% in the IFM) compared to the total volume of the bay only ca. 0.2% of the total leak rate could be the result of diffusion into the soil.

Soil isoprene uptake was measured both at the whole mesocosm level and in the small soil chambers (Pegoraro et al., in review). Isoprene consumption was observed as decrease in concentration that always followed the form of an exponential decay function. The proportionality constant k (deposition velocity) of such exponential decay function was defined as "soil activity factor" to include all physical and biological components responsible for uptake of isoprene by soil. The soil activity factor k (m h^{-1}) was used to calculate the *soil isoprene uptake flux* as nmol of isoprene flux to the soil per m^2 of ground area per second: $F_{\mathrm{soil}} = -k^{*}C$ (nmol m^{-2} s^{-1}); and *gross isoprene production* (GIP, nmol m^{-2} s^{-1}) was then calculated as:

$$\mathrm{GIP} = \mathrm{NIE} - F_{\mathrm{soil}}.$$

Data analysis

To study the response of GIP and NEE to controlled and uncontrolled environmental variables, we used midday averages (10:45 AM–3:45 PM). This allowed us to exclude the morning periods in which the push–pull fans were flushing the bays and thus we may consider the system as a perfectly closed system.

Data were analysed using both bivariate correlation and multiple regression between midday averages of GIP as the dependent variable and PPFD, air temperature, soil moisture and VPD as independent variables. The multiple regression model used was log(GIP) = PPFD + T_{air} + SM + VPD (VPD was used as dummy variable with coding: 0 for low VPD and 1 for high VPD). Analyses were done for the ambient and the elevated CO_2 treatment independently. All assumptions were checked using SAS software and it was necessary to perform a logarithmic transformation on the dependent variable to ensure homogeneity of variance. In addition, this transformation allowed us to perform a simpler analysis since the relationships between the predictor variables and GIP were linear. The bivariate correlations revealed which predictor variables were significantly related to GIP. Then, we applied a linear multiple regression model using all significant variables as predictors. Beta weights (standardised multiple regression coefficients) were also calculated.

The response of NEE and GIP to CO_2 was fitted to a non-linear function (proc NLIN in SAS). The difference between CO_2 treatments was tested by comparing the individual and combined curves (F-test) following Mead and Curnow (1983) as recommended by Potvin et al. (1990). Differences in the response of GIP to temperature and light between VPD treatments were tested in the same way. All statistical tests were considered significant at 5% level of probability. All analyses were performed using SAS software.

As can be seen in Fig. 1 the ecosystem took a few days to adjust to the new VPD conditions, when a VPD treatment was applied (3 days each). Therefore, when analysing the effect of high VPD on GIP we only used the midday average of one measurement day (the last day of treatment) to exclude the days in which the system was adjusting to the new conditions. As a result there was no replicate in time for any VPD cycle and thus no statistic has been performed on the data. As described in the legend of Fig. 4, the error bars only represent the variability of the GIP around the midday average data.

Results

Over the course of the experiment, GIP showed a rapid response to short term (day-to-day) changes in all environmental parameters (i.e. [CO_2], PPFD, air temperature, VPD and soil moisture), whereas, in the short term, NEE was sensitive mainly to the variation of light and soil moisture (Fig. 1). In well-watered conditions, elevated [CO_2] caused a decrease in the GIP of ca. 30% compared to the isoprene emissions in ambient [CO_2], whereas NEE was stimulated, being ca. 153% higher in the elevated [CO_2] mesocosm than in the ambient [CO_2] mesocosm. This was partly the result of a larger LAI developed in elevated [CO_2] than in ambient [CO_2]. Therefore, when considering fluxes per unit of leaf area, the decrease of GIP (nnmol m_{leaf}^{-2} s^{-1}) caused by elevated [CO_2] increased to ca. 58%, whereas the stimulation of NEE (μmol m_{leaf}^{-2} s^{-1}) was only ca. 72%. GIP showed a very rapid response to changes in the

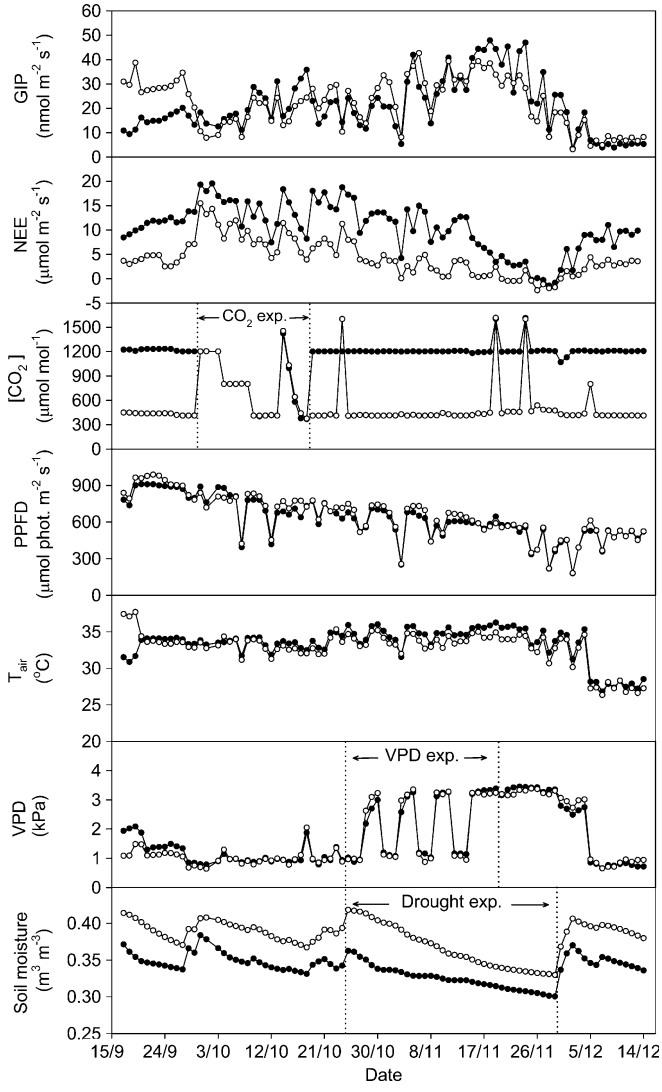

Fig. 1 Central daytime averages (10:45 am to 3:45 pm) of gross isoprene production (GIP) and net ecosystem exchange (NEE), and environmental variables: $[CO_2]$, photosynthetic photon flux density (PPFD), air temperature (T_{air}), VPD and soil moisture, for the 430 and 1,200 µmol mol^{-1} CO_2 treatments (*white* and *black circles*, respectively) over the experimental period (17/9/2002 to 14/12/2002). The *vertical dotted lines* correspond to the boundaries of the CO_2, VPD and drought experiments

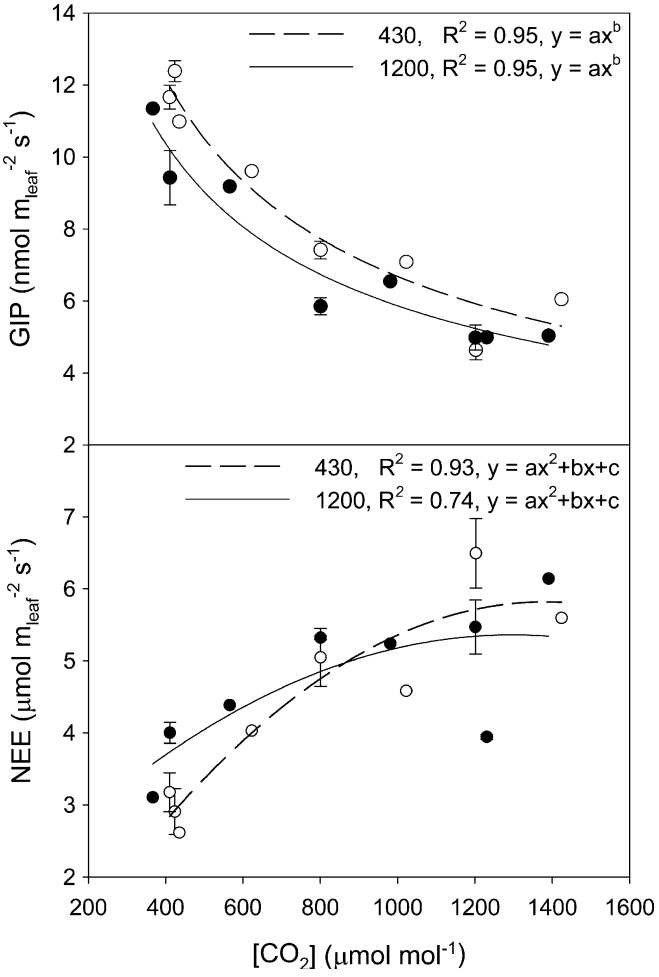

Fig. 2 Relationship between gross isoprene production (GIP) and net ecosystem exchange (NEE) expressed on a leaf area basis, and measurement $[CO_2]$, for the ambient (*white dots*) and the elevated (*black dots*) CO_2 treatments. Lines are fitted regressions with coefficients: **a** ambient treatment: $a = 431.6310$, $b = -0.6223$; elevated treatment: $a = 640.8200$, $b = -0.6615$; **b** ambient treatment: $a = -3.1598$, $b = 0.0087$, $c = -0.2090$; elevated treatment: $a = -2.0732$, $b = 0.0054$, $c = 1.8775$

$[CO_2]$ (Fig. 1), with rates increasing almost coincidentally with decreasing $[CO_2]$. Again, when corrected for the difference in LAI between the two CO_2 treatments (data not shown), GIP from the ambient CO_2 treatment was significantly higher ($P < 0.001$) than GIP from the elevated CO_2 treatment over the whole duration of the CO_2 experiment (Fig. 2). As expected, NEE rates increased with increasing $[CO_2]$. However, although the ambient and elevated CO_2 growth treatments appeared to cause a difference in the instantaneous response of NEE measured at ambient and elevated atmospheric $[CO_2]$, the two regression curves were not significantly different ($P > 0.01$). At ambient atmospheric $[CO_2]$, the trees in the elevated CO_2 treatment showed higher NEE rates than the trees grown in the ambient CO_2 treatment, and at elevated atmospheric $[CO_2]$, the NEE fluxes in the elevated CO_2 growth treatment appeared to be lower than fluxes in the ambient CO_2 treatment.

Over the 3 month period of the experiment, midday average PPFD progressively decreased from ca. 900 (mid September) to ca. 600 (mid December) µmol photons m^{-2} s^{-1} (Fig. 1), with the exception of a few days during which rainstorms and heavy cloudiness caused a significant drop in the available light. These occasional drops in radiation also caused the air temperature inside the bays to deviate from the set point (Fig. 1). With the exception of these days and of the first three and last 10 days, air temperatures remained essentially constant around the set point, rising only slightly during the drought period.

Although the daily onset of isoprene emission from leaves was triggered by the increase in PPFD in the early

morning (at around 7:30 AM with the onset of a PPFD of ca. 100 µmol photons m^{-2} s^{-1}, data not shown), GIP showed a stronger response to temperature than to light (Fig. 3). Furthermore, the response of GIP to both light and temperature was significantly ($P < 0.005$) more pronounced in high than in low VPD conditions in both the ambient and elevated CO_2 treatments. However, the light response curve for the elevated CO_2 growth treatment showed a clear saturation at PPFD of only 750 µmol photons m^{-2} s^{-1} under high VPD conditions and 850 µmol photons m^{-2} s^{-1} under low VPD conditions; there was less evidence of GIP saturation with light in the trees from the ambient CO_2 growth treatment.

During the high VPD (ca. 3 kPa) phases in well-watered conditions, isoprene emission fluxes per unit of leaf area were always stimulated in both CO_2 treatments (Fig. 4). Although during the first VPD cycle GIP was always lower in the elevated CO_2 treatment than in the ambient CO_2 treatment, the stimulating effect of high VPD on the isoprene emission in this treatment was stronger (ca. 34%) than for the ambient CO_2 treatment (ca. 29%). In the following cycles, the combination of high VPD with water stress accentuated the stimulation of GIP in the elevated CO_2 treatment, and the difference in GIP between the two CO_2 treatments tended to become smaller. In the last cycle, when soil moisture was significantly reduced and water stress was affecting NEE, the stronger stimulation on the GIP from the elevated CO_2 treatment was observed also at low VPD (Fig. 4). Although soil moisture was always ca. 10% lower in the elevated CO_2 treatment than in the ambient CO_2 treatment (Fig. 1d), it did not seem to have an effect on isoprene emission. Under low VPD conditions the increase in GIP due to water limitation was evident in both CO_2 treatments only at the time of the last low VPD phase, when the water stress was severe (Fig. 4). In contrast, under high VPD conditions GIP started to increase from the first high VPD phase of the drought period on October 28, when soil moisture was still high, 0.41 and 0.35 m^3 m^{-3} for the ambient and the elevated CO_2 treatment, respectively. However, from November 20 (soil moisture = 0.34 m^3 m^{-3}) to November 22 (soil moisture = 0.31 m^3 m^{-3}) for the ambient and the elevated CO_2 treatment, respectively, GIP decreased dramatically until the end of the drought. Because of the contrasting effects that water stress and VPD had on GIP and NEE (Fig. 1), GIP increased with decreasing NEE (Fig. 5), until the magnitude of the soil water deficit was severe, and as a result the cost in carbon emitted as isoprene with respect to the assimilated carbon also increased from a ca. 2.5% to ca. 0.6% in well-watered conditions to a maximum of ca. 60 and 40% for the ambient and the elevated CO_2 treatments, respectively. Although the overall effect of the drought and high VPD has been of increasing GIP in both treatments

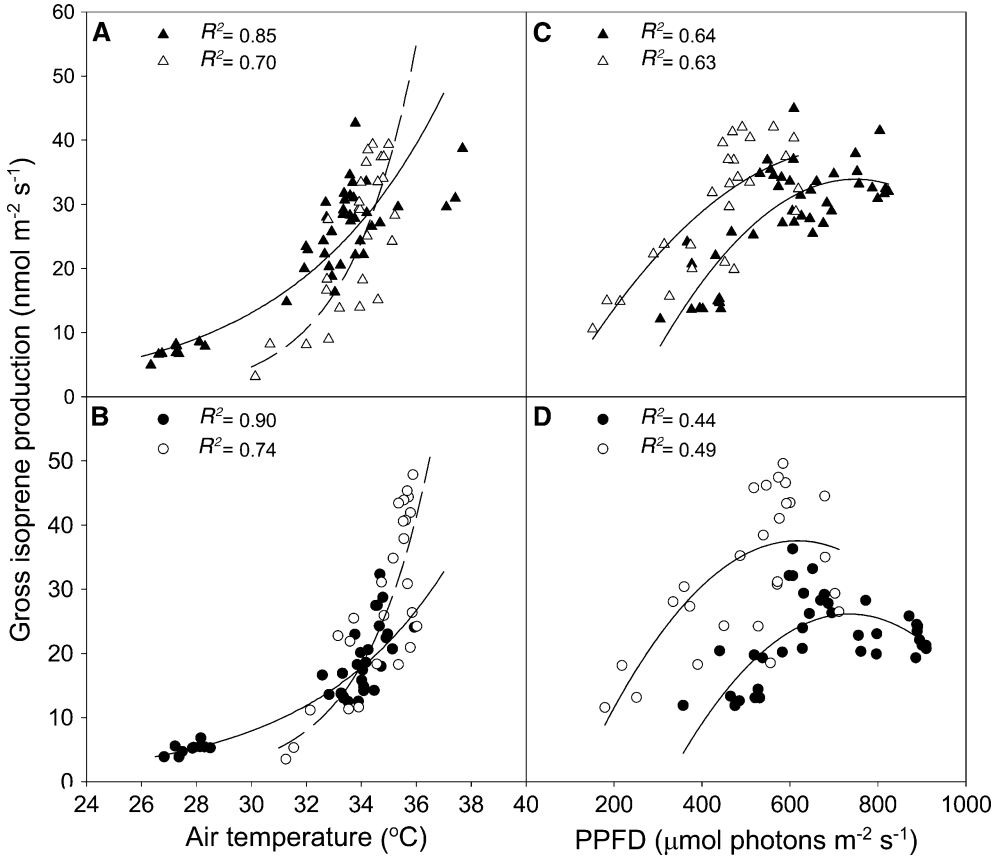

Fig. 3 Relationship between gross isoprene production and the two non-controlled environmental variables: air temperature and PPFD, for the ambient (**a** and **c**) and the elevated (**b** and **d**) CO_2 treatments. Data are grouped for the low (ca. 1 kPa) (*black dots*) and the high (ca. 3 kPa) (*white dots*) VPD phases

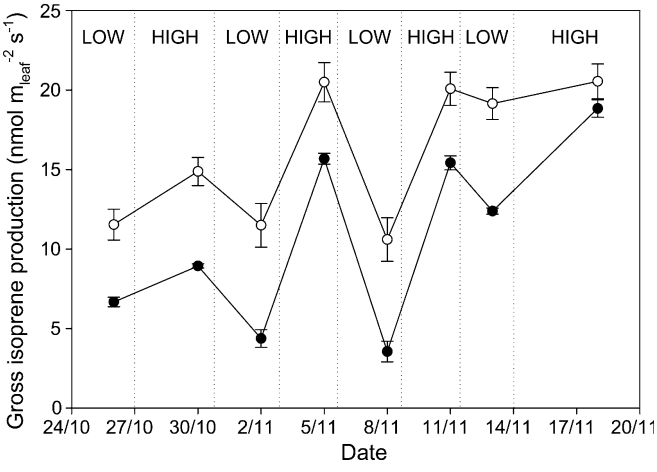

Fig. 4 Gross isoprene production expressed on a leaf area basis in the ambient (*white circles*) and elevated (*black circles*) CO_2 treatments during the four cycles of low and high (ca. 1–3 kPa) VPD. *Symbols* represent central daytime averages (10:45 am to 3:45 pm), with *standard error bars* indicating the variation around the average

(Fig. 1), as the drought progressed isoprene fluxes increased more in the elevated CO_2 treatment than in the ambient CO_2 treatment. As a result, the difference between the two treatments decreased gradually with the decrease in soil water availability and when the stress was severe, in the last 7 days of the drought, the difference in isoprene fluxes between the two CO_2 treatments was only 3%.

Linear multiple regression model

Using multiple regression analysis, logarithmically transformed GIP data were regressed on the linear combination of PPFD, air temperature, soil moisture and VPD. Parameter estimates, standard errors, *P* values and standardised parameters are presented in Tables 1 and 2 for the ambient and the elevated CO_2 treatments, respectively. The equation containing the four variables accounted for 89% ($P < 0.0001$, adjusted $R^2 = 0.88$) and 91% ($P < 0.0001$, adjusted $R^2 = 0.90$) of the variance in the GIP for the ambient and elevated CO_2 treatments, respectively. When comparing the observed values against the predicted values obtained by running the linear model using PPFD, air temperature, soil moisture and VPD as descriptive variables, they

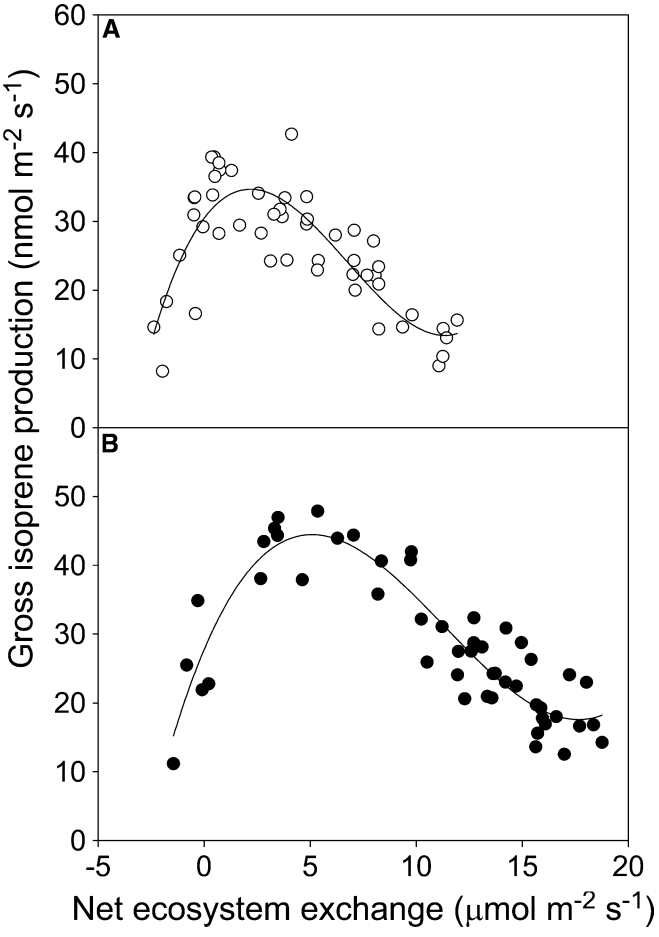

Fig. 5 Relationship between gross isoprene production and net ecosystem exchange for the ambient (**a**) and elevated (**b**) CO_2 treatments

showed a very good linear relationship with an R^2 of 0.79 and 0.86 for the ambient and the elevated CO_2 treatments, respectively (Fig. 6).

Discussion

The unique facility of the Biosphere 2 IFM gave us the unprecedented opportunity to observe the sensitivity of ecosystem level isoprene fluxes to changes in selected and controlled environmental variables such as [CO_2], VPD and soil moisture, and the effect of their interac-

Table 1 Parameter estimates, standard errors, *t* values, *P* values and standardised parameters estimates (β values), for the linear multiple regression model used on logarithm transformed gross isoprene production (GIP) values in the 430 μmol mol^{-1} CO_2 treatment

430 μmol mol^{-1} CO_2	Estimate	SE	*t* value	*Pr*(>|t|)	Standardised estimate
Intercept	0.375	2.334e^{-01}	1.61	0.1132	0
PPFD	4.932e^{-04}	8.875e^{-05}	5.56	< 0.0001	0.362
T_{air}	6.583e^{-02}	5.730e^{-03}	11.49	< 0.0001	0.699
SM	−3.985e^{+00}	4.926e^{-01}	−8.09	< 0.0001	−0.392
VPD (dummy)	−4.373e^{-02}	3.140e^{-02}	−1.39	0.1686	−0.088

PPFD is photosynthetic photon flux density, T_{air} is air temperature, SM is soil moisture and VPD is vapour pressure deficit

Table 2 Parameter estimates, standard errors, t values, P values and standardised parameters estimates (β values), for the linear multiple regression model used on logarithm transformed gross isoprene production (GIP) in the 1,200 μmol mol^{-1} CO_2 treatment

1,200 μmol mol^{-1} CO_2	Estimate	SE	t value	P (>|t|)	Standardised estimate
Intercept	−0.159	3.331e^{-01}	−0.48	0.6321	0
PPFD	1.705e^{-04}	9.982e^{-05}	1.71	0.0926	0.098
T_{air}	8.370e^{-02}	6.120e^{-03}	13.67	<.0001	0.757
SM	−4.494e^{+00}	7.446e^{-01}	−6.03	<0.0001	−0.303
VPD (dummy)	1.871e^{-01}	3.431e^{-02}	1.41	0.1633	0.082

PPFD is photosynthetic photon flux density, T_{air} is air temperature, SM is soil moisture and VPD is vapour pressure deficit

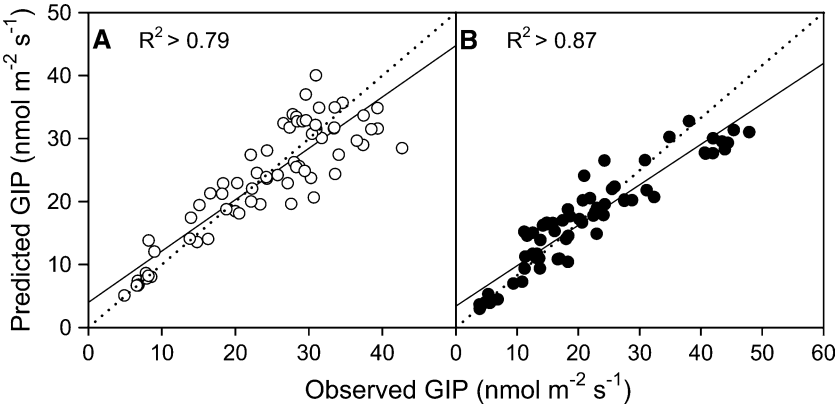

Fig. 6 Linear multiple regression between observed gross isoprene production (GIP) and predicted values of GIP obtained from the linear regression model of logarithm transformed GIP as a function of photosynthetic photon flux density, air temperature, soil moisture and VPD, for the ambient (**a**) and the elevated (**b**) CO_2 treatments ($n = 68$). The *dotted line* represents the 1:1 line

tion. As found in previous studies (e.g. Monson and Fall 1989; Loreto and Sharkey 1990; Sharkey et al. 1991; Loreto et al. 2001; Scholefield et al. 2004; Rosenstiel et al. 2003), exposure to elevated atmospheric [CO_2] reduced ecosystem isoprene production presumably by reducing substrate availability as a result of metabolic competition for phosphoenolpyruvate (Rosenstiel et al. 2003). In this experiment, elevated [CO_2] also inhibited isoprene emission. Furthermore, isoprene fluxes measured in the elevated CO_2 treatment were always lower than those measured in the ambient CO_2 treatment when compared at the same [CO_2], indicating that the inhibition caused by growth in elevated CO_2 conditions is a long-term adaptation of the plant metabolism. Our results indicate that in the short term, both water stress and high VPD counteracted the [CO_2] effect by stimulating isoprene emission. The same response was observed at leaf level (Pegoraro et al. 2004b). Furthermore, the stimulating effect of water limitation was strongest in the elevated CO_2 treatment. As observed from leaf level data (Pegoraro et al. 2004b), the stimulation of isoprene emission was probably the result of the decrease in intercellular [CO_2] (C_i) caused by stomata closure, which led to a stronger decrease of the inhibitory effect in the elevated CO_2 treatment. Although the decrease in transpiration led to an increase in leaf temperature (data not shown), this was not large enough (ca. 4°C in both CO_2 treatments) to explain the large increase in emission rates. The difference in the increase in isoprene emission during the drought from the two CO_2 treatments was also replicated during the VPD cycles, supporting the hypothesis that the stimulation of isoprene emission is linked mainly to a decrease in C_i. Furthermore, toward the middle of the experiment, the stimulation effect was larger with the combination of low soil moisture and high VPD, which is consistent with the higher sensitivity of stomata to VPD in situations of water limitation.

Although GIP and NEE showed a similar response to light and temperature, their response to CO_2 and VPD was opposite in sign, with NEE being stimulated by elevated CO_2 and depressed by high VPD, while GIP was inhibited by elevated CO_2 and stimulated by high VPD. The difference in response between isoprene production and the photosynthetic process was also evident during the water stress experiment, with GIP being stimulated in the short term and declining only when the stress was severe, whereas NEE started to decrease from the beginning. This incomplete coupling between isoprene synthesis and photosynthesis derives from the existence of alternative extra-chloroplastic, slow-turnover, sources and chloroplastic sources of carbon that the plant can use for isoprene production (Karl et al. 2002; Affek and Yakir 2003; Schnitzler et al. 2004). There is now ample evidence that part of the carbon contained in the isoprene molecule is extra-chloroplastic in origin, derived from xylem transported carbon (Schnitzler et al. 2004) and leaf internal carbon pools such as starch (Karl et al. 2002; Affek and Yakir 2003).

Previous studies have found that normally, under unstressed conditions, ca. 80% of the carbon in isoprene is derived from fresh photosynthate and only ca. 20% is derived from alternative carbon sources (Sharkey et al. 1991; Delwiche and Sharkey 1993; Karl et al. 2002; Affek and Yakir 2003), whereas under stressed conditions, the contribution of alternative carbon to isoprene production can increase up to 30% and even more (Funk et al. 2004). It has been observed that isoprene emissions can continue unchanged up to several days even when photosynthetic carbon assimilation is reduced to near zero as a result of stomatal closure (Fang et al. 1996; Pegoraro et al. 2004a). A recent study by Loreto et al. (2004) showed that there is no cross-talking between the two carbon pools normally used in the pathway of isoprene biosynthesis. However, as isoprene production represents only a small percentage of the freshly assimilated carbon, it is possible that when photosynthesis is depressed, isoprene may be formed from previously assimilated carbon still available inside the chloroplast or from respiratory CO_2 recycled in leaves. In the present study, even though NEE was reduced during water stress, isoprene maintained high fluxes and continued to respond quickly to changes in [CO_2]. As a result, the opposite response of GIP and NEE to water limitation led to a drastic increase in the fraction of fixed carbon lost as isoprene emission as water stress progressed. Although it is possible that the plant may use both the alternative extra-chloroplastic and chloroplastic carbon sources to provide the necessary carbon supply for maintaining high isoprene emission rates, the prolonged depression of photosynthetic carbon flow may have ultimately drained these alternative carbon reservoirs. It is likely, that the drop in GIP at the end of the drought period was a consequence of the ultimate depletion of the available carbon pool for isoprene production.

It has been suggested that future increases in atmospheric [CO_2] may not only enhance biomass accumulation in agriforest plantations, but also reduce isoprene production and thereby mitigate, to some extent, the negative air-quality impacts of this trace gas on regional atmospheric chemistry (Rosenstiel et al. 2003). However, our results show that soil water stress and high VPD have the potential to counteract the effect of elevated [CO_2], increasing isoprene production while decreasing CO_2 assimilation. As some future climate scenarios suggest, we may expect that future climate change will bring global increases in mean temperature and localised reductions in precipitation in many regions of the world (Houghton et al. 2001). Our results suggest that in such scenarios the potential exists for a complex pattern of change in the isoprene fluxes, with stimulation possible in response to higher temperature and lower water availability, and possible inhibition as a result of elevated [CO_2] in the absence of warmer, drier conditions. Realistic estimates of regional isoprene emission fluxes are difficult to obtain and so far they mostly rely on modelling efforts (Guenther et al. 1995). Because of practical limitations, most investigations trying to further our understanding of the biochemical mechanisms that may couple isoprene to environmental variables associated with climate change, have been small scale, short-term (few weeks) experiments mostly using potted plants. The complexity of ecological interactions makes it difficult to extrapolate from individuals to communities, and to predict from short-term to the long-term responses. Large scale facilities capable of precise manipulation of selected environmental variables represent a unique tool to complement small-scale experiments, helping to deduce key mechanisms and thereby reduce much of the detail needed for the process of scaling-up (Osmond et al. 2004). One of the major limits of this large facility is probably the limitation on the ability to replicate experiments. However, repetition of experiments is possible and has been performed for example to assess the system variability (Lin et al. 1998; Rosenthal 1998; Rosenthal et al. 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).

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