

Effect of elevated CO₂ concentration and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought

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Abstract. To further our understanding of the influence of global climate change on isoprene production we studied the effect of elevated [CO₂] and vapour pressure deficit (VPD) on isoprene emission rates from leaves of *Populus deltoides* Bartr. during drought stress. Trees, grown inside three large bays with atmospheres containing 430, 800, or 1200 μmol mol⁻¹ CO₂ at the Biosphere 2 facility, were subjected to a period of drought during which VPD was manipulated, switching between low VPD (approximately 1 kPa) and high VPD (approximately 3 kPa) for several days. When trees were not water-stressed, elevated [CO₂] inhibited isoprene emission and stimulated photosynthesis. Isoprene emission was less responsive to drought than photosynthesis. As water-stress increased, the inhibition of isoprene emission disappeared, probably as a result of stomatal closure and the resulting decreases in intercellular [CO₂] (C_i). This assumption was supported by increased isoprene emission under high VPD. Drought and high VPD dramatically increased the proportion of assimilated carbon lost as isoprene. When measured at the same [CO₂], leaves from trees grown at ambient [CO₂] always had higher isoprene emission rates than the leaves of trees grown at elevated [CO₂], demonstrating that CO₂ inhibition is a long-term effect.

Keywords: Biosphere 2 Laboratory, carbon loss, cottonwood, elevated CO₂, intercellular CO₂ concentration, isoprene production, photosynthesis, *Populus deltoides*, stomatal conductance, water-stress.

Introduction

Many studies have been published in recent years on the response of trees to elevated atmospheric [CO₂] and great progress has been made in the mechanistic understanding of the physiological responses of different species (e.g. Medlyn *et al.* 1999). Models suggest that future increases in temperature and decreases in precipitation will cause the

terrestrial carbon sink to become a carbon source later this century (Cox *et al.* 2000). However, such models do not deal with the emission of volatile organic compounds (VOCs) by plants and the effect that this might have on the carbon cycle and on atmospheric chemistry (Fehsenfeld *et al.* 1992; Lerdau *et al.* 1997; Fuentes *et al.* 2000; Monson and Holland 2001; Guenther 2002). Whereas isoprene emissions have

Abbreviations used: *A*, photosynthetic rate; B2L, Biosphere 2 Laboratory; C_i, intercellular CO₂ concentration; DMAPP, dimethylallyl-diphosphate; FID, flame ionisation detector; g_s, leaf stomatal conductance; IFM, Intensive Forestry Mesocosm; PEP, cytosolic phosphoenolpyruvate; SOC, soil organic carbon; θ_v, soil volumetric water content; VPD, vapour pressure deficit; VOCs, volatile organic compounds.

profound effects on atmospheric chemistry because of the high reactivity of isoprene, many environmental variables, such as light and temperature, exert strong controls on its biosynthesis and emission (Harley *et al.* 1999; Fuentes *et al.* 2000; Niinemets *et al.* 2004).

It is known that atmospheric CO₂ concentration can affect isoprene emission from plants (Monson and Fall 1989; Guenther *et al.* 1991; Sharkey *et al.* 1991; Rosenstiel *et al.* 2003; Scholefield *et al.* 2004). For example, Guenther *et al.* (1991) found that isoprene emission rates from leaves of *Eucalyptus globulus* Labill. exposed to approximately 600 µmol mol⁻¹ atmospheric [CO₂] were lower than isoprene emission rates from leaves exposed to 100 µmol mol⁻¹ CO₂. Sharkey *et al.* (1991) found contrasting responses by *Populus tremuloides* Michx. (30–40% decrease) and *Quercus rubra* L. (100% increase) exposed to an increase in atmospheric [CO₂] from 400 to 650 µmol mol⁻¹. Rosenstiel *et al.* (2003) showed that isoprene emission rates from *Populus deltoides* Marsh. clones grown for almost 2 years at 800 and 1200 µmol mol⁻¹ atmospheric [CO₂] were reduced by 21 and 41%, respectively, compared with plants grown at 430 µmol mol⁻¹ CO₂. Moreover, it has been observed that isoprene emission is already inhibited at ambient [CO₂] when compared with lower atmospheric levels of CO₂ (Monson and Fall 1989; Sharkey *et al.* 1991).

The results from previous studies on the effect of water stress on isoprene emission (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996; Guenther *et al.* 1999; Bruggemann and Schnitzler 2002; Pegoraro *et al.* 2004) suggest that isoprene emission is much less sensitive than photosynthesis to drought. Although isoprene emission seems to be independent of stomatal dynamics (Fall and Monson 1992), stomatal closure caused by water stress or high atmospheric water vapour pressure deficit (VPD) may lead to decreases in intercellular [CO₂] (C_i), leading to increases in isoprene production. Furthermore, the reduced transpiration may increase leaf temperature, which also induces higher isoprene emission rates (Fang *et al.* 1996; Harley *et al.* 1999). Despite great progress, our understanding of the effect of water stress on isoprene emission is still limited. Results are variable among studies and no general pattern has been observed. Moreover, most studies have been done on potted plants (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996; Bruggemann and Schnitzler 2002; Pegoraro *et al.* 2004) and very few have been done on field-grown plants under natural drought conditions (e.g. Guenther *et al.* 1999). Although experiments on potted seedlings provide useful mechanistic information about processes they do not always yield results that are applicable to forest ecosystems (e.g. Medlyn *et al.* 1999).

To study the effect of atmospheric [CO₂] and VPD, and their interaction with drought on isoprene emission in an agriforest plantation, an experiment was set up inside the controlled environment research facility of the Intensive Forestry Mesocosm (IFM) of Columbia

University's Biosphere 2 Laboratory (B2L, Oracle, AZ). The specific objectives of this study were: (1) to investigate the short-term solitary and interactive effects of elevated [CO₂], VPD, and drought on isoprene emission; (2) to study the long-term (permanent) effects of plant acclimation to elevated CO₂ on isoprene emission; and (3) to calculate the isoprene:carbon dioxide flux ratio for this species under different CO₂, VPD and soil moisture conditions. It was hypothesised that: (I) plants growing in elevated atmospheric [CO₂] undergo an acclimation of the metabolic processes linked to photosynthesis and respiration, so that the inhibition of isoprene production caused by elevated [CO₂] becomes a long-term feature, and (II) high VPD and water stress, by decreasing leaf stomatal conductance (g_s) and consequently decreasing C_i levels, induce higher isoprene emission rates as a consequence of the reduced CO₂ inhibitory effect. Compared with studies conducted on potted plants, the ability to perform this experiment in the IFM allowed us the opportunity to study the response of fully-grown plants to variations in selected and fully controlled environmental variables, which would never have been possible in the outside world. Thus, this large-scale facility allowed us to undertake an unprecedented manipulation study over large forest stands.

Materials and methods

Plant material

The study was conducted in the Intensive Forestry Mesocosm (IFM) facility at the Biosphere 2 Laboratory (B2L) between 21 October and 30 November 2002. This facility consists of three experimental bays at three atmospheric [CO₂]: 430, 800 and 1200 µmol mol⁻¹. Cottonwood clones (*Populus deltoides* Bartr.) were grown from cuttings planted at the start of the 1998 growing season. Thereafter, trees were coppiced at the end of each growing season, through to the end of winter of 2001. At the time of this experiment (growing season 2002) the aboveground shoots were 6 months old and approximately 6 m tall. The soil was 1 m deep and consisted of a mixture of bare soil (60%) and organic matter (40%). Presently, the textural classification of the soil is a silt-loam (Torbert and Johnson 2001). When the soil was analysed in 2004, it contained 2–3% soil organic carbon (SOC) and a C:N ratio of 8.32, similar to that of an agricultural system.

Growth conditions

Plants inside the three bays were grown under the following environmental conditions: average total daily photosynthetic photon flux density (PPFD) of 15.1 ± 3.5 mol m⁻² d⁻¹ at the top of the canopy; daylength of approximately 11 h, day/night air temperatures of 32/26°C and a VPD of either 1 or 3 kPa for the low and high VPD settings, respectively. In each bay, PPFD was measured at different heights (3, 6 and 9 m above ground level) in the canopy with 12 sensors (Apogee Instruments, Logan, UT) installed at four locations (NE, NW, SE and SW). Air temperature and relative humidity were measured with a weather station with thermocouples and a hydrometer (HT205W, Rotronics, La Roche sur Foron, Haute-Savoie, France) mounted at approximately 9 m above the soil surface in each bay and shielded from solar heating. VPD was calculated from these data as the difference between the partial pressure of water vapour of saturated air at ambient temperature and the measured partial pressure of water vapour. All data were measured every 15 s, averaged and stored every 15 min using dataloggers (Campbell-CR10x, Campbell Scientific Inc., Logan, UT).

Soil volumetric water content (θ_v) was determined at a depth of 10 cm in four locations in each bay. Previous studies on the below ground biomass of the stands found that most of the fine root biomass was concentrated in the top 10 cm of soil (G Barron-Gafford, personal communication). Soil samples of approximately 20 g were taken at depths of 10 cm using a soil auger. The soil samples were placed in pre-weighed tins, which were then sealed and transported to the laboratory where they were weighed. Samples were then placed in a forced-draft oven at 100°C for 5 d, removed and weighed again. The bulk density of the top 10 cm of the soil was measured and θ_v ($\text{m}^3 \text{m}^{-3}$) was determined as:

$$\theta_v = \frac{M_f - M_d}{M_d} \times \rho_s \times \frac{1}{\rho_w} \quad (1)$$

where M_f is fresh mass (g), M_d is dry mass (g), ρ_s is the bulk density of the soil (1.1 g cm^{-3}) and ρ_w is the density of water (1 g cm^{-3}).

Experimental design

The three bays were closed all day with no exchange with the outside air with the exception of a 2-h period at dawn to facilitate the expulsion of night-time respired CO₂. This was necessary only if the CO₂ inside the bay increased to such high concentrations that the trees were unable to bring down the [CO₂] to the desired target concentration on the next day. From 23 October (day 0) to 29 November (day 37) water was withheld in the three bays. The three bays were subjected to two VPD levels, which were imposed in three alternate cycles of 6 d during the drought: first cycle, day 2 to day 7; second cycle, day 8 to day 13; and third cycle, day 20 to day 25. Each cycle consisted of one low VPD level set at approximately 1 kPa and one high VPD level set at approximately 3 kPa (Table 1). At the start of the experiment (day 0), VPD was set to low and it was changed to high on day 5. Starting from day 5, each level was maintained for a period of 3 d until day 25 when the VPD level was left on high for the rest of the experiment to accentuate the drought stress on the plants.

Sampling protocol

Before the beginning of the gas exchange measurements, five trees were randomly chosen in each bay and followed over the whole experiment.

From each tree, one fully expanded leaf was randomly selected from the same position (middle canopy) and orientation (south facing) in the canopy and tagged for subsequent measurements. To avoid large diurnal sampling biases, all gas exchange measurements and collection of air samples for the determination of isoprene emission were made between 1030 and 1430 hours.

Leaf gas-exchange measurements

Photosynthetic rate (A), stomatal conductance (g_s) and intercellular [CO₂] (C_i) were measured using an open-path gas exchange measurement system LI-6400 (Li-Cor, Lincoln, NE). All measurements were made using the same standard cuvette conditions: leaf temperature of 32°C and PPFD of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and VPD similar to that in the ambient growing conditions. Leaf temperature and light conditions were chosen to match as closely as possible the average outside conditions at the time of measurements to reduce the equilibration time to the cuvette conditions. After a leaf was placed in the cuvette, a minimum of 10 min was allowed for equilibration, and all measurements were made after steady rates of exchange of CO₂ and H₂O were obtained.

For measurements of isoprene emission rates, the air exiting the cuvette was collected inside a Teflon® bag connected to the exhaust of the leaf cuvette. Isoprene concentration inside the bags was determined in the laboratory by gas chromatography (GC; SRI 310, Buck Scientific, East Norwalk, CT) with a custom-made inlet system capable of vacuum sample collection and isoprene cryofocusing. A 300-cm³ sample of the air contained in the bag was trapped in a first loop packed with 60/80 mesh Tenax TA (Sigma Aldrich, Dorset, UK) kept at 4°C to reduce the sample volume and at the same time to eliminate excess amounts of water that could skew the isoprene detection. The sample was then cryogenically concentrated in a second loop kept under liquid nitrogen and transferred to the GC column (0.25 mm ID × 30 m MXT-624 capillary column, RESTEK Corporation, Bellefonte, PA) in a flow of ultra high purity helium. Eluted isoprene from the column was measured with a flame ionisation detector (FID). Additional details of the analytical system are given by Greenberg *et al.* (2003).

A summary of the different experiments and measurements conducted is presented in Table 1. In order to study the effect of elevated [CO₂] on isoprene emission and its interaction with drought,

Table 1. Summary of the experiments conducted at Biosphere 2 Laboratory facility in the Intensive Forestry Management biomes of *Populus deltoides*
Growth and measurement [CO₂] ($\mu\text{mol mol}^{-1}$), VPD (high: 3 kPa, low: 1 kPa), soil moisture ($\text{m}^3 \text{m}^{-3}$) and dates (given as days from the beginning of the experiment) are given

Experiment	Growth [CO ₂] ($\mu\text{mol mol}^{-1}$)	Measurement [CO ₂] ($\mu\text{mol mol}^{-1}$)	VPD (kPa)	Soil moisture ($\text{m}^3 \text{m}^{-3}$)	Measurement date (Days)
I. CO ₂ and drought (37 days)	430 800 1200	430 800 1200	high	Decrease gradually over the course of the experiment	6
					12
					24
					29
					33
					35
II. CO ₂ , VPD and drought	430 800 1200	430 800 1200	Cycle (low/high)	I wet ($\theta > 0.2$) II dry ($0.14 < \theta < 0.2$) III very dry ($\theta < 0.14$)	0
					6
					9
					12
					22
					24
III. CO ₂ acclimation	430 1200	430/1200 1200/430	low high high	$0.16 < \theta < 0.18$ $\theta < 0.14$	9–10
					12–13
					33–34

leaf gas-exchange measurements were made on seven occasions over 31 d between day 6 and day 37 for the three CO₂ treatments. All measurements were made on days with conditions of high VPD to accentuate the drought effects. The first measurements were made in conditions of non-limiting soil water content ($\theta_v > 0.2 \text{ m}^3 \text{ m}^{-3}$).

To investigate the interactive effects of elevated [CO₂], VPD and water stress on isoprene emission, leaf gas-exchange measurements were made during three cycles of alternating conditions of low and high VPD. During the first and second cycles, measurements were made over 9 d between day 4 and day 13 ($\theta_v > 0.14 \text{ m}^3 \text{ m}^{-3}$). To observe the effect of drought on isoprene emission and its interaction with elevated [CO₂] and VPD, two additional measurements during another cycle were taken on day 22 and 25, when the trees were assumed to be water stressed ($\theta_v < 0.14 \text{ m}^3 \text{ m}^{-3}$).

To understand whether plants had acclimated to elevated [CO₂] and to see if the effects of [CO₂] on isoprene emission had become long-term (permanent), leaf gas-exchange measurements were made at both ambient (430 $\mu\text{mol mol}^{-1}$) and elevated (1200 $\mu\text{mol mol}^{-1}$) [CO₂] in the lowest (430 $\mu\text{mol mol}^{-1}$) and highest (1200 $\mu\text{mol mol}^{-1}$) CO₂ treatment mesocosms. Leaves were placed in the cuvette and always measured first at the growth [CO₂]. To determine the interaction of CO₂ and VPD, gas-exchange measurements were made on two different days: on day 10, the third day of the second low VPD cycle and on day 13, the third day of the second high VPD cycle. Moreover, to observe the effect of drought, a third set of measurements was made on day 34, when the plants were assumed to be water stressed ($\theta_v < 0.14 \text{ m}^3 \text{ m}^{-3}$).

Statistical analysis

All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC). When looking at the effect of elevated [CO₂] on isoprene emission during the drought period, the overall mean comparison of *A*, *g_s*, isoprene emission rate and θ_v between CO₂ treatments were analysed with a multivariate analysis of variance (MANOVA). The experimental unit was the individual tree ($n = 5$). To take into account the overall effect during the course of the experiment, data were first analysed by a two-way ANOVA with repeated measures with drought, treatment, time and their interactions as factors. When this test was significant for a treatment at a 5% level of probability, a single ANOVA was used to test differences on each date to understand how and when the CO₂ treatment affected the specific variables under study. When analysing the interactions of VPD and elevated [CO₂] on isoprene emission rates during the drought the three factors, cycles, VPD and CO₂ treatments, were first tested for significance using a three-way ANOVA. For each cycle, *A*, isoprene emission rate, *g_s*, and *C_i* were first analysed using a two-way ANOVA in which CO₂, VPD treatment and their interaction, were considered. Within each cycle, significance for high and low VPD of *A*, isoprene emission rate, *g_s*, and *C_i* in the three CO₂ treatments was tested using a two-way ANOVA. When looking at the effect of acclimation to [CO₂] on isoprene emission and its interaction with VPD and θ_v , linear and non-linear regressions were used to determine the relationships between the different physiological variables. Variation around the mean is reported as one standard error (SE).

Results

Effect of elevated atmospheric [CO₂] on CO₂ uptake and isoprene emission

At the beginning of the experiment (day 0), θ_v was $0.27 \pm 0.01 \text{ m}^3 \text{ m}^{-3}$ and decreased gradually until it reached a value of $0.10 \pm 0.01 \text{ m}^3 \text{ m}^{-3}$ at the end of the experiment (day 37; Fig. 1A). Water limitation reduced *A* when θ_v was less than $0.2 \text{ m}^3 \text{ m}^{-3}$, whereas the decline in isoprene

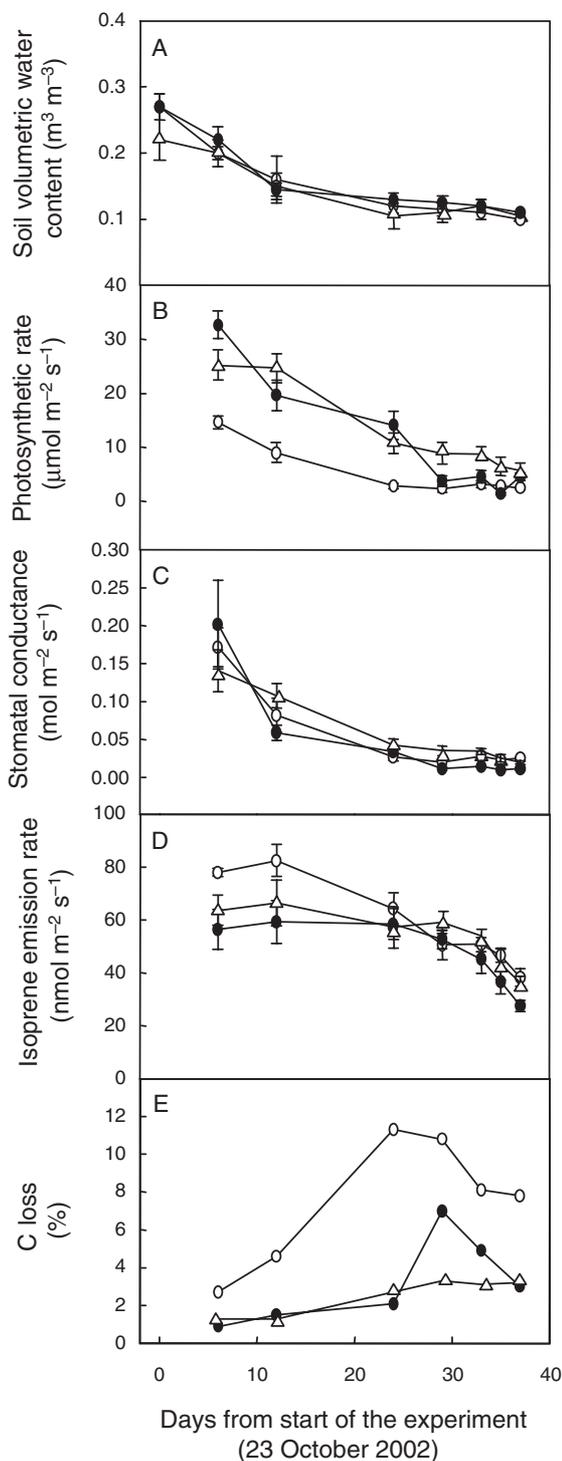


Fig. 1. Time course of (A) soil volumetric water content ($\text{m}^3 \text{ m}^{-3}$), (B) photosynthetic rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), (C) stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$), (D) isoprene emission rate ($\text{nmol m}^{-2} \text{ s}^{-1}$) and (E) percentage of assimilated carbon lost as isoprene (%), for the three [CO₂] growth treatments (○, 430; △, 800 and ●, 1200 $\mu\text{mol mol}^{-1}$ CO₂) measured at high VPD (approximately 3 kPa) during the course of the drought experiment. Values are means ± 1 SE ($n = 5$).

emission began only when θ_v was less than $0.14 \text{ m}^3 \text{ m}^{-3}$. On day 6 of the experiment, A of leaves grown at $430 \mu\text{mol mol}^{-1}$ of CO₂ were significantly lower ($P < 0.01$) than those grown in the 800 (approximately 72% higher) and in the 1200 (approximately 123% higher) $\mu\text{mol mol}^{-1}$ CO₂ treatments (Fig. 1B). During the course of the drought, A decreased by approximately 84, 65 and 89%, for the 430, 800, and 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments, respectively. Stomatal conductance g_s showed a similar trend but there were no statistical differences between CO₂ treatments over the course of the experiment ($P > 0.05$ in all cases; Fig. 1C). In all CO₂ treatments, g_s declined steadily from days 12 to 29, with a reduction of approximately 89, 75 and 94% for the 430, 800 and 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments, respectively, and from that point remained essentially constant to the end of the experiment.

On day 6, isoprene emission rates were significantly greater ($P < 0.01$) in the $430 \mu\text{mol mol}^{-1}$ CO₂ treatment than in the 800 or the 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments (approximately 19% and 28% lower, respectively; Fig. 1D). Isoprene emission rates changed little for leaves grown in the 800 and 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments from days 6 to 29 whereas in the $430 \mu\text{mol mol}^{-1}$ CO₂ treatment they decreased remarkably until rates were similar to emission rates of the two elevated CO₂ treatments by day 24. From day 33 to the end of the experiment, isoprene emission rates continued to decline slowly reaching minima of 38.3, 36.4 and $27.5 \text{ nmol m}^{-2} \text{ s}^{-1}$ in the 430, 800 and 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments, respectively.

Over the course of the drought, the ratio of the carbon lost as isoprene to carbon assimilated, i.e. the percentage of carbon used in isoprene production compared with the amount of assimilated carbon (C_{iso}/C_A), changed significantly, particularly in the $430 \mu\text{mol mol}^{-1}$ CO₂ treatment (Fig. 1E). At the beginning of the experiment, when soil moisture was not limiting ($\theta_v > 0.2 \text{ m}^3 \text{ m}^{-3}$) C_{iso}/C_A was small (approximately 1–3%) and differed slightly among the CO₂ treatments, with the $430 \mu\text{mol mol}^{-1}$ being the highest. As the drought progressed, in the $430 \mu\text{mol mol}^{-1}$ CO₂ treatment, the combination of water limitation and high VPD conditions strongly depressed A leading to up to a 4-fold increase in the C_{iso}/C_A by day 24. From day 33, because isoprene emission rates started to decline steadily in all CO₂ treatments, whereas A remained essentially constant, C_{iso}/C_A decreased through the end of the experiment. In the 800 and 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments, because of higher A and smaller isoprene emission rates, C_{iso}/C_A increased less and more slowly than in the $430 \mu\text{mol mol}^{-1}$ CO₂ treatment.

Effect of elevated [CO₂] and VPD on CO₂ uptake and isoprene emission

In the first VPD cycle, in wet conditions ($\theta_v > 0.2 \text{ m}^3 \text{ m}^{-3}$) and during the low VPD phase, photosynthetic rates were

similar in the two elevated CO₂ treatments and significantly greater (approximately 75%) than in the ambient CO₂ treatment ($P < 0.001$; Fig. 2A). In high VPD, photosynthetic rates for the three CO₂ treatments were lower compared with the low VPD phase and significantly different from each other ($P < 0.05$). During the second cycle, although θ_v had decreased to $0.17 \text{ m}^3 \text{ m}^{-3}$ (dry conditions, Fig. 2B), in the low VPD phase, A showed similar values to those measured during the first cycle. During the high VPD phase, photosynthetic rates measured in the 430 and 1200 $\mu\text{mol mol}^{-1}$ treatments were strongly reduced (approximately 40%) compared with the wet phase (Fig. 2A), whereas rates in the 800 $\mu\text{mol mol}^{-1}$ CO₂ treatment did not change. During the third cycle, in very dry conditions ($\theta_v < 0.14 \text{ m}^3 \text{ m}^{-3}$), A again showed a similar pattern to the previous cycles, with leaf photosynthetic rates measured in the elevated CO₂ treatment being significantly greater than those measured in the ambient CO₂ treatment ($P < 0.05$; Fig. 2C). However, compared with the second cycle, A was drastically depressed, particularly in the ambient CO₂ treatment. In low VPD conditions photosynthetic rates were reduced by approximately 59, 40 and 32% in the 430, 800 and 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments, respectively, while in the high VPD they were reduced by approximately 68, 56 and 28% in the 430, 800 and 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments, respectively.

In the first VPD cycle, isoprene emission rates measured at both low and high VPD were significantly different between the 430 and the 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatments ($P < 0.001$) and always decreased with increasing [CO₂] (Fig. 2D). At low VPD, isoprene emission rates in the 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatment were approximately 47% lower than isoprene emissions measured in the 430 $\mu\text{mol mol}^{-1}$ CO₂ treatments. At high VPD, isoprene emission rates for each CO₂ treatment were significantly higher ($P < 0.001$) than those measured at low VPD. At this time, isoprene emission rates in the 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatment were only approximately 28% lower than isoprene emissions measured in the 430 $\mu\text{mol mol}^{-1}$ CO₂ treatment. During the low VPD conditions of the second VPD cycle, in dry conditions ($0.2 > \theta_v > 0.14 \text{ m}^3 \text{ m}^{-3}$), isoprene emission rates increased by approximately 27% in the 430 $\mu\text{mol mol}^{-1}$ CO₂ treatment compared with those measured in the first VPD cycle, reaching similar rates to those measured at high VPD conditions (Fig. 2E), whereas no change was detected in the 800 $\mu\text{mol mol}^{-1}$ and isoprene emission rates only increased by approximately 17% in the 1200 $\mu\text{mol mol}^{-1}$ CO₂ treatment. During the high VPD conditions, isoprene emission rates remained essentially the same in all three CO₂ treatments compared with those measured in the first cycle. By the third cycle, in very dry conditions ($\theta_v < 0.14 \text{ m}^3 \text{ m}^{-3}$), isoprene emission rates were not significantly different between CO₂ treatments during both the high and low VPD treatments and between VPD

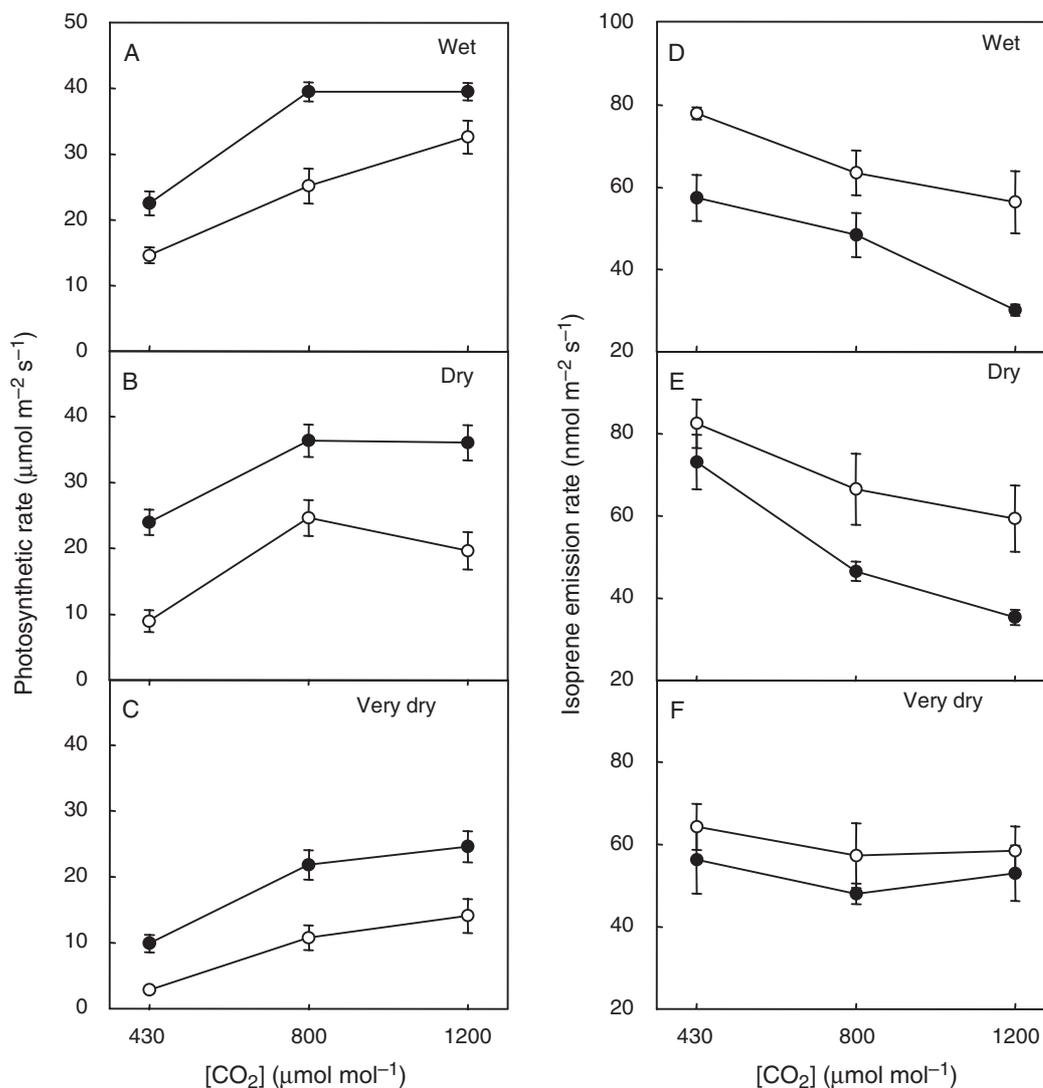


Fig. 2. Effect of $[\text{CO}_2]$ growth treatment (430, 800 and $1200 \mu\text{mol mol}^{-1}$) on (A–C) photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and (D–F) isoprene emission rate ($\text{nmol m}^{-2} \text{s}^{-1}$) measured in high (O, approximately 3 kPa) and low (●, approximately 1 kPa) VPD during three cycles corresponding to a soil water content of 0.22, 0.17 and $0.13 \text{ m}^3 \text{ m}^{-3}$: (A, D) wet, (B, E) dry and (C, F) very dry, respectively. Symbols represent means \pm SE ($n = 5$).

treatments ($P > 0.05$ in all cases). During the low VPD setting, drought appeared to have a strong contrasting effect on isoprene emission mainly in the 430 and $1200 \mu\text{mol mol}^{-1}$ CO_2 treatments; compared with the second VPD cycle (dry conditions) isoprene emission rates decreased by approximately 23% in the $430 \mu\text{mol mol}^{-1}$ CO_2 treatment whereas they increased by 50% in the $1200 \mu\text{mol mol}^{-1}$ CO_2 treatment. During the high VPD setting, isoprene emissions decreased in the three CO_2 treatments: by approximately 22 and 14% in the 430 and $800 \mu\text{mol mol}^{-1}$ CO_2 treatments, respectively, and only 1.5% in the $1200 \mu\text{mol mol}^{-1}$ CO_2 treatment. During all measurements over the period of the three VPD cycles, within each CO_2 treatment, the higher values of isoprene emission obtained during the

high VPD phases were associated with lower values of C_i (Fig. 3).

In wet conditions and during the low VPD phase, isoprene emission represented a carbon loss of 1–2% of the assimilated carbon in photosynthesis (C_{iso}/C_A), with the largest loss in the $430 \mu\text{mol mol}^{-1}$ CO_2 treatment and the smallest in the $1200 \mu\text{mol mol}^{-1}$ CO_2 treatment (Fig. 4A). At this time, the loss was only slightly larger at high VPD than at low VPD conditions (Fig. 4B). Over the three VPD cycles, at low VPD, C_{iso}/C_A increased with decreasing θ_v in all CO_2 treatments and the carbon loss doubled when water stress was most severe. At high VPD, C_{iso}/C_A for all CO_2 treatments showed the same pattern, although it dramatically increased particularly in the $430 \mu\text{mol mol}^{-1}$

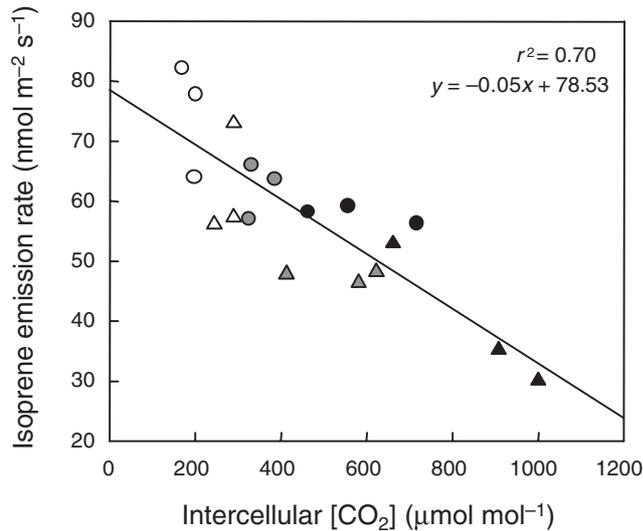


Fig. 3. Relationship between intercellular [CO₂] ($\mu\text{mol mol}^{-1}$) and isoprene emission rate ($\text{nmol m}^{-2} \text{s}^{-1}$) measured at low (approximately 1 kPa; triangles) and high (approximately 3 kPa; circles) VPD during the course of the drought experiment. Each symbol represents the mean ($n = 5$) of trees grown at three different atmospheric [CO₂]: 430, 800 and $1200 \mu\text{mol mol}^{-1}$, white, grey and black symbols, respectively.

CO₂ treatment. At the end of the water stress period the $C_{\text{iso}}/C_{\text{A}}$ was approximately four times higher than in wet conditions.

Effect of CO₂ acclimation and VPD on CO₂ uptake and isoprene emission

Photosynthesis

At the time of the second VPD cycle, with values of $\theta_v > 0.14 \text{ m}^3 \text{ m}^{-3}$, when leaves were measured at their growth [CO₂], photosynthetic rates of leaves grown at $1200 \mu\text{mol mol}^{-1}$ CO₂ were significantly higher ($P < 0.01$) than those of trees grown at $430 \mu\text{mol mol}^{-1}$ CO₂, both at high and low VPD (Fig. 5A). In both CO₂ treatments, elevated [CO₂] measurement concentration always stimulated A compared with rates measured at ambient [CO₂] measurement concentration. However, when compared at the same [CO₂] ('measurement concentration') in low VPD, leaves grown at $430 \mu\text{mol mol}^{-1}$ CO₂ always exhibited higher A than leaves grown at $1200 \mu\text{mol mol}^{-1}$ CO₂. High VPD caused a strong decrease in A in both CO₂ treatments, particularly that of the ambient CO₂ treatment (approximately 63%). Consequently, differences between A of leaves grown at ambient and elevated [CO₂] treatments disappeared when measured at the same [CO₂] ($P > 0.05$).

Towards the end of the experiment when values of θ_v were less than $0.14 \text{ m}^3 \text{ m}^{-3}$, i.e. when the effect of drought was most severe, photosynthetic rates were measured again at high VPD conditions and rates were further reduced by approximately 65 and 77% in the ambient and elevated CO₂ treatments, respectively, (Fig. 5A)

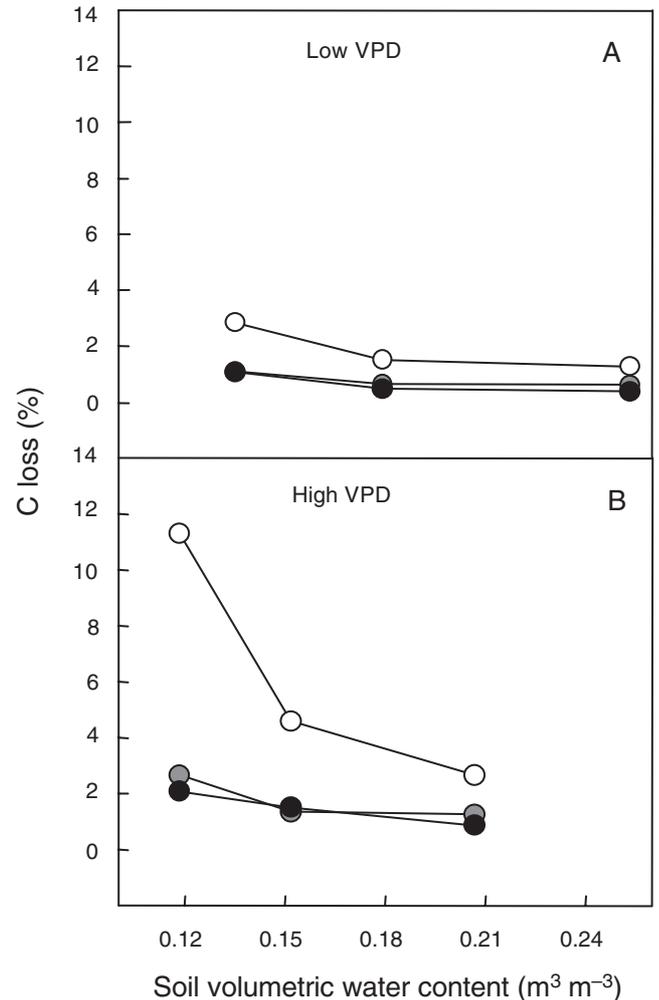


Fig. 4. Effect of (A) low (approximately 1 kPa) and (B) high (approximately 3 kPa) VPD on the proportion of assimilated carbon lost as isoprene (%) and the interaction with soil volumetric water content ($\text{m}^3 \text{ m}^{-3}$) during the course of the drought experiment. Each carbon loss value was calculated using the mean ($n = 5$) of trees grown at three different atmospheric [CO₂]: 430, 800 and $1200 \mu\text{mol mol}^{-1}$, white, grey and black symbols, respectively.

Isoprene emission

As seen in Fig. 1, in wet conditions, isoprene emission rates of leaves grown and measured at elevated [CO₂] were always significantly lower than those of leaves grown and measured at ambient [CO₂] ($P > 0.05$). When leaves of the ambient and elevated CO₂ treatments were measured in low VPD conditions and at both ambient and elevated [CO₂], isoprene emission rates were significantly lower in the elevated CO₂ treatment than in the ambient CO₂ treatment ($P < 0.001$) (Fig. 5B). When measured at high VPD, isoprene emission rates were stimulated in both CO₂ treatments at both ambient and elevated CO₂ measurement concentrations compared with those measured in low VPD conditions. The stimulation effect was particularly strong in the elevated CO₂ treatment where emissions doubled

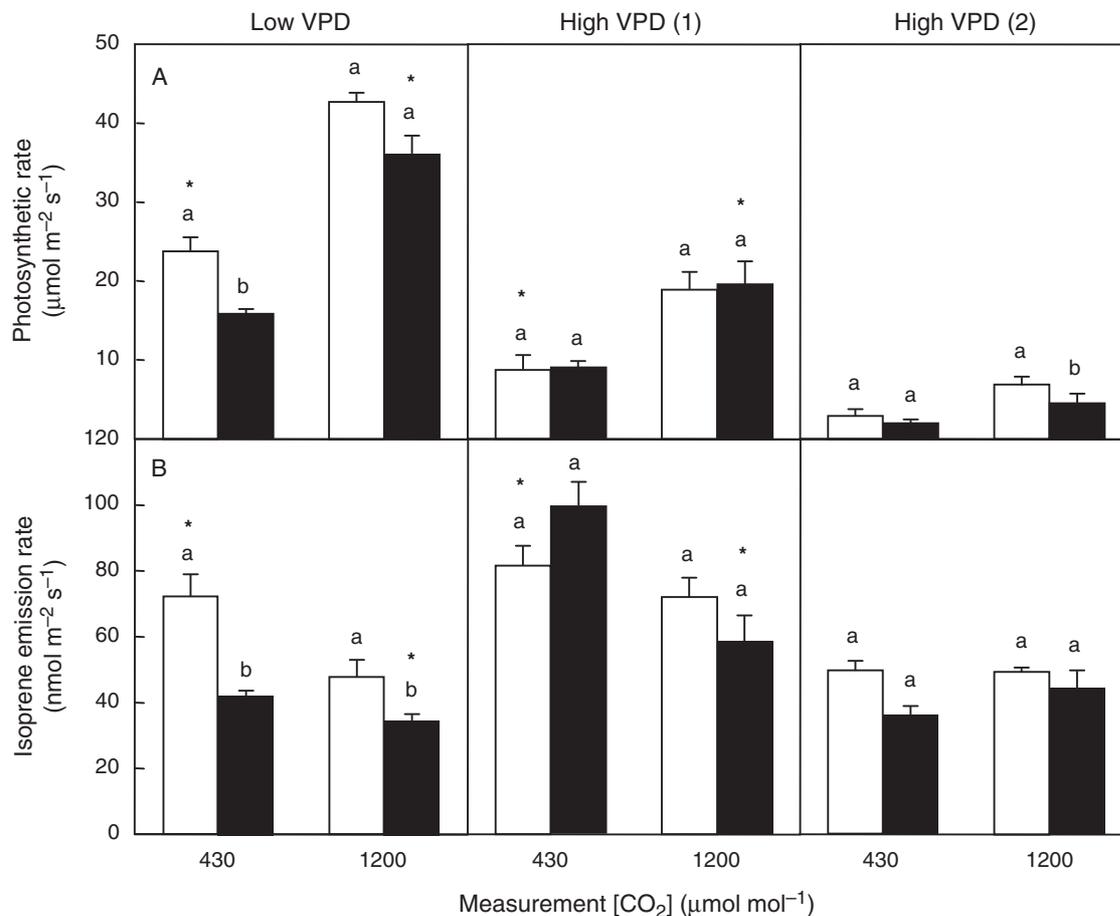


Fig. 5. Effect of CO_2 growth concentration (430 and $1200 \mu\text{mol mol}^{-1}$, white and black bars, respectively) on (A) leaf photosynthetic rates and (B) isoprene emission rates measured at 430 and $1200 \mu\text{mol mol}^{-1}$ $[\text{CO}_2]$. Measurements were made in low VPD (approximately 1 kPa), high VPD (1) (approximately 3 kPa) with soil volumetric water content (θ_v) = $0.15 \text{ m}^3 \text{ m}^{-3}$ and HIGH VPD (2) with soil volumetric water content = $0.11 \text{ m}^3 \text{ m}^{-3}$. Bars represent means ± 1 SE ($n = 5$). Bars at the same measurement $[\text{CO}_2]$ with different letters are significantly different at 5% (least significant difference test). In each panel, bars of the ambient and elevated CO_2 treatment marked with * are significantly different at 5% (least significant difference test).

when measured at $430 \mu\text{mol mol}^{-1}$ CO_2 and increased by 68% when measured at $1200 \mu\text{mol mol}^{-1}$ CO_2 , while in the ambient $[\text{CO}_2]$ treatment, leaf isoprene emissions increased only by 13% when measured at $430 \mu\text{mol mol}^{-1}$ CO_2 and by 50% when measured at $1200 \mu\text{mol mol}^{-1}$ CO_2 . Similar to the effect on A, at high VPD there were no statistically significant differences in isoprene emission rates between CO_2 treatments measured at both ambient and elevated $[\text{CO}_2]$ ($P > 0.05$ in all cases). When measured again at high VPD, but in limiting soil moisture conditions ($\theta_v = 0.11 \text{ m}^3 \text{ m}^{-3}$), compared with the previous measurement isoprene emission rates were reduced by approximately 35% in the ambient CO_2 treatment at both 430 and $1200 \mu\text{mol mol}^{-1}$ CO_2 measurement concentrations, and by approximately 57 and 24% in the elevated CO_2 treatment at 430 and $1200 \mu\text{mol mol}^{-1}$ CO_2 measurement concentrations, respectively.

Discussion

Isoprene emission is generally believed to decrease with increases in atmospheric $[\text{CO}_2]$ (e.g. Monson and Fall 1989; Guenther *et al.* 1991; Sharkey *et al.* 1991; Rosenstiel *et al.* 2003; Centritto *et al.* 2004; Rapparini *et al.* 2004). Our experiment with cottonwoods from an agriforest plantation inside the controlled environment research facility of the B2L, confirmed this negative response and also demonstrates that this inhibition may become a permanent feature in plants growing in a CO_2 -rich atmosphere. Rosenstiel *et al.* (2003) found that although isoprene emission is generally considered closely linked to photosynthesis, an increase in atmospheric $[\text{CO}_2]$ alters plant metabolism by stimulating CO_2 fixation and reducing the availability of cytosolic phosphoenolpyruvate (PEP), necessary for the synthesis in the chloroplast of dimethylallyl-diphosphate (DMAPP), the immediate precursor in isoprene biosynthesis. This

may help explain the contrasting response of assimilation and isoprene emission in plants acclimated to elevated atmospheric [CO₂]. As documented in previous field studies (Rey and Jarvis 1998), our results show that although *A* was stimulated in elevated CO₂, there was a permanent metabolic change as a result of plant acclimation leading to a reduced photosynthetic efficiency; plants grown at elevated [CO₂] always showed lower *A* than plants growing at ambient [CO₂] when measured at the same [CO₂]. This metabolic acclimation may include an alteration of the PEP partitioning in favour of the photosynthetic process, resulting in a permanent reduction in the necessary substrate for isoprene production. As our results indicate, isoprene emission also showed a permanent change in its response to elevated [CO₂]. When measured at the same [CO₂], the leaves of trees grown at 1200 μmol mol⁻¹ CO₂ always displayed reduced isoprene emission rates compared with leaves of trees grown at 430 μmol mol⁻¹ CO₂. This result supports our first hypothesis that when plants acclimate to growth in elevated [CO₂], inhibition of isoprene emission becomes a long-term feature.

However, for the first time it was demonstrated that high VPD can counteract the CO₂ effect by enhancing isoprene emission rates in ambient and elevated [CO₂]. It appears that the inhibitory effect of elevated [CO₂] on isoprene emission was compensated for by the stomatal closure as a result of the higher water vapour concentration gradient between the leaves and the atmosphere. The observed stimulation of isoprene emission was related to stomatal closure in the sense that this resulted in a decrease in *C*_i, which in turn would decrease the inhibitory effect of elevated CO₂ on isoprene emission (Monson and Fall 1989; Rosenstiel *et al.* 2003; Rapparini *et al.* 2004). Therefore, these results generally support our second hypothesis that under high VPD or water stress conditions, isoprene emission from plants may be stimulated as a consequence of the reduced CO₂ inhibition effect.

Although isoprene emission from leaves of large trees of *Populus deltoides* in B2L decreased when water stress was severe, it appeared to be considerably less sensitive to drought than *A* and *g*_s. These results are similar to those obtained from a previous study (Pegoraro *et al.* 2004) on potted plants of *Quercus virginiana* Mill. For relatively wet soil conditions, the isoprene emission rates measured in elevated [CO₂] were always lower than those measured at ambient [CO₂], but this difference tended to disappear with the progression of drought. There have been many studies on the response of tree physiology to elevated CO₂, soil moisture stress and high VPD, demonstrating that high VPD and low soil moisture tend to reduce *g*_s diminishing the effect of elevated CO₂ and decreasing *A* (e.g. Field *et al.* 1995; Curtis 1996; Will and Teskey 1997). In our experiment, the depression of *A* and the increase in isoprene emission caused by water stress and high VPD resulted in a major increase in carbon loss.

At the beginning of the experiment, when soil water content was not limiting, *C*_{iso}/*C*_A was similar to that observed in other studies (approximately 2%; Monson and Fall 1989; Sharkey *et al.* 1991; Harley *et al.* 1994; Baldocchi *et al.* 1995; Fang *et al.* 1996). As soil water content decreased, during the high VPD periods *C*_{iso}/*C*_A reached values that exceeded 10%. These values were similar to those observed for other species during drought, although carbon losses exceeding 50% have been observed when *A* is reduced to almost zero (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996; Harley *et al.* 1996; Pegoraro *et al.* 2004). *C*_{iso}/*C*_A values were higher for the ambient CO₂ treatment than for the elevated CO₂ treatment as a result of higher isoprene emission rates and the increase in the percentage of the carbon loss, mainly caused by major reductions of *A*.

Towards the end of the experiment, the declines in isoprene emission rates observed in all three CO₂ treatments were probably the result of the long period of depression of the photosynthetic process. Although most of the carbon in the isoprene molecule comes from recent photosynthates (Sharkey *et al.* 1991; Delwiche and Sharkey 1993; Karl *et al.* 2002), it has been shown that other sources can be used for leaf isoprene formation (Kreuzwieser *et al.* 2002; Schnitzler *et al.* 2004). Under stress conditions plants may use slow-turnover alternative carbon sources that increase their carbon contribution with increasing decline in *A* (Funk *et al.* 2004). However, when water stress is severe and causes prolonged depression of *A*, it is possible that the decline in isoprene emission reflects the depletion of these alternative carbon pools.

Populus deltoides, like almost all fast-growing tree species, emits large quantities of isoprene. Following the resolutions of the Kyoto protocol, forestation on large spatial scales with such fast-growing species has been encouraged as a way to fight the increase in atmospheric [CO₂]. However, continued expansion of plantations of high-isoprene emitting species can result in a significant influence on regional atmospheric chemistry, increasing ozone pollution, perturbing biogeochemical cycles and further contributing to global warming by enhancing the lifetime of methane, a powerful greenhouse gas (Rosenstiel *et al.* 2003). Although increased atmospheric [CO₂] enhances biomass production and reduces ecosystem isoprene emissions (Rosenstiel *et al.* 2003), more specific studies are needed to understand the exact contribution of the different environmental and metabolic control variables on isoprene emissions from different species. The results from the present study show that drought and high VPD have the opposite effect of elevated [CO₂], increasing isoprene emission and decreasing photosynthetic rates. Future climate scenarios suggest global increases in mean temperature and localised reduction in precipitation in many regions of the world (Houghton *et al.* 2001). As a result, future increases in global temperature, VPD and drought may shift this balance strongly in

favour of isoprene emission, in turn depressing biomass production.

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