

Isoprene emission from terrestrial ecosystems in response to global change: minding the gap between models and observations

BY RUSSELL K. MONSON^{1,2,*}, NICOLE TRAHAN^{1,2},
TODD N. ROSENSTIEL^{1,2,†}, PATRICK VERES^{2,3}, DAVID MOORE^{2,4},
MICHAEL WILKINSON¹, RICHARD J. NORBY⁵, ASTRID VOLDER⁶,
MARK G. TJOELKER⁷, DAVID D. BRISKE⁸, DAVID F. KARNOSKY⁹
AND RAY FALL^{2,3}

¹*Department of Ecology and Evolutionary Biology,* ²*Cooperative Institute for Research in Environmental Sciences, and* ³*Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, USA*

⁴*National Centre for Atmospheric Research, Boulder, CO 80301, USA*

⁵*Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA*

⁶*Department of Horticultural Sciences, and* ⁸*Rangeland Ecology and Management, Texas A&M University, College Station, TX 77840, USA*

⁷*Department of Forest Science, Texas A&M University, College Station, TX 77843, USA*

⁹*School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA*

Coupled surface–atmosphere models are being used with increased frequency to make predictions of tropospheric chemistry on a ‘future’ earth characterized by a warmer climate and elevated atmospheric CO₂ concentration. One of the key inputs to these models is the emission of isoprene from forest ecosystems. Most models in current use rely on a scheme by which global change is coupled to changes in terrestrial net primary productivity (NPP) which, in turn, is coupled to changes in the magnitude of isoprene emissions. In this study, we conducted measurements of isoprene emissions at three prominent global change experiments in the United States. Our results showed that growth in an atmosphere of elevated CO₂ inhibited the emission of isoprene at levels that completely compensate for possible increases in emission due to increases in aboveground NPP. Exposure to a prolonged drought caused leaves to increase their isoprene emissions despite reductions in photosynthesis, and presumably NPP. Thus, the current generation of models intended to predict the response of isoprene emission to future global change probably contain large errors. A framework is offered as a foundation for constructing new isoprene emission models based on the responses of leaf biochemistry to future climate change and elevated atmospheric CO₂ concentrations.

Keywords: volatile organic compound; hydrocarbon; 2-methyl-1,3-butadiene; air pollution; net primary productivity

* Author for correspondence (russell.monson@colorado.edu).

† Present address: Department of Biology, Portland State University, Portland, OR 97201, USA. One contribution of 18 to a Discussion Meeting Issue ‘Trace gas biogeochemistry and global change’.

1. Introduction

The emission of isoprene (2-methyl-1,3-butadiene) from terrestrial ecosystems provides one of the principal controls over oxidative photochemistry in the lower atmosphere, especially above continental regions (Fehsenfeld *et al.* 1992; Crutzen *et al.* 1999; Monson & Holland 2001; Monson 2002). Global isoprene emissions may be in the range 450–600 Tg C yr⁻¹ or approximately 40% of the global biogenic non-methane volatile organic compound (NMVOC) emissions (Guenther *et al.* 1995; Potter *et al.* 2001; Levis *et al.* 2003; Naik *et al.* 2004; Tao & Jain 2005; Guenther *et al.* 2006). Increases in isoprene emissions have occurred over the past few decades in certain geographical areas, while at the same time legislative actions have reduced anthropogenic NMVOC emissions (Chameides *et al.* 1988; Constable *et al.* 1999; Purves *et al.* 2004; Tao & Jain 2005). This has caused the production of isoprene by urban and suburban forests to dominate controls over local and regional air quality. The oxidation of isoprene in the troposphere occurs in a matter of hours and results in the production of a variety of more stable, but pernicious compounds, including ozone (O₃), organic nitrates and organic acids (Fehsenfeld *et al.* 1992; Fuentes *et al.* 2000). Globally, isoprene emissions from terrestrial ecosystems are thought to cause an increase in O₃ concentration, a decrease in hydroxyl radical (OH) concentration and an increase in the tropospheric lifetime of methane (CH₄) (Wang *et al.* 1998; Poisson *et al.* 2000; Roelofs & Lelieveld 2000). Thus, the biogenic emission of this one compound has far-reaching effects on air quality and global tropospheric chemistry.

Once in the atmosphere, isoprene and other reactive NMVOCs contribute to O₃ production or destruction, depending on the concentration of nitrogen oxides (NO_x). When NO is present at sufficiently high concentrations (more than 5–30 pptv), the oxidation of NMVOCs produces NO₂ and, following photolysis of NO₂, supports the net production of O₃. At low NO concentrations, isoprene has the potential to react directly with O₃ (Brasseur *et al.* 1999). The capacity for a mole of NO_x to catalyse the formation of O₃ in the presence of NMVOCs is called the ozone production efficiency (OPE). The emission of biogenic isoprene can increase the OPE (Lin *et al.* 1988). The presence of isoprene tends to reduce the concentration of tropospheric hydroxyl radical (OH) which slows the conversion of NO₂ to HNO₃ and enhances the photochemical efficiency of each mole of NO_x in producing O₃. The interactions between isoprene and NO_x in producing O₃ are complex, and highly dependent on the relative atmospheric concentrations of both. Whether NO_x or hydrocarbons ultimately limit the rate of O₃ production is strongly influenced by dynamics in the higher-order chemistry of NO_x compounds and their dependence on the oxidative relationship between NMVOCs and OH.

Interest has recently been expressed in estimating the effect of future global change scenarios on atmospheric chemistry, particularly those involving dynamics in tropospheric O₃ concentration (Brasseur *et al.* 1998; Johnson *et al.* 1999, 2001; Sanderson *et al.* 2003; Zeng & Pyle 2003; Fiore *et al.* 2005; Lathiere *et al.* 2005; Liao *et al.* 2006). These studies show that predictions of future changes in oxidative chemistry must be coupled to predictions of changes in the surface emissions of reactive biogenic hydrocarbons, including isoprene. Most global or regional models of present or future isoprene emissions are based on relationships among climate

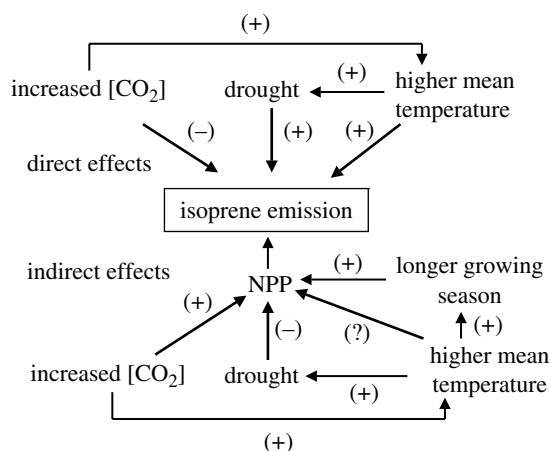


Figure 1. Conceptual model showing the direct and indirect effects of global changes in climate and atmospheric composition on isoprene emission rate. The indirect effects are transmitted through influences on NPP and are the typical effects used in current models of global isoprene emission. The direct effects are transmitted directly through influences on metabolism.

change, increases in atmospheric CO₂ concentration and changes in net primary productivity (NPP; e.g. Constable *et al.* 1999; Tao & Jain 2005; Liao *et al.* 2006). The fundamental logic of such models is that changes in NPP will produce more or less biomass capable of emitting isoprene, and changes in climate (principally temperature) will stimulate or inhibit emissions per unit of biomass. These models tend to ignore the discovery that there are direct effects of changes in the atmospheric CO₂ concentration on isoprene emission that tend to work in the opposite direction to that of stimulated NPP (Sanadze 1964; Monson & Fall 1989; Rosenstiel *et al.* 2003; Centritto *et al.* 2004; Rapparini *et al.* 2004; Scholefield *et al.* 2004; Possell *et al.* 2005; figure 1). Progress has been made in the past few years on the biochemical mechanisms underlying this direct response (Rosenstiel *et al.* 2003, 2004), and it is now possible to propose strategies for incorporating these effects into surface emission models. Furthermore, we now understand that most of the current generation of models use erroneous logic in assuming that the response of isoprene emission to temperature is based solely on the instantaneous effects of temperature on the enzyme isoprene synthase (e.g. the model originally proposed by Guenther *et al.* 1991, 1993). Several more recent studies have shown that the capacity for isoprene emission also responds to slower day-to-day changes in mean temperature, probably involving changes in the expression levels of isoprene synthase, not only its catalytic potential (Sharkey *et al.* 1999; Geron *et al.* 2000; Pétron *et al.* 2001; Funk *et al.* 2003). In the most recent global emissions model by Guenther *et al.* (2006), an effort has been made to modify the instantaneous algorithm to allow for some short-term acclimation of the key temperature-sensitive coefficients. While this recent effort represents a step towards providing some adjustments for recent weather conditions, it is accomplished by non-mechanistic ‘tuning’, rather than informed knowledge of underlying biochemical causes. Finally, we now understand that there are complex responses of isoprene emission to drought cycles, often involving lags in the responses between photosynthesis rate and isoprene emission rate, but typically involving an

eventual decrease in isoprene emissions as the stress becomes severe (Tingey *et al.* 1981; Sharkey & Loreto 1993; Fang *et al.* 1996; Bruggemann & Schnitzler 2002; Funk *et al.* 2005). The response to drought is generally missing from most of the current generation of emission models. One exception is the effort by Guenther *et al.* (2006) to define an isoprene emission factor that is sensitive to changes in soil water content. The latter effort, while important for recognizing the effects of water stress on isoprene emission rate, forces the emission rate factor downward by an empirically determined amount in response to declining soil water content; a response that, as we show in this paper, may not be universal.

In this paper, we describe recent measurements that we have made at the sites of three prominent global change experiments in the United States, all involving entire ecosystems in close-to-natural settings. We use these measurements to make the case that the magnitude of the direct effects of increased atmospheric CO₂ concentration, day-to-day changes in mean temperature and drought on the rate of forest isoprene emissions are probable to be as great as changes due to NPP and the response of isoprene synthase to instantaneous changes in temperature. Thus, the current generation of models that are founded on changes in these two factors alone are missing significant drivers of the response of isoprene emissions to future global changes. We also describe a conceptual model to explain the important biochemical controls on these direct effects in the hope that it will stimulate the development of new model schemes capable of quantifying isoprene emissions within the context of future global change.

2. Material and methods

(a) General approach

During the spring and summer of 2006, we conducted investigations at three prominent global change experimental sites in the USA: the warming and rainfall manipulation (WaRM) experiment in Texas; the Oak Ridge FACE experiment in Tennessee; and the Aspen FACE experiment in Wisconsin.

(i) The WaRM experiment

The WaRM experiment is located on a remnant post oak savannah site near Texas A&M University, College Station, Texas. This facility was constructed in 2003 to investigate the combined effects of altered precipitation distribution and warming on tree and grass dominant species of the southern oak savannah. The research infrastructure includes eight permanent 18×9×4.5 m (*L*×*W*×*H*) rainout shelters covered with clear polyethylene film. An overhead irrigation system in each shelter simulates a long-term ambient and redistributed (40% of summer rainfall redistributed to autumn and spring) precipitation regime. Two sets of five species combinations, post oak (*Quercus stellata* Wangenh.), a C₄ grass little bluestem (*Schizachyrium scoparium* (Michx.) Nash) and an invasive tree eastern red cedar (*Juniperus virginiana* L.), are grown in monoculture and tree–grass mixtures in 2×2 m plots beneath each of the rainout shelters. One set of plots in each shelter is warmed with overhead infrared lamps (100 W m⁻²) that increase canopy and soil (depth of 3 cm) temperature by approximately 1.5 and 0.6°C, respectively. We concentrated our measurements on post oak leaves (the only isoprene emitter in

the plots). No significant main effects of the various precipitation ($F=1.83$, $p=0.19$) and warming treatments ($F=0.07$, $p=0.79$) on oak isoprene emissions were found. Thus, we lumped oak trees from all treatments together and examined the potential for variation in isoprene emissions during pre- and post-watering periods, which provided an experiment on the effects of seasonal drought on isoprene emissions. We conducted the pre-watering measurements during 17–18 May 2006, which was 19–20 days since water had been added to the plots. We conducted the post-watering measurements on 19–20 May 2006, which was 1–2 days after 34 mm of water had been added to the plots (added on the evening of 18 May). In response to this watering event, soil water content increased from 11.4 to 16.9%, as measured with time domain reflectometry (TDR) integrated across the top 20 cm of the soil. The ambient temperature during the campaign in May 2006 ranged from 28 to 34°C. We repeated the measurements in August 2006, 3 days before and after a 19 mm rainfall event; in response to this latter watering event, soil water content increased from 7 to 13.2% in the upper 20 cm of the soil. The ambient temperature during the campaign in August 2006 ranged from 35 to 37°C. During both campaigns, days were generally cloud free. Both the May and August measurement campaigns occurred during the dry phase of the experimental redistribution treatment. This intensified an already severe summer drought by moving 40% of the May–September precipitation to the cooler months. Further details of the WaRM experiment can be found at <http://rangeland.tamu.edu/research/nigec/index.html>.

(ii) *The Oak Ridge FACE experiment*

The Oak Ridge free-air CO₂ enhancement (FACE) site is located on the Oak Ridge National Environmental Research Park in Tennessee and consists of five 25 m diameter plots which were established in 1997 using the design of [Hendrey et al. \(1999\)](#). The trees in each ring are sweetgum (*Liquidambar styraciflua* L.) and were planted in 1988. Since the trees were originally planted, natural understory growth from the surrounding oak-hickory forest has also become established in the plots. The atmospheric CO₂ concentration in each plot is maintained at ambient or elevated levels using 24 vent pipes and an automatic control system. The CO₂ treatments were initiated in May 1998. The set point for the elevated CO₂ plots was 565 ppmv during the day (there was no CO₂ supplementation during the night). The average daytime CO₂ concentration during the measurement campaign was 556 ppmv in the elevated CO₂ rings and 393 ppmv in the ambient CO₂ rings. We conducted measurements of isoprene emission at the Oak Ridge site during the period 10–23 June 2006. The maximum daily temperature during the campaign ranged from 28 to 32°C. Days were generally cloud free. The only measurable precipitation (0.5 cm) during the campaign fell on the evening of 19 June, halfway through the campaign. We noticed no obvious changes in the photosynthesis rate or isoprene emission rate of the leaves when comparing measurements before and after this small precipitation event. Further details of this site and the experimental treatments can be found at <http://face.ornl.gov>.

(iii) *The Aspen FACE experiment*

Full-season treatments at the Aspen FACE experiment were initiated in 1998 near Rhinelander, Wisconsin, and consist of twelve 30 m diameter treatment rings, including three ambient CO₂ (control) rings, three rings with elevated

CO₂, three rings with elevated O₃ and three rings with elevated CO₂ and O₃ together. The set point for the elevated CO₂ plots was 560 ppmv during the day (there was no CO₂ supplementation during the night). We concentrated our measurements on the ambient and elevated CO₂ rings. Details about the control and elevated CO₂ treatments are provided in Karnosky *et al.* (2003). The trees used in this experiment belong to five cloned lines of the species *Populus tremuloides* (Michx.) with known differences in O₃ tolerance. In this paper, we will not focus on differences in isoprene emission rate among clones, but rather on general responses of this species to elevated CO₂. We used all of the five clones in our sampling scheme and balanced the number of each clone used among the treatments so as not to skew the results from any single treatment due to favoured sampling of one clone over another. We conducted studies at the Aspen FACE site during 11–21 July 2006. The maximum daily temperature during the campaign ranged from 25 to 37°C. Days were generally cloud free. The only measurable precipitation (0.28 cm) during the campaign fell on the early morning of 14 July, 2 days into the campaign. We noticed no obvious changes in the photosynthesis rates or isoprene emission rates of the leaves following this small precipitation event. Further details of the Aspen FACE experiment can be found at <http://www.aspenface.mtu.edu/>.

(b) *Isoprene emission measurements*

Isoprene emission rate was measured on individual leaves using a portable gas-exchange system (LiCor, Inc., model 6400, Lincoln, Nebraska, USA) connected to a chemiluminescence continuous isoprene detector (Hills Scientific, model FIS, Boulder, Colorado, USA). In the studies in Texas and Wisconsin, we used leaves still attached to the trees. In the studies in Tennessee, we used leaves attached to branches (50–70 cm long) that had been cut from the trees using a pole pruner and immediately re-cut under water; the cut end of the branch was kept immersed in tap water during the gas-exchange measurements. We conducted initial experiments and determined that photosynthesis rates, stomatal conductance and isoprene emission rates were stable in leaves on the cut branches for at least 2 h after cutting. This was the approximate maximum time required to conduct the measurements of isoprene emission rate from the leaves. We also compared measurements of photosynthesis rate, isoprene emission rate and stomatal conductance on 10 leaves still attached to trees, to 10 leaves cut from the trees and found no significant differences. Branches used in our measurements were selected from the top 25% of the tree crowns in order to focus on leaves from sun-lit microenvironments. We restricted our measurements to the period that began 3 h after sunrise and ended 3 h before sunset in order to avoid the dynamic influences of early morning and late-day environments. Fortunately, during all four field campaigns, we were able to make our measurements during windows of local weather that were stable, warm and without significant rain; this allowed for rather stable physiological responses in the trees and good comparability among trees from different experimental treatments.

The gas-exchange measurements were made on leaves maintained at 30°C for the May Texas campaign, and 32°C for the remainder of the campaigns, with the incident photosynthetic photon flux density (PPFD) at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the chamber CO₂ concentration maintained at 400 ppmv, unless otherwise noted.

On some days, it was not possible to keep the leaf temperature within these set ranges due to hot weather. In those cases when the leaf temperature strayed more than 2°C from the set point, we recorded the actual leaf temperature and used a previously published model relating leaf temperature to isoprene emission rate (Guenther *et al.* 1993) to calculate the isoprene emission rate for the set-point temperature. In applying this model, we assumed $Q_{10}=2$ for the temperature dependence of isoprene emission rate. In practice, this temperature correction was only a problem for several days during the August Texas campaign and 2 days during the July Rhinelander campaign. The ambient air source that was delivered to the leaf chamber was taken from the outlet of a clean-air generator (Aadco, Inc., model 737). The chemiluminescence isoprene detector was calibrated several times each day using a standard gas cylinder containing 6.8 ppmv isoprene with the balance being high-purity synthesized air (79% N₂, 21% O₂). Calibration curves were conducted at five isoprene concentrations from 0 to 400 ppbv.

(c) Sampling design and statistical analysis

To evaluate the impact of precipitation, warming and seasonal drought treatments on isoprene emission rates in the Texas WaRM experiment, we measured leaves on 17 post oak trees during the pre-watering period and 30 trees during the post-watering period in May 2006; the trees were distributed in the 2×2 m plot treatments in four polyethylene plastic shelters. In August 2006, we conducted measurements on leaves from 60 trees both pre- and post-watering events in six polyethylene plastic shelters, also distributed throughout the plot treatments. We treated the shelters as the fundamental unit of replication, $n=4$ in May and $n=6$ in August. Treatment and seasonal effects were assessed via a mixed model four-way factorial ANOVA, using Tukey–Kramer *post hoc* tests ($\alpha=0.05$) to compare treatment means.

In order to assess the influence of growth at elevated CO₂ on leaf gas-exchange rates in sweetgum trees in the Oak Ridge FACE experiment, we conducted measurements on leaves on 4–8 branches in two of the control rings (rings 3 and 4) and two of the elevated CO₂ rings (rings 1 and 2) (branches were not accessible from the remaining experimental ring during our measurements). We averaged the measurements from each ring and treated the ring as the fundamental treatment unit (thus $n=2$ for the Oak Ridge analysis). In making observations on aspen trees in the Rhinelander FACE experiment, we used 4–6 trees in each ring and conducted measurements on three leaves from each tree. All leaves were averaged to provide a mean rate for isoprene emission, net photosynthesis or stomatal conductance (I_s , A or g_s , respectively) for each of the three rings for each treatment (thus $n=3$ for the Rhinelander analysis). In all analyses, means from each treatment were evaluated for differences using the Student's *t*-test with $p=0.05$ as the threshold of significance.

3. Results

Oak leaves from the various temperature and precipitation treatments of the WaRM experiment in Texas exhibited a slight increase in isoprene emission rate (I_s) when the atmospheric CO₂ concentration (and thus the intercellular CO₂

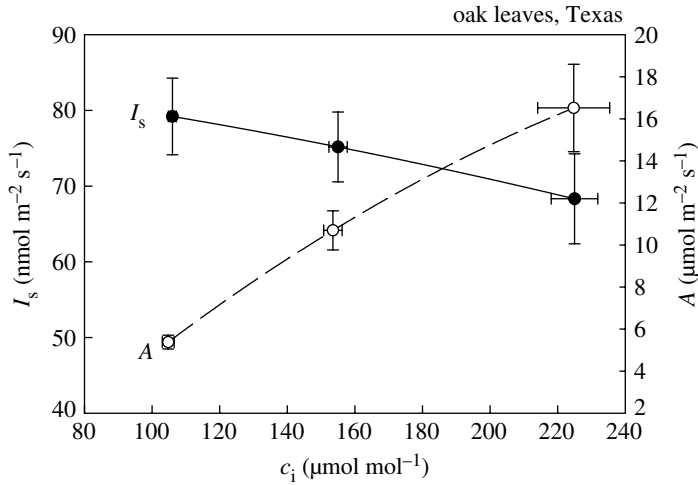


Figure 2. Leaf isoprene emission rate (I_s) and net CO_2 assimilation rate (A) as a function of instantaneous changes in intercellular CO_2 mole fraction (c_i) in oak leaves from the College Station, Texas, global change experiment. Values are mean \pm s.e. ($n=4$).

Table 1. Results of the four-way factorial, mixed model ANOVA intended to test for individual and interactive effects of date (isoprene emission rate in May versus August), water (isoprene emission rate prior to and following a watering event), precipitation (isoprene emission rate in control plots versus plots with redistributed seasonal rainfall) and temperature (isoprene emission rate in control plots versus plots with warming lamps).

effect	F -value	probability
date	289.4	<0.0001
water	9.91	0.0046
precipitation	1.83	0.19
date \times water	3.61	0.0704
date \times precipitation	7.94	0.0099
water \times precipitation	0.12	0.7293
date \times water \times precipitation	1.02	0.3232
temperature	0.07	0.7911
date \times temperature	0.15	0.6988
water \times temperature	0.05	0.8212
date \times water \times temperature	0.10	0.7571
precipitation \times temperature	0.10	0.7497
date \times precipitation \times temperature	1.30	0.2658
water \times precipitation \times temperature	0.23	0.6398
date \times water \times precipitation \times temperature	0.64	0.4321

concentration, c_i) was decreased instantaneously from ambient values (figure 2). Net CO_2 assimilation rates (A) decreased as c_i was decreased.

We observed significant main effects of date (May versus August) and time relative to a major watering event (pre- or post-watering) on I_s from post oak leaves in the WaRM experiment (table 1). We did not observe a main effect of treatment due to seasonal redistribution of precipitation, but we did observe an

Table 2. Summary of the significant date×precipitation and date×watering regime interactions. (Significance was determined by the Tukey–Kramer *post hoc t*-test.)

effect	date	pre- or post-watering event	precipitation treatment	I_s (nmol m ⁻² s ⁻¹)	s.e.	d.f.	<i>t</i> value	<i>p</i> value
date×water	Aug	post		14.1	3.60	17.8	3.91	0.001
date×water	Aug	pre		35.4	3.46	17.4	10.22	0.000
date×water	May	post		93.8	4.16	15.3	22.56	0.000
date×water	May	pre		99.1	5.37	37.7	18.44	0.000
date×precip	Aug		control	27.8	3.50	16.8	7.94	0.000
date×precip	Aug		redistributed	21.6	3.56	18.4	6.08	0.000
date×precip	May		control	87.7	4.73	25.2	18.54	0.000
date×precip	May		redistributed	105.3	4.88	26.4	21.57	0.000

interaction of this treatment effect with date. We did not observe a significant effect of the warming treatment on I_s . We also observed significant interactions in the date×water (pre- versus post-watering event) and date×precipitation (control versus seasonally redistributed precipitation) treatments (table 2). Thus, overall, we observed that I_s was higher in the plots with redistributed rainfall during May (plots with less summer rain had higher I_s), and there was a differential influence of a major watering event depending on whether it occurred in August or May (the decrease in I_s following a watering event was greater in August than in May). Rates of I_s were relatively high for the oak leaves, especially during May, when compared with the sweetgum and aspen leaves we measured later in the summer (see figures 4a and 5a, respectively).

We observed a significant increase in A during May in response to the relaxation of drought by the watering treatment ($p < 0.05$; figure 3). There was a slight upward trend in the mean c_i following relaxation of the drought in May, but this did not prove significant ($p > 0.05$). During August, we observed no significant change in A following relaxation of drought, despite seeing significant decreases in I_s and increases in c_i . The mean increase in c_i following relaxation of the drought was 13 $\mu\text{mol mol}^{-1}$.

We conducted measurements of I_s , A and g_s at both normal (400 ppmv) and elevated (600 ppmv) atmospheric CO₂ concentrations in both treatments for trees at the Oak Ridge FACE experiment. When assessed at normal ambient CO₂, leaves from trees in the elevated CO₂ rings had lower I_s when compared with leaves in the control rings ($p < 0.05$; figure 4). Rates for A and g_s were not significantly different in leaves from the two treatments. When assessed at elevated atmospheric CO₂, leaves from trees in the elevated CO₂ rings also exhibited lower mean I_s when compared with leaves from the control rings (23.2 and 32.1 nmol m⁻² s⁻¹, respectively), but the difference in these means was not quite statistically significant ($p = 0.069$; data not shown). As in the measurements at normal ambient CO₂, rates for A and g_s were also not significantly different in leaves from the two treatments when measured at elevated CO₂.

In measuring I_s for leaves of *P. tremuloides* at the Aspen FACE site, we only made measurements at the approximate atmospheric CO₂ concentration of the growth rings (e.g. 400 ppmv for leaves from the control rings and 550 ppmv for leaves from the elevated CO₂ rings). Leaves from the elevated CO₂ rings

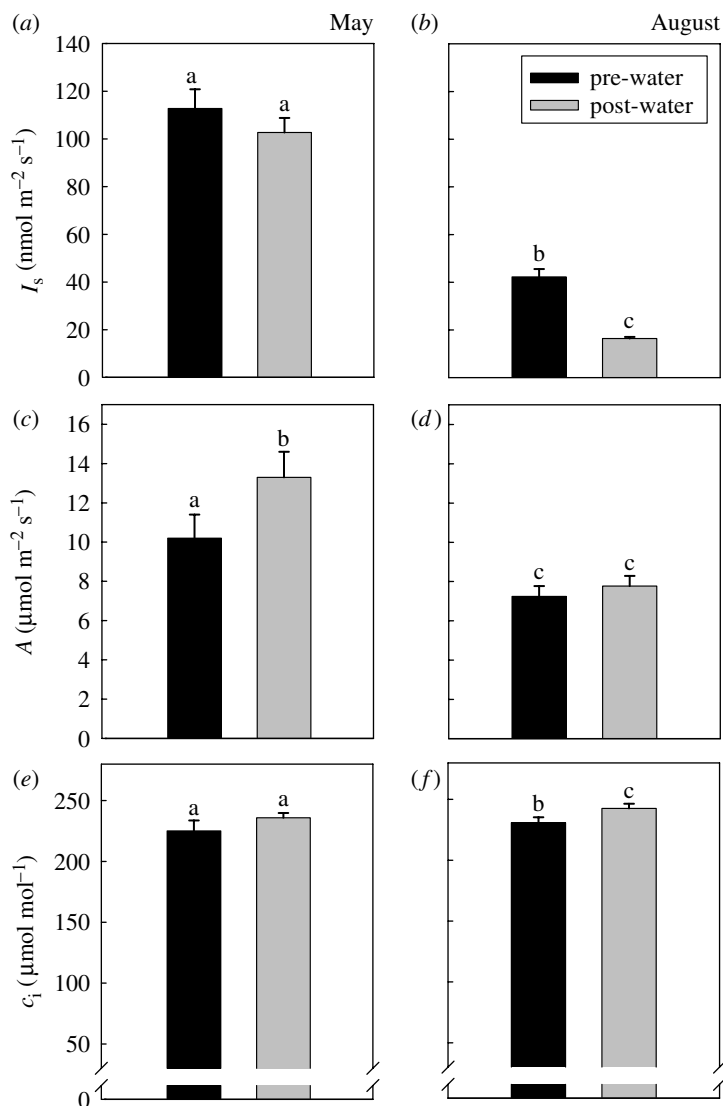


Figure 3. (a,b) Isoprene emission rate (I_s), (c,d) net CO₂ assimilation rate (A) and (e,f) intercellular CO₂ mole fraction (c_i) in leaves of oak from the WaRM experiment in College Station, Texas. (a,c,e) Before and after a major simulated precipitation event in mid-May 2006. (b,d,f) Before and after a major simulated precipitation event in mid-August 2006. Values marked by the same letter within a row of panels were not significantly different ($p > 0.05$); those marked by different letters were significantly different.

exhibited lower I_s when measured at 550 ppmv when compared with leaves from the control leaves measured at 400 ppmv ($p = 0.05$; figure 5). Mean rates of A tended on average to be higher for leaves grown and measured at elevated CO₂ concentrations, but the replicate measurements were highly variable, and the means proved not to be statistically different when formally tested ($p = 0.11$). Mean values of g_s were similar in magnitude and not statistically different in leaves from the control and elevated CO₂ treatments ($p > 0.05$).

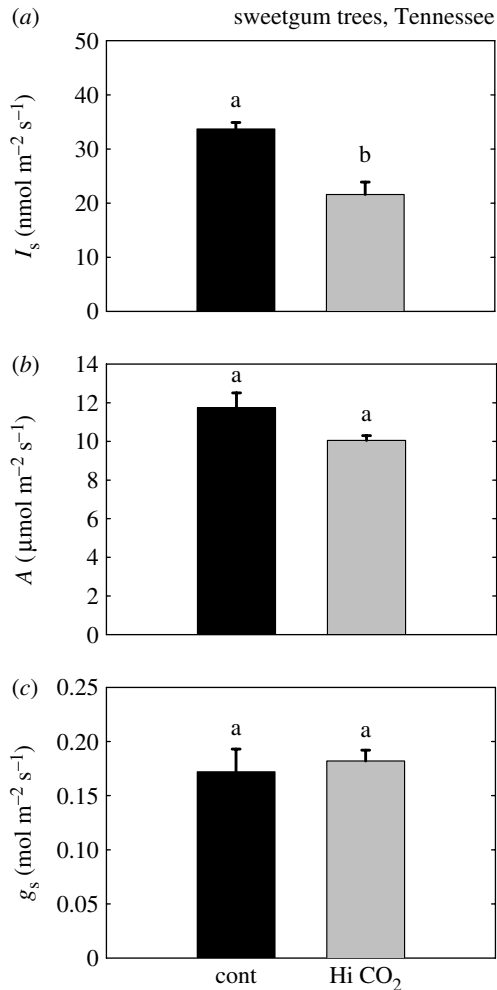


Figure 4. (a) Isoprene emission rate (I_s), (b) net CO₂ assimilation rate (A) and (c) stomatal conductance (g_s) in sweetgum trees growing in control rings (Cont) and elevated CO₂ rings (Hi CO₂) at the Oak Ridge FACE experiment in Tennessee. Measurements were made at the same CO₂ concentration (400 ppmv) for both treatments. Values marked by the same letter within a panel were not significantly different ($p > 0.05$); those marked by different letters were significantly different.

We were not able to make observations across a long enough time span to detect changes in I_s in response to changes in temperature due to weather fronts that moved through the study location, except for a short period of hot weather that developed in the middle of our observations at the Aspen FACE site. Here, the high temperature extremes only lasted for 2 days, and thus we were not able to validate our observations across several replicate hot and cold weather periods. However, we did observe that for trees in the control CO₂ rings, the mean I_s at a constant measurement leaf temperature of 32°C was higher ($32.4 \pm 1.0 \text{ nmol m}^{-2} \text{ s}^{-1}$, mean \pm s.e., $n=38$ leaves) during the 2 days of the hot weather (mean daily maximum temperature = $35.3 \pm 1.25^\circ\text{C}$, mean \pm s.e.) when compared with the mean I_s ($25.8 \pm 0.7 \text{ nmol m}^{-2} \text{ s}^{-1}$, mean \pm s.e., $n=53$ leaves) during the 3 days of

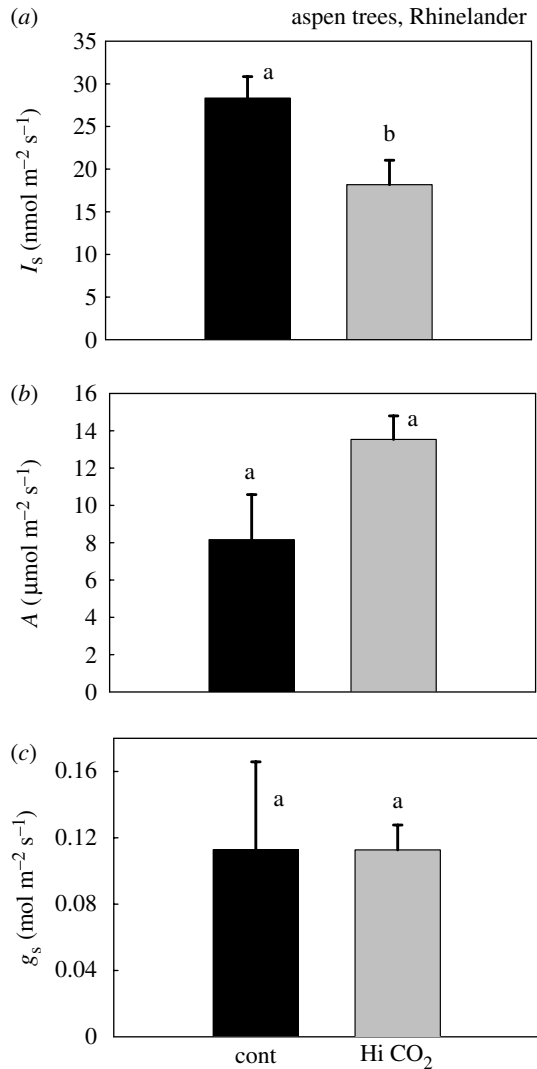


Figure 5. (a) Isoprene emission rate (I_s), (b) net CO₂ assimilation rate (A) and (c) stomatal conductance (g_s) in aspen trees growing in control rings (Cont) and elevated CO₂ rings (Hi CO₂) at the Aspen FACE experiment in Wisconsin. Values marked by the same letter within a panel were not significantly different ($p > 0.05$); those marked by different letters were significantly different.

cooler weather (mean daily maximum temperature = $28.7 \pm 0.2^\circ\text{C}$, mean \pm s.e.) (means different at $p < 0.05$). We did not have the same opportunity to observe a response to transient hot weather in trees in the elevated CO₂ rings.

4. Discussion

The isoprene emission rate (I_s) from terrestrial forest ecosystems in future climatic and atmospheric regimes will be determined by complex interactions among several driving variables (Monson *et al.* 1995; figure 1). Most researchers

who have made predictions of I_s in future global change scenarios relied upon indirect coupling between NPP and I_s , as altered by changes in the atmospheric CO_2 concentration or changes in precipitation, to guide their models (e.g. Constable *et al.* 1999; Naik *et al.* 2004; Tao & Jain 2005; Liao *et al.* 2006). Additionally, when climate warming is considered as a driver of future I_s , it is typically considered within the context of an Arrhenius-type model, which describes the instantaneous coupling of higher temperature to increased isoprene synthase catalysis rate (*sensu* Guenther *et al.* 1991, 1993, 1995). In this study, we focused on direct interactions between increased atmospheric CO_2 concentration and drought on I_s , and the influence of warmer temperatures produced by day-to-day changes in weather, rather than instantaneous second-to-second, or even hour-to-hour, increases in temperature, as are normally studied. Our intent was to fill in some of the observational gaps needed to produce a more complete modelling context for the response of I_s to future global change.

Our studies of oak trees growing in the WaRM experiment in Texas produced two novel results. First, I_s was higher during the spring than during the mid-summer. It is more typical for I_s to increase with temperature as the growing season progresses and the weather becomes warmer (e.g. Monson *et al.* 1994), the opposite pattern to what we observed. In fact, there is evidence that in past studies the seasonal increase in I_s is a direct response to the seasonal increase in temperature (Mayrhofer *et al.* 2005; Wiberley *et al.* 2005). It is not clear at the present time what the biochemical interactions are that control the response that we observed, but it is significant that we could not predict it based on our current understanding of seasonality in I_s . We do, however, point out that the local weather was relatively hot during both the May and August campaigns (32.4 ± 0.7 and $36.1 \pm 0.8^\circ\text{C}$, respectively) and it might be that the progressive drought over the summer caused a decline in I_s , masking any increase in I_s that might have been due to a small seasonal increase in the mean temperature. Second, we observed that drought during the middle of the summer caused a doubling in I_s , when compared with well-watered periods. Past studies have revealed that I_s is less sensitive to water stress than A , and that I_s tends to remain stable, or is modestly enhanced during periods of acute water stress (e.g. Fang *et al.* 1996; Bruggemann & Schnitzler 2002; Pegoraro *et al.* 2005, 2006). In one comprehensive analysis, drought was shown to have resulted in a significant decrease in I_s in a red oak forest in the northeastern United States (Funk *et al.* 2005). It is possible that the result we observed is similar to that of Funk *et al.* (2005), if indeed the decline in I_s in post oak from May to August was caused by chronic drought. However, we observed that relief from the drought in August caused a further decrease in I_s , not an increase; this was an unexpected result. The effect of reduced I_s following relief from drought could not be explained by the small increase in c_1 that we observed, as has been hypothesized in past studies of the effect of water stress on I_s (Pegoraro *et al.* 2004). The increase in c_1 that we observed would cause only small changes in I_s through the direct CO_2 response that has been described in past studies (figure 2). Rather, we hypothesize that there is an active upregulation of isoprene biosynthesis during periods of drought. Downregulation of I_s during the middle of the growing season, when days are hotter, and upregulation during drought are processes not currently included in models of the response of I_s to future global change. The magnitude of the changes observed in our study however—a twofold reduction through the growing season and another twofold reduction following significant precipitation—is high enough

to have significant ramifications for model predictions of the coupling between I_s and future climate changes. The general lack of correlation (in sign or magnitude) of the effects of drought on I_s versus the effects on photosynthesis rate (also see Funk *et al.* 2005) further amplifies the conclusion that sensitivity of NPP to climate may not be the best basis for describing sensitivity of I_s to climate.

Our observations in both a sweetgum forest in Tennessee and aspen stands in Wisconsin, revealed evidence of an active downregulation of I_s during growth in an atmosphere of increased CO_2 concentration. These results are among the first to show a consistent downregulation of I_s in response to growth at elevated CO_2 , and they emphasize that while many past studies show an instantaneous inhibition of elevated CO_2 on I_s when measured in a leaf cuvette, a response in the similar direction is evident on whole forest stands exposed to elevated CO_2 under natural field growth conditions. The response to growth CO_2 , however, is probably based on a mechanism that is different than that for instantaneous changes in the atmospheric CO_2 concentration; this is supported by the fact that we observed downregulation when leaves were measured at the same instantaneous CO_2 concentration in sweetgum trees. In a past study, Rosenstiel *et al.* (2003) showed that instantaneous exposure of poplar leaves to elevated CO_2 causes an inhibition of I_s , probably resulting from increased activities of the enzyme phosphoenolpyruvate carboxylase (PEPc), which shifts patterns of cytosolic and chloroplastic substrate use and limits the availability of pyruvate substrate for chloroplastic isoprene biosynthesis. It is possible that growth at elevated CO_2 causes an upregulation in expression of the PEPc gene and concomitant reduction in I_s , as has been observed when poplar trees are grown with NO_3^- as their only nitrogen source (Rosenstiel *et al.* 2004). Past studies have shown that mitochondrial densities increase when trees of several species (including sweetgum) are grown at elevated CO_2 (Griffin *et al.* 2001), and it is possible that higher mitochondrial densities are accompanied by increased expression of the PEPc gene; the PEPc enzyme is known to provide substrate to support mitochondrial respiration.

Increases in the mean global temperature, while moving in a direction that could cause increased isoprene emission, may not be as important as increases in the frequency of extremely hot days. Past studies have shown that I_s can be regulated up or down depending on recent day-to-day weather patterns (Sharkey *et al.* 1999; Geron *et al.* 2000; Pétron *et al.* 2001), and even within a single day (Geron *et al.* 2000; Mayrhofer *et al.* 2005). Although it is somewhat anecdotal, we observed a 26% increase in I_s during the 2 days of extremely hot weather that occurred during the Aspen FACE campaign. This increase is presumably due to an upregulation of the genes underlying the isoprene biosynthetic pathway.

Figure 6 provides a cellular and biochemical context for considering the effects of elevated CO_2 , drought and periods of hot weather on I_s . High growth [CO_2] is proposed to cause an upregulation in the expression levels of PEPc and mitochondrial density, both of which would increase the channelling of PEP to the production of oxaloacetate (OAA) and decrease the channelling of pyruvate to chloroplastic DMAPP production, and thus I_s . We leave open the possibility that high growth [CO_2] also causes the downregulation of expression of isoprene synthase. High instantaneous [CO_2] is proposed to cause an increase in the photosynthetic production of glyceraldehyde 3-phosphate (G3P), but a concomitant increase in the activity of PEPc, which would also inhibit isoprene

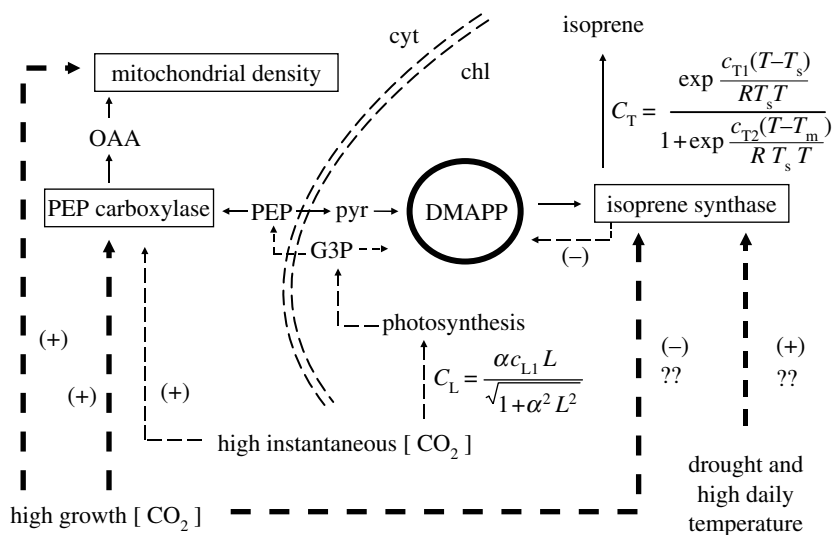


Figure 6. Proposed relationships between the environmental forcing variables of high growth [CO₂], high instantaneous [CO₂], high-temperature weather episodes and drought, and the metabolic processes and metabolite pools thought to influence isoprene emissions. The details of the model are discussed in the text. Abbreviations: PEP, phosphoenolpyruvate; pyr, pyruvate; G3P, glyceraldehyde 3-phosphate; cyt, cytosol; chl, chloroplast; and DMAPP, dimethylallyl diphosphate.

emission by decreasing the channelling of PEP to the chloroplastic and production of chloroplastic DMAPP. Drought and periods of hot weather are proposed to increase the expression of isoprene synthase, although this has not been clearly established. Also shown are the mathematical models from Guenther *et al.* (1991, 1993) which are commonly used to predict the responses of isoprene emission rate to instantaneous changes in temperature and light intensity.

Researchers who model the response of biogenic VOC emissions and their associated oxidative photochemistry to changes in climate and atmospheric CO₂ concentration have focused almost exclusively on influences coupled through NPP (Constable *et al.* 1999; Naik *et al.* 2004; Tao & Jain 2005; Liao *et al.* 2006). It is true that numerous modelling efforts have predicted changes in the amount and distribution of NPP during future global change (e.g. Cramer *et al.* 2001). However, models that reflect these effects as the only driving forces of future trends in tropospheric chemistry are missing a large part of the relevant dynamics. Even within the restricted context of the global change experiments we investigated, erroneous predictions are probable to emerge with sole reliance on the indirect effects of NPP. At the Oak Ridge FACE site, increases in forest NPP over the 8 years of the experiment to date have occurred through increases in fine root production, not the aboveground, isoprene-emitting shoot production (Norby *et al.* 2004). Thus, the only effect of increased atmospheric CO₂ on isoprene emission in this forest is probable to be the direct effects, transmitted through the influences of elevated CO₂ on leaf metabolism per unit leaf area and not through the indirect effects of increases in leaf area. The trees grown in elevated [CO₂] at the Aspen FACE site have exhibited an approximate 15% increase in leaf area index, when compared with trees in the control rings

(Karnosky *et al.* 2003). The inhibitory effect we observed due to the direct influence of elevated $[\text{CO}_2]$ on isoprene emission is larger in magnitude than the potential stimulation in isoprene emission predicted by the increase in leaf area index. The direct influences we observed in our experiments are large—often in the range of $2\times$ effects—and generally greater than the changes in aboveground NPP or increase in leaf area index. Without inclusion of these effects in the current array of models being used to predict changes in atmospheric chemistry due to global change, one has to question the relevance of the predictions themselves. Other challenges lay in the potential for individualistic responses among different tree species and forest types. The potential for species with different growth and biomass allocation strategies, and different tolerances of environmental stress, to respond to global change creates immense challenges in their own rights. To a large extent, the modelling has ‘raced ahead’ of our mechanistic understanding of how isoprene emissions will respond to the fundamental drivers of global change. As a result, our understanding of the fundamental mechanisms controlling the direct responses of I_s to global change needs be addressed to allow for the development of strategies and the inclusion of this knowledge in predictive models.

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References

- Brasseur, G. P., Kiehl, J. T., Mueller, J.-F., Schneider, T., Granier, C., Tie, X. X. & Hauglustaine, D. 1998 Past and future changes in global tropospheric ozone: impact on radiative forcing. *Geophys. Res. Lett.* **25**, 3807–3810. (doi:10.1029/1998GL900013)
- Brasseur, G. P., Orlando, J. J. & Tyndall, G. S. 1999 *Atmospheric chemistry and global change*, p. 654. New York, NY: Oxford University Press.
- Bruggemann, N. & Schnitzler, J.-P. 2002 Comparison of isoprene emission, intercellular isoprene concentration and photosynthetic performance in water-limited oak (*Quercus pubescens* Willd. and *Quercus robur* L.) saplings. *Plant Biol.* **4**, 456–463. (doi:10.1055/s-2002-34128)
- Centritto, M., Nascetti, P., Petrilli, L., Raschi, A. & Loreto, F. 2004 Profiles of isoprene emission and photosynthetic parameters in hybrid poplars exposed to free-air CO_2 enrichment. *Plant Cell Environ.* **27**, 403–412. (doi:10.1111/j.1365-3040.2003.01156.x)
- Chameides, W. L., Lindsay, R. W., Richardson, J. & Kiang, C. S. 1988 The role of biogenic hydrocarbons in urban photochemical smog—Atlanta as a case study. *Science* **241**, 1473–1475. (doi:10.1126/science.3420404)
- Constable, J. V. H., Guenther, A. B., Schimel, D. S. & Monson, R. K. 1999 Modelling changes in VOC emission in response to climate change in the continental United States. *Global Change Biol.* **5**, 791–806. (doi:10.1046/j.1365-2486.1999.00273.x)
- Cramer, W. *et al.* 2001 Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. *Global Change Biol.* **4**, 357–373. (doi:10.1046/j.1365-2486.2001.00383.x)
- Crutzen, P. J., Lawrence, M. G. & Poschl, U. 1999 On the background photochemistry of tropospheric ozone. *Tellus A* **51**, 123–146. (doi:10.1034/j.1600-0870.1999.t01-1-00010.x)

- Fang, C., Monson, R. & Cowling, E. 1996 Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long-term drying cycles. *Tree Physiol.* **16**, 441–446.
- Fehsenfeld, F. C. *et al.* 1992 Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochem. Cycles* **6**, 389–430.
- Fiore, A. M., Horowitz, L. W., Purves, D. W., Levy, H., Evans, M. J., Wang, Y. X., Li, Q. B. & Yantosca, R. M. 2005 Evaluating the contribution of changes in isoprene emissions to surface ozone trends over the eastern United States. *J. Geophys. Res.* **110**, D12 303. (doi:10.1029/2004JD005485)
- Fuentes, J. D. *et al.* 2000 Biogenic hydrocarbons in the atmospheric boundary layer: a review. *Bull. Am. Meteorol. Soc.* **81**, 1537–1576. (doi:10.1175/1520-0477(2000)081<1537:BHITAB>2.3.CO;2)
- Funk, J. L., Jones, C. G., Baker, C. J., Fuller, H. M., Giardina, C. P. & Lerdau, M. T. 2003 Diurnal variation in the basal emission rate of isoprene. *Ecol. Appl.* **13**, 269–276.
- Funk, J. L., Jones, C. G., Gray, D. W., Throop, H. L., Hyatt, L. A. & Lerdau, M. T. 2005 Variation in isoprene emission from *Quercus rubra*: sources, causes, and consequences for estimating fluxes. *J. Geophys. Res.* **110**, D04 301. (doi:10.1029/2004JD005229)
- Geron, C., Guenther, A., Sharkey, T. & Arnts, R. R. 2000 Temporal variability in basal isoprene emission factor. *Tree Physiol.* **20**, 799–805.
- Griffin, K. L. *et al.* 2001 Plant growth in elevated CO₂ alters mitochondrial number and chloroplast fine structure. *Proc. Natl Acad. Sci. USA* **98**, 2473–2478. (doi:10.1073/pnas.041620898)
- Guenther, A. B., Monson, R. K. & Fall, R. 1991 Isoprene and monoterpene emission rate variability: observations with eucalyptus and emission rate algorithm development. *J. Geophys. Res.* **96**, 10 799–10 808.
- Guenther, A. B., Zimmerman, P. R., Harley, P. C., Monson, R. K. & Fall, R. 1993 Isoprene and monoterpene emission rate variability—model evaluations and sensitivity analyses. *J. Geophys. Res. Atmos.* **98**, 12 609–12 617.
- Guenther, A. *et al.* 1995 Global-model of natural volatile organic-compound emissions. *J. Geophys. Res. Atmos.* **100**, 8873–8892. (doi:10.1029/94JD02950)
- Guenther, A., Karl, T., Harley, P., Wiedinmyer, C., Palmer, P. I. & Geron, C. 2006 Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmos. Chem. Phys.* **6**, 3181–3210.
- Hendrey, G. R., Ellsworth, D. S., Lewin, K. F. & Nagy, J. 1999 A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂. *Global Change Biol.* **5**, 293–309. (doi:10.1046/j.1365-2486.1999.00228.x)
- Johnson, C. E., Collins, W. J., Stevenson, D. S. & Derwent, R. G. 1999 Relative roles of climate and emissions changes on future tropospheric oxidant concentrations. *J. Geophys. Res.* **104**, 18 631–18 645. (doi:10.1029/1999JD900204)
- Johnson, C. E., Stevenson, D. S., Collins, W. J. & Derwent, R. G. 2001 Role of climate feedback on methane and ozone studied with a coupled ocean–atmosphere–chemistry model. *Geophys. Res. Lett.* **28**, 1723–1726. (doi:10.1029/2000GL011996)
- Karnosky, D. F. *et al.* 2003 Tropospheric O₃ moderates responses of temperate hardwood forests to elevated CO₂: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Funct. Ecol.* **17**, 289–304. (doi:10.1046/j.1365-2435.2003.00733.x)
- Lathiere, J., Hauglustaine, D. A., De Noblet-Ducoudre, N., Krinner, G. & Folberth, G. A. 2005 Past and future changes in biogenic volatile organic compound emissions simulated with a global dynamic vegetation model. *Geophys. Res. Lett.* **32**, L20 818. (doi:10.1029/2005GL024164)
- Levis, S., Wiedinmyer, C., Bonan, G. B. & Guenther, A. 2003 Simulating biogenic volatile organic compound emissions in the community climate system model. *J. Geophys. Res.* **108**, D4 659. (doi:10.1029/2002JD003203)
- Liao, H., Chen, W. T. & Seinfeld, J. H. 2006 Role of climate change in global predictions of future tropospheric ozone and aerosols. *J. Geophys. Res.* **111**, D12 304. (doi:10.1029/2005JD006852)

- Lin, X., Trainer, M. & Liu, S. C. 1988 On the nonlinearity of the tropospheric ozone production. *J. Geophys. Res.* **93**, 15 879–15 888.
- Mayrhofer, S., Teuber, M., Zimmer, I., Louis, S., Fischbach, R. J. & Schnitzler, R. P. 2005 Diurnal and seasonal variation of isoprene biosynthesis-related genes in Grey poplar leaves. *Plant Physiol.* **139**, 474–484. (doi:10.1104/pp.105.066373)
- Monson, R. K. 2002 Volatile organic compound emissions from terrestrial ecosystems: a primary biological control over atmospheric chemistry. *Israel J. Chem.* **42**, 29–42. (doi:10.1560/OJJC-XQAA-JX0G-FXJG)
- Monson, R. K. & Fall, R. 1989 Isoprene emission from aspen leaves—influence of environment and relation to photosynthesis and photorespiration. *Plant Physiol.* **90**, 267–274.
- Monson, R. K. & Holland, E. A. 2001 Biospheric trace gas fluxes and their control over tropospheric chemistry. *Annu. Rev. Ecol Syst.* **32**, 547–576. (doi:10.1146/annurev.ecolsys.32.081501.114136)
- Monson, R. K., Harley, P. C., Litvak, M. E., Wildermuth, M., Guenther, A. B., Zimmerman, P. R. & Fall, R. 1994 Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. *Oecologia* **99**, 260–270. (doi:10.1007/BF00627738)
- Monson, R. K., Lerdau, M. T., Sharkey, T. D., Schimel, D. S. & Fall, R. 1995 Biological aspects of constructing volatile organic compound emission inventories. *Atmos. Environ.* **29**, 2989–3002. (doi:10.1016/1352-2310(94)00360-W)
- Naik, V., Delire, C. & Wuebbles, D. J. 2004 Sensitivity of global biogenic isoprenoid emissions to climate variability and atmospheric CO₂. *J. Geophys. Res.* **109**, D06 301. (doi:10.1029/2003JD004236)
- Norby, R. J., Ledford, J., Reilly, C. D., Miller, N. E. & O'Neill, E. G. 2004 Fine-root production dominates response of a deciduous forest to atmospheric CO₂ enrichment. *Proc. Natl Acad. Sci. USA* **101**, 9689–9693. (doi:10.1073/pnas.0403491101)
- Pegoraro, E., Rey, A., Bobich, E. G., Barron-Gafford, G., Grieve, K. A., Malhi, Y. & Murthy, R. 2004 Effect of elevated CO₂ concentration and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought. *Funct. Plant Biol.* **31**, 1137–1147. (doi:10.1071/FP04142)
- Pegoraro, E., Rey, A., Barron-Gafford, G., Monson, R., Malhi, Y. & Murthy, R. 2005 The interacting effects of elevated atmospheric CO₂ concentration, drought and leaf-to-air vapour pressure deficit on ecosystem isoprene fluxes. *Oecologia* **146**, 120–129. (doi:10.1007/s00442-005-0166-5)
- Pegoraro, E., Rey, A., Abrell, L., Vanharen, J. & Lin, G. H. 2006 Drought effect on isoprene production and consumption in biosphere 2 tropical rainforest. *Global Change Biol.* **12**, 456–469. (doi:10.1111/j.1365-2486.2006.01112.x)
- Pétron, G., Harley, P., Greenberg, J. & Guenther, A. 2001 Seasonal temperature variations influence isoprene emission. *Geophys. Res. Lett.* **28**, 1707–1710. (doi:10.1029/2000GL011583)
- Poisson, N., Kanakidou, M. & Crutzen, P. J. 2000 Impact of non-methane hydrocarbons on tropospheric chemistry and the oxidizing power of the global troposphere: three-dimensional modelling results. *J. Atmos. Chem.* **36**, 157–230. (doi:10.1023/A:1006300616544)
- Possell, M., Hewitt, C. N. & Beerling, D. J. 2005 The effects of glacial atmospheric CO₂ concentrations and climate on isoprene emission by vascular plants. *Global Change Biol.* **11**, 60–69. (doi:10.1111/j.1365-2486.2004.00889.x)
- Potter, C. S., Alexander, S. E., Coughlan, J. C. & Klooster, S. A. 2001 Modeling biogenic emissions of isoprene: exploration of model drivers, climate control algorithms, and use of global satellite observations. *Atmos. Environ.* **35**, 6151–6165. (doi:10.1016/S1352-2310(01)00390-9)
- Purves, D. W., Caspersen, J. P., Moorcroft, P. R., Hurtt, G. C. & Pacala, S. W. 2004 Human-induced changes in US biogenic volatile organic compound emissions: evidence from long-term forest inventory data. *Global Change Biol.* **10**, 1737–1755. (doi:10.1111/j.1365-2486.2004.00844.x)
- Rapparini, F., Baraldi, R., Miglietta, F. & Loreto, F. 2004 Isoprenoid emission in trees of *Quercus pubescens* and *Quercus ilex* with lifetime exposure to naturally high CO₂ environment. *Plant Cell Environ.* **27**, 381–391. (doi:10.1111/j.1365-3040.2003.01151.x)

- Roelofs, G.-J. & Lelieveld, J. 2000 Tropospheric ozone simulation with a chemistry–general circulation model: influence of higher hydrocarbon chemistry. *J. Geophys. Res.* **105**, 22 697–23 012. (doi:10.1029/2000JD900316)
- Rosenstiel, T. N., Potosnak, M. J., Griffin, K. L., Fall, R. & Monson, R. K. 2003 Increased CO₂ uncouples growth from isoprene emission in an agriforest ecosystem. *Nature* **421**, 256–259. (doi:10.1038/nature01312)
- Rosenstiel, T. N., Ebbets, A. L., Khatri, W. C., Fall, R. & Monson, R. K. 2004 Induction of poplar leaf nitrate reductase: a test of extrachloroplastic control of isoprene emission rate. *Plant Biol.* **6**, 12–21. (doi:10.1055/s-2003-44722)
- Sanadze, G. A. 1964 Light-dependent excretion of isoprene by plants. *Photosynth. Res.* **2**, 701–707.
- Sanderson, M. G., Jones, C. D., Collins, W. J., Johnson, C. E. & Derwent, R. G. 2003 Effect of climate change on isoprene emissions and surface ozone levels. *Geophys. Res. Lett.* **30**, 18. (doi:10.1029/2003GL017642)
- Scholefield, P. A., Doick, K. J., Herbert, B. M. J., Hewitt, C. N. S., Schnitzler, J. P., Pinelli, P. & Loreto, F. 2004 Impact of rising CO₂ on emissions of volatile organic compounds: isoprene emission from *Phragmites australis* growing at elevated CO₂ in a natural carbon dioxide spring. *Plant Cell Environ.* **27**, 393–401. (doi:10.1111/j.1365-3040.2003.01155.x)
- Sharkey, T. D. & Loreto, F. 1993 Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia* **95**, 328–333. (doi:10.1007/BF00320984)
- Sharkey, T., Singaas, E., Lerdau, M. & Geron, C. 1999 Weather effects on isoprene emission capacity and applications in emissions algorithms. *Ecol. Appl.* **9**, 1132–1137. (doi:10.2307/2641383)
- Tao, Z. & Jain, A. K. 2005 Modeling of global biogenic emissions for key indirect greenhouse gases and their response to atmospheric CO₂ increases and changes in land cover and climate. *J. Geophys. Res.* **110**, D21 309. (doi:10.1029/2005JD005874)
- Tingey, D. T., Evans, R. & Gumpertz, M. 1981 Effects of environmental conditions on isoprene emission from live oak. *Planta* **152**, 565–570. (doi:10.1007/BF00380829)
- Wang, Y. H., Jacob, D. J. & Logan, J. A. 1998 Global simulation of tropospheric O₃–NO_x–hydrocarbon chemistry. 3. Origin of tropospheric ozone and effects of non-methane hydrocarbons. *J. Geophys. Res.* **103**, 10 757–10 767. (doi:10.1029/98JD00156)
- Wiberley, A. E., Linskey, A. R., Falbel, T. G. & Sharkey, T. D. 2005 Development of the capacity for isoprene emission in kudzu. *Plant Cell Environ.* **28**, 898–905. (doi:10.1111/j.1365-3040.2005.01342.x)
- Zeng, G. & Pyle, J. A. 2003 Changes in tropospheric ozone between 2000 and 2100 modeled in a chemistry–climate model. *Geophys. Res. Lett.* **30**, 1392. (doi:10.1029/2002GL016708)