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Leaf isoprene emission rate as a function of atmospheric CO_2 concentration

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Abstract

There is considerable interest in modeling isoprene emissions from terrestrial vegetation, because these emissions exert a principal control over the oxidative capacity of the troposphere. We used a unique field experiment that employs a continuous gradient in CO_2 concentration from 240 to 520 ppmv to demonstrate that isoprene emissions in *Eucalyptus globulus* were enhanced at the lowest CO_2 concentration, which was similar to the estimated CO_2 concentrations during the last Glacial Maximum, compared with 380 ppmv, the current CO_2 concentration. Leaves of *Liquidambar styraciflua* did not show an increase in isoprene emission at the lowest CO_2 concentration. However, isoprene emission rates from both species were lower for trees grown at 520 ppmv CO_2 compared with trees grown at 380 ppmv CO_2. When grown in environmentally controlled chambers, trees of *Populus deltoides* and *Populus tremuloides* exhibited a 30–40% reduction in isoprene emission rate when grown at 800 ppmv CO_2, compared with 400 ppmv CO_2. *P. tremuloides* exhibited a 33% reduction when grown at 1200 ppmv CO_2, compared with 600 ppmv CO_2. We used current models of leaf isoprene emission to demonstrate that significant errors occur if the CO_2 inhibition of isoprene is not taken into account. In order to alleviate these errors, we present a new model of isoprene emission that describes its response to changes in atmospheric CO_2 concentration. The model logic is based on assumed competition between cytosolic and chloroplastic processes for pyruvate, one of the principal substrates of isoprene biosynthesis.

Keywords: atmospheric chemistry, CH_4, climate change, forests, global change, O_3

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Introduction

Isoprene (C_5H_8, 2-methyl-1,3-butadiene) is the base unit of the isoprenoid family of organic chemical compounds (e.g., monoterpenes and carotenoids) and is emitted to the atmosphere from a variety of both natural and anthropogenic sources (Harley *et al*., 1999; Monson & Holland, 2001; Lerdau & Gray, 2003). At the global scale, biogenic emissions of isoprene dominate the flux of biogenic volatile organic compounds (BVOCs) from vegetation to the atmosphere, and are arguably the dominant control over tropospheric photochemistry above continental regions (Fehsenfeld *et al*., 1992; Monson, 2002). Global isoprene emissions account for an estimated 500 Tg C yr^{-1}, $\sim$ 45% of the total flux of BVOC (Guenther *et al*., 1995; Potter *et al*., 2001; Shim *et al*., 2005). The emission of isoprene affects the regional and global tropospheric hydroxyl radical (OH) concentration (Fehsenfeld *et al*., 1992), has the potential to modify regional ozone pollution (Pierce *et al*., 1998), and can lead to secondary aerosol formation (Claeys *et al*., 2004). Hence, accurate estimates for the regional and global flux of isoprene from forest ecosystems are

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required to understand controls over the earth's albedo, and because of its interactions with OH, controls over the lifetime of the important climate greenhouse gas CH_4 (Houweling *et al*., 1998; Poisson *et al*., 2000; Valdes *et al*., 2005).

Biogenic emissions of isoprene were first reported by Sanadze (1957), who later showed that isoprene emissions are sensitive to changes in photosynthetic photon flux density (PPFD) and atmospheric CO_2 concentration ($[CO_2]$) (Sanadze, 1964). Subsequent studies revealed that a tight connection exists between isoprene emissions and photosynthetic CO_2 assimilation (Tingey *et al*., 1981; Monson & Fall, 1989; Loreto & Sharkey, 1990, 1993; Delwiche & Sharkey, 1993). This metabolic connection was clarified following the discovery that photosynthetically produced glyceraldehyde 3-phosphate (G3P) is required in the chloroplastic methyl erythritol 4-phosphate (MEP) pathway, which is responsible for biogenic isoprene production (Lichtenthaler *et al*., 1997). The dependence of isoprene biosynthesis on photosynthetic G3P, however, cannot explain the response of isoprene emission rate (I_s) to changes in atmospheric $[CO_2]$, which tends to vary inversely with $[CO_2]$; opposite to that observed for photosynthetic CO_2 assimilation rate (Sanadze, 1964; Monson & Fall, 1989; Loreto & Sharkey, 1990). Evidence has accumulated recently that the CO_2 response of I_s is due to changes in the partitioning of phosphoenol pyruvate (PEP) between the cytosol and chloroplast, which in turn influences the availability of the second initial substrate in the MEP pathway, pyruvate (Rosenstiel *et al*., 2003). The CO_2-dependence of I_s has implications for both the response of emissions to future changes in the atmospheric $[CO_2]$, and the response of leaf-level emissions to changes in the intercellular $[CO_2]$ (C_i) that might occur during drought, changes in incident PPFD, the leaf-to-air water vapor pressure deficit, and temperature.

Recently, the results of several modeling studies suggest that an increase in the flux of isoprene from the biosphere can be expected due to increases in net primary productivity (NPP) and global temperatures (Brasseur *et al*., 1998; Johnson *et al*., 1999, 2001; Zeng & Pyle, 2003; Lathiere *et al*., 2005; Tao & Jain, 2005; Liao *et al*., 2006). However, these models do not account for the decrease in I_s with increasing atmospheric $[CO_2]$ (Sanadze, 1964; Monson & Fall, 1989; Rosenstiel *et al*., 2003; Centritto *et al*., 2004; Scholefield *et al*., 2004; Possell *et al*., 2005), a feedback that works in the opposite direction to the positive effects of temperature and NPP under future global change scenarios (Monson *et al*., 2007). To our knowledge, there has only been one study that attempts to quantify the effects of $[CO_2]$ on I_s with a view to improving predictive models (Possell *et al*., 2005), and only two that have tried to introduce the CO_2-effect into global scale models of I_s in a projected future atmosphere (Arneth *et al*., 2007, 2008). The inclusion of the CO_2 response of I_s in the current generation of atmospheric chemistry models has been hampered by two important factors: (1) lack of knowledge about the influences of changes in atmospheric $[CO_2]$ (C_a) over longer time scales vs. changes in C_i over shorter time scales, and (2) lack of a quantitative framework to describe leaf-level responses of I_s to long-term and short-term CO_2 influences. Therefore, to improve our understanding in these areas, we investigated both long-term and short-term effects of different CO_2 concentrations on I_s using leaves from aspen (*Populus tremuloides*), cottonwood (*Populus deltoides*), sweetgum (*Liquidambar styraciflua*), and eucalyptus (*Eucalyptus globulus*) trees. We used these results to develop a quantitative algorithm that can be used to scale I_s at the leaf level to changes in atmospheric and intercellular $[CO_2]$, and can be incorporated into larger-scale models that aim to predict regional or global patterns in I_s.

Materials and methods

Growth of trees in controlled-environment chambers

Twenty-four 3-year-old cottonwood trees were grown in controlled-environment (light, temperature and $[CO_2]$) growth chambers (Conviron, model PGR 15, Winnipeg, Canada) at the University of Colorado. Twelve trees were placed in an 'ambient' CO_2 chamber (400 ± 10 ppmv) and 12 trees were placed in an 'elevated' CO_2 chamber (800 ± 15 ppmv) for 8 weeks. All trees were defoliated before being placed in the chambers so that new leaves developed in the treatment $[CO_2]$. The trees were moved within the chambers weekly to minimize the effects of within chamber heterogeneity. The trees were grown in 10 L pots containing commercial potting soil, and fertilized regularly with half-strength Scotts' solution (21 : 18 : 18; Scotts-Sierra Horticultural Products Company, Maryville, OH, USA). The photoperiod was 14 h with PPFD of $700\ \mu mol\ m^{-2}\ s^{-1}$ measured approximately 30 cm from the lamps, which represents a distance approximately 25% through the vertical length of average tree crown. Leaf gas exchange measurements were made on leaves that were growing within this region of the crown. Day/night air temperatures were kept at 25/15 °C.

In a second experiment, 24 2-year-old aspen trees were grown in the chambers at different atmospheric $[CO_2]$ using the same PPFD and temperature regimes described above. We conducted the studies with aspen over two growth cycles, one in which we grew the trees at 400 or 800 ppmv CO_2, and one in which we grew

them at 600 or 1200 ppmv CO_2. The same trees were used in the two cycles, with the 400/800 ppmv experiment being conducted first. Between cycles, the trees were defoliated, and the trees from the 400 ppmv treatment were allowed to regrow at 600 ppmv CO_2; those from the 800 ppmv experiment were allowed to regrow at 1200 ppmv CO_2. The trees were allowed to grow for 8 weeks in each treatment before new measurements were made. At the end of the second experiment, we defoliated all trees once again, and returned five of the trees from each treatment to 400 ppmv for 8 weeks of regrowth. In this third experiment, we noted no significant difference in I_s between those trees that had been previously grown at the high CO_2 treatments (800 and 1200 ppmv) and those that had been grown at the low CO_2 treatments (400 and 600 ppmv), indicating a lack of significant treatment carry-over effects from one experiment to the next ($n = 5$; $P = 0.4$). However, there was a significant difference between I_s at 400 ppmv CO_2 in the first experiment and I_s at 400 ppmv CO_2 in the third experiment ($P < 0.05$), indicating that the results were affected by time during the experiment. The exact cause of this time-dependent effect was not analyzed further. However, because of its existence, we analyzed results between the two CO_2 treatments within each experiment separately, but did not compare the results of the first experiment (400 and 800 ppmv CO_2) directly with those of the second experiment (600 and 1200 ppmv CO_2).

Lysimeter CO_2 Gradient

The Lysimeter CO_2 Gradient (LYCOG; Temple, TX, USA) is a unique controlled-environment facility for exposing plants to a continuous gradient in atmospheric [CO_2]. The facility consists of two tunnel-shaped chambers aligned parallel to each other. Each chamber consists of 10 compartments aligned in series. Pure CO_2 is added to air entering the superambient chamber to elevate the initial [CO_2] to $\sim$ 520 ppmv. Ambient air ($\sim$ 380 ppmv) is introduced to the entrance of the subambient chamber. Photosynthesis by the enclosed vegetation depletes CO_2 in the air supply such that by the end of the tunnels, the [CO_2] is approximately ambient at the end of the superambient chamber and approximately 240 ppmv at the end of the subambient chamber. The CO_2 gradient within the chambers is maintained by automatic adjustment of the rate of air flow through each chamber compartment. The chambers transmitted approximately 90% of the incident PPFD. For a more complete description of the LYCOG site and apparatus, see Polley *et al*. (2008).

Nine 2-year-old sweetgum trees and nine 2-year-old eucalyptus trees were defoliated and placed in the LYCOG chambers in early June 2007. Three trees of each species were placed in high [CO_2] in the superambient chamber. Six trees of each species were also placed in the subambient chamber, three trees in the $\sim$ 380 ppmv [CO_2] compartment and three trees in the $\sim$ 240 ppmv [CO_2] compartment. The trees were watered regularly and new leaves were allowed to develop for 8 weeks in their respective [CO_2] before leaf gas exchange measurements were carried out. During the 8-week growth period, better than 80% of the daytime 20-min readings of [CO_2] in air exiting the chambers fell within $\pm$ 25 ppmv of the desired CO_2 set points.

Gas exchange measurements

Measurements of leaf-level gas exchange (including I_s) to changes in C_i were made during August 2007 after trees had been individually removed from each chamber and measured over the subsequent 4 h. All measurements were conducted according to the protocols described below during the period of the day between 09:00 and 160:0 hours to avoid the dynamic influences of early morning and late day environments on I_s. Additional measurements were conducted between dawn and dusk to determine the effect of different [CO_2] on model estimates of the diurnal flux of isoprene from sweetgum trees.

To investigate both the long-term (weeks to months) and short-term (seconds to minutes) effects of changing [CO_2] on I_s, CO_2 assimilation rate and leaf conductance, gas exchange measurements were made at 25 °C leaf temperature and 1500 $\mu mol\, m^{-2} s^{-1}$ PPFD for trees from the growth-chamber experiments and 30 °C and 1000 $\mu mol\, m^{-2} s^{-1}$ PPFD, for trees from the CO_2 gradient. Gas exchange measurements conducted on trees from the growth chamber experiments were carried out by removing the trees from the chambers and subjecting a portion of an intact leaf to a range of [CO_2] in the leaf cuvette. Similarly, gas exchange measurements in the LYCOG experiments were conducted after trees were removed from their respective chamber compartments. Here, measurements were carried out only at [CO_2] corresponding to the [CO_2] at which the leaves developed.

Leaf gas exchange measurements were conducted using a portable photosynthesis system and 'broadleaf' cuvette (model 6400, LiCor Inc., Lincoln, NE, USA). The cuvette was coupled to either a chemiluminescence fast isoprene sensor (model FIS; Hills Scientific, Boulder, CO, USA) or to a proton transfer reaction-mass spectrometer (PTR-MS; Ionicon GmbH, Innsbruck, Austria) to measure isoprene concentration in the cuvette air. Air delivered to the portable photosynthesis system was scrubbed of ambient VOCs and ozone using a clean air generator (model 737, Aadco Inc., Cleves, OH, USA). The FIS was

calibrated each day by serial dilution of a 6 ppmv isoprene gas standard. Calibration curves were conducted at five isoprene concentrations from 0 to 400 ppbv. The PTR-MS was operated at 125 Townsend (1.25×10–$17\ V\ cm^{-1}$) to reduce compound fragmentation.

Statistical analysis

To evaluate the effects of different $[CO_2]$ on I_s, one-way analysis of variance (ANOVA) was conducted on data collected from sweetgum and eucalyptus trees. A one-way ANOVA was also conducted on I_s collected from all aspen trees grown at 400 ppmv CO_2 to determine if there were significant treatment carry-over effects between experiments. Student's *t*-tests were carried out on data collected from the controlled environment chambers (aspen and cottonwood) to determine if differences in I_s between CO_2 treatments were significant. Statistical analysis was carried out using SAS statistical software (Version 9.1).

Results

Response of I_s to changes in CO_2 concentration during leaf growth (long-term response)

Differences in the atmospheric $[CO_2]$ that persist across time scales of weeks to months, the same time scale across which leaves develop and grow, are likely to exert a control over I_s through adjustments in gene expression, and the production of a metabolic system uniquely 'tuned' to the atmospheric CO_2 regime. We refer to this type of adjustment as the 'long-term response'. For trees grown at the LYCOG project, I_s was approximately 30% and 18% lower, respectively, for eucalyptus and sweetgum trees grown at 520 ppmv CO_2, compared with trees grown at 240 ppmv CO_2 (Fig. 1). Eucalyptus leaves also exhibited significantly lower I_s when grown at ambient CO_2 compared with 240 ppmv CO_2 ($n = 3$; $P < 0.05$), an effect that was not observed for sweetgum leaves.

When aspen trees were grown in controlled-environment growth chambers at 400 and 800 ppmv CO_2, I_s was lower for trees grown at the highest $[CO_2]$ ($n = 11$; $P < 0.05$; Fig. 2a). Aspen trees grown at 600 ppmv exhibited higher I_s than those grown at 1200 ppmv ($n = 10$; $P < 0.05$; Fig. 2b). Cottonwood trees grown at 400 ppmv CO_2 exhibited significantly higher isoprene emission rates than those grown at 800 ppmv ($n = 10$; $P < 0.05$; Fig. 2c). There was a significant time-dependent effect in the aspen experiment, which caused a decrease in I_s with increasing tree age. Thus, the isoprene emission rates of the trees grown in the 600 and 1200 ppmv CO_2 treatments (later in the experimental cycle) were, on average, lower than those of the trees grown in the 400 and 800 ppmv CO_2 treatments.

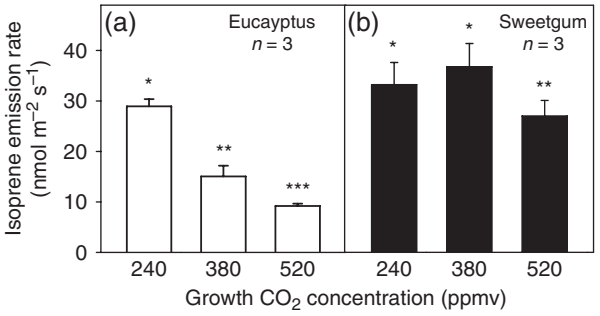

Fig. 1 Isoprene emission rates (I_s) for eucalyptus and sweetgum trees grown at different CO_2 concentrations in the LYCOG experiment. Error bars = ± 1 SE. A different number of asterisks next to each data bar indicates differences that are significant at $P < 0.05$.

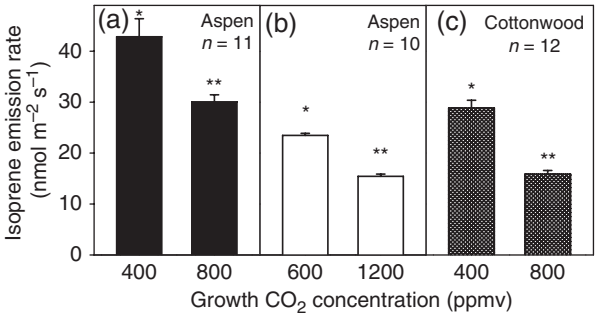

Fig. 2 Isoprene emission rates for aspen and cottonwood trees grown at different CO_2 concentrations. Error bars = ± 1 SE. A different number of asterisks next to each bar indicates differences that are significant at $P < 0.05$.

We used observations of the diurnal pattern of I_s from a sweetgum leaf grown at 520 ppmv CO_2 in the LYCOG experiment, along with the G95 algorithm (the combined model for describing the light and temperature dependence of I_s; Guenther *et al*., 1995), to predict the effect that growth at high CO_2 has on model estimates of the diurnal variability in I_s (Fig. 3). The diurnal pattern of I_s was predicted for a leaf grown at ambient CO_2 using its basal emission rate (measured at 30 °C and 1000 μmol $m^{-2}\ s^{-1}$ PPFD) along with measurements of leaf temperature and PPFD recorded throughout a typical day during the Texas measurements (Fig. 3a). These predicted measurements were then compared with actual measurements of I_s made during the same day on a leaf that had been grown in the elevated-CO_2 chamber (i.e., from the 520 ppmv chamber; Fig. 3b). We interpreted the difference between the two to be the error that would occur if one ignored the 'growth CO_2 effect' and assumed that the G95 algorithm correctly predicts I_s for the high-CO_2 growth condition. We

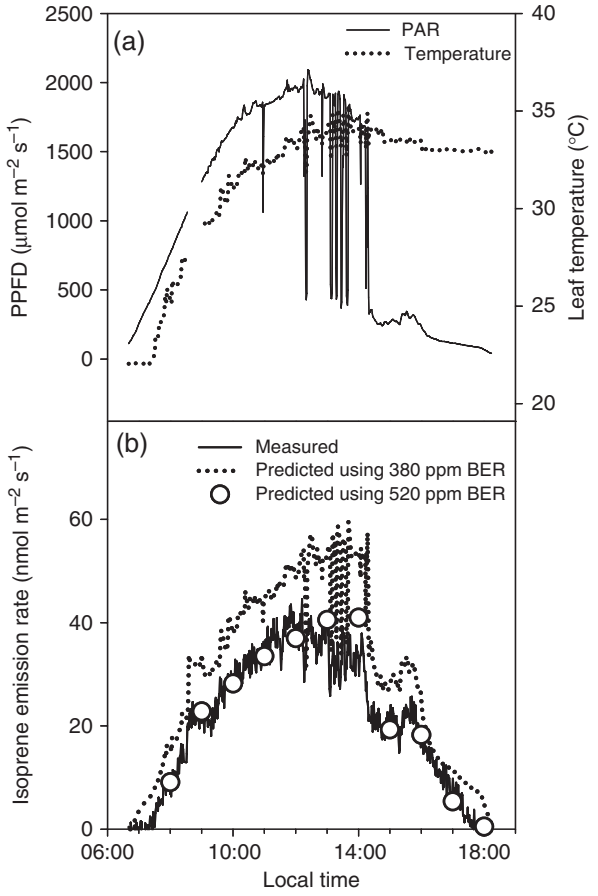

Fig. 3 Effect of using basal emission rates of trees grown at present-day CO_2 concentrations to predict the diurnal flux of isoprene from vegetation grown under elevated CO_2. Panel (a) shows the diurnal trend in environmental variables (solid line, PPFD; dotted line, leaf temperature). Panel (b) shows the diurnal trend in I_s for sweetgum trees growing at 520 ppmv CO_2 (solid line), modeled I_s predicted using the average basal emission rate calculated from trees growing at 380 ppmv (dotted line) and 520 ppmv CO_2 (open circles).

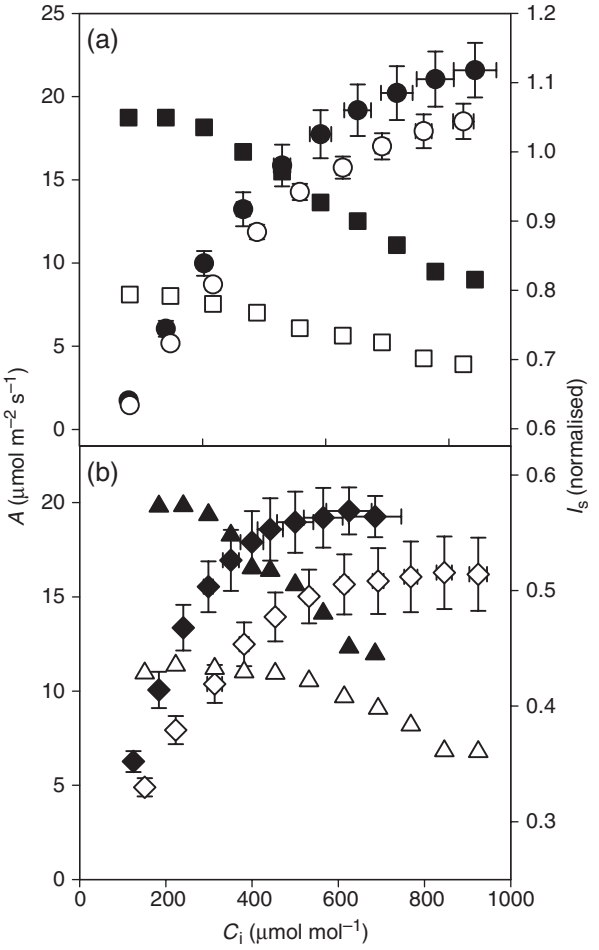

Fig. 4 Carbon assimilation rates (A) and normalized isoprene emission rates measured when A : C_i curves were conducted on aspen trees grown at different CO_2 concentrations. Isoprene emission rates were normalized by calculating the ratio of isoprene emission rates measured at growth conditions for tress growing at 400 ppmv CO_2. Panel (a) shows gas exchange measurements for 12 trees grown at 400 ppmv (A, closed circles; I_s, closed squares; $n = 12$) and 800 ppmv CO_2 (A, open circles; I_s, open squares; $n = 12$). Panel (b) shows gas exchange measurements for trees grown at 600 ppmv (A, closed diamonds; I_s, closed triangle; $n = 10$) and 1200 ppmv CO_2 (A, open diamonds; I_s, open triangles; $n = 10$). Error bars = $\pm$ 1 SE.

observed a diurnal pattern in I_s over the day with low values in the morning, which increased with PPFD and temperature. The estimated I_s was approximately 15–18% greater when the growth CO_2 effect was ignored, compared with observed values. When measured I_s was compared with model values calculated using a basal emission rate for the trees grown in the 520 ppmv CO_2 chamber, model estimates agreed closely with measured values.

Response of I_s to changes in CO_2 concentration during a single day (short-term response)

When the atmospheric [CO_2] is changed across the time scale of seconds or minutes, rather than days or weeks, the response of I_s is driven by adjustments in existing metabolic components, rather than the production of new metabolic components. We refer to this type of response as the 'short-term response'. We measured the response of photosynthetic CO_2 assimilation rate (A) and I_s to changes in C_i; the C_i value is more relevant to short-term leaf metabolism, than the atmospheric [CO_2], as it eliminates from the analysis effects caused by stomatal responses to CO_2. In order to provide

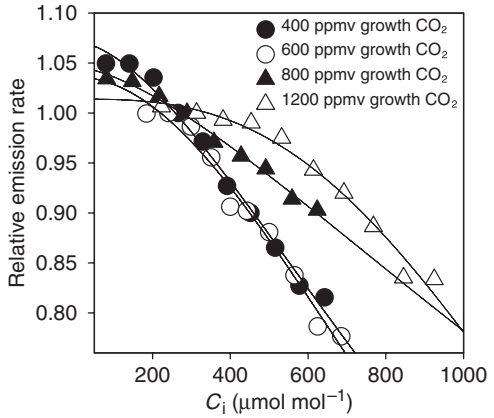

Fig. 5 Response of normalized isoprene emission rate to C_i for aspen trees grown in four different CO_2 concentrations ([CO_2]). Isoprene emission rates were normalized by assigning emissions measured at short-term [CO_2] of 400 ppmv, a value of 1.0 for trees from each of the long-term CO_2 treatments. Curves were fit to the data using the best fit of Eqn (1).

context for the observed C_i values, we note that the average growth C_i was 265 (400 ppmv growth CO_2), 351 (600 ppmv growth CO_2), 559 (800 ppmv growth CO_2), and 925 (1200 ppmv growth CO_2) ppmv CO_2.

I_s was reduced when leaves were subjected to short-term increases in [CO_2] (200–1200 ppmv), but the decrease was not linear. At C_i values between 200 and 400 ppmv, I_s was less sensitive to changes in C_i, compared with the response between 400 and 800 ppmv. At C_i values above 800 ppmv, I_s again became less sensitive to changes in [CO_2] resulting in an overall sigmoidal-shaped response pattern. Long-term effects of growth at elevated CO_2 appeared to influence the trend in I_s recorded during short-term changes in CO_2. Generally, the higher the growth CO_2, the less sensitive the I_s from aspen leaves were to short-term variations in C_i (Fig. 5).

Modeling the response of isoprene emission to elevated CO_2

The relationship between isoprene emission rate and C_i, for the short-term effect, resembled an inverse sigmoidal response surface (Fig. 4). Sigmoidal relations between the velocity of an enzyme-catalyzed reaction and the concentration of a substrate or allosteric effector often reflect second-order influences involving competing or cooperative substrates (Cook & Cleland, 2007). In such systems, reaction velocity is typically affected by exponential amplification. Building on this past knowledge, and starting from existing models (e.g., the Hill equation), we designed an empirically based relationship that fits the isoprene-C_i response pattern:

$$C_{Ci} = I_{s\,max} - \left[\frac{I_{s\,max}(C_i)^h}{(C^*)^h + (C_i)^h}\right], \quad (1)$$

where C_{Ci} is the overall C_i scaling factor, intended to scale I_s to the progressive inhibitory effects of increasing C_i, $I_{s\,max}$ is the estimated asymptote at which further decreases in C_i have a negligible effect on the isoprene emission rate (I_s), C* and *h* are Hill-type scaling coefficients used to calibrate the sigmoidal slope of the relationship between I_s and C_i. The terms C* and *h* are derived empirically, with C* carrying units of ppmv (analogous to the K_m of the Michaelis–Menten model of enzyme kinetics) and *h* is unitless. The model ignores the fact that as C_i decreases toward zero, I_s eventually decreases from its low C_i maximum (Loreto & Sharkey, 1990); however, this effect generally occurs at extremely low C_i (<100 ppmv in the study by Loreto & Sharkey, 1990), beyond the range relevant to plants, except those under the most severe stress.

The model presented in Eqn (1) is intended to complement the scaling coefficients C_L and C_T (Guenther *et al*., 1991, 1993, 1995) which are typically used to adjust a normalized (basal) emission rate (I_{sb}) (sometimes called the 'emission factor') to incident light intensity (C_L) and prevailing leaf temperature (C_T). In combination with these previously defined coefficients, the new scaling coefficient, C_{Ci}, can be used to scale I_{sb} (typically determined at a leaf temperature of 30 °C, an incident PPFD of 1000 μmol m^{-2} s^{-1} and, in this case, the C_i that occurs at an ambient atmospheric [CO_2] of 400 ppmv) to instantaneous combinations of these factors that differ from the 'basal' state:

$$I_s = I_{sb}(C_T \times C_L \times C_{Ci}). \quad (2)$$

A discussion of the general derivation of Eqn (1) is given in Appendix A.

The values of C_{Ci} and its component factors are affected by differences in [CO_2] during growth (Fig. 5); in other words, the shape of the shorter term response is influenced by the longer term response. This means that one set of empirically determined parameters in Eqn (1) will not suffice, but rather the model will need to be 'tuned' to the prevailing growth [CO_2]. Overall, the model described the response of the standardized I_s to C_i for trees grown over a range of four atmospheric [CO_2] relatively well (Fig. 5). As discussed above, the measured responses are ideally sigmoidal. The model, which has a form that predicts an overall sigmoidal response, predicted a response surface that was not obviously sigmoidal within the range of C_i observations used for the curve fits of Fig. 5. The asymptotic shape of the response predicted by Eqn

Table 1 Empirically determined parameter values for Eqn (1) from aspen trees grown at four different CO_2 concentrations

	Growth CO_2 concentration			
Parameter	400 ppmv	600 ppmv	800 ppmv	1200 ppmv
$I_{s\,max}$	1.072	1.036	1.046	1.014
h	1.7000	2.0125	1.5380	2.8610
C*	1218	1150	2025	1525

(1) at high C_i is not predicted to occur until higher C_i values and lower standardized I_s than those observed in our experiments. Thus, the form of the model is not a perfect fit to the patterns in the observed data, but provides a good description. Further observations at C_i greater than those measured during these experiments are required to evaluate whether the sigmoidal nature of Eqn (1) is truly justified.

We have determined the sets of parameter values that best accommodate Eqn (1) to the normalized C_i response of aspen leaves grown at the four different [CO_2] in the growth-chamber experiment (Table 1). There is a clear effect due to tree aging in the experiment in that the values for h were higher, C* lower, and $I_{s\,max}$ lower, for the trees grown at 600 and 1200 ppmv, compared with those grown at 400 and 800 ppmv, respectively. Taking this effect into account, from the results presented in Table 1 and Fig. 5, we can state that $I_{s\,max}$ tends to decrease and C* tends to increase as growth [CO_2] increases; the effect on h was variable.

We also assessed the utility in deploying Eqn (1) to predict the longer term, growth CO_2 responses (Fig. 6). In a previous study, Possell *et al*. (2005) provided a regression model describing the normalized I_s for a variety of species in response to a range of [CO_2], when grown and measured at the same [CO_2]. We have used this regression as a reference curve after a reduction of the [CO_2] by the multiplier 0.7, to reflect estimated C_i, rather than C_a. We then plotted the mean normalized I_s for aspen trees grown at each of the four growth chamber [CO_2] vs. the estimated C_i. When applied to the latter dataset, Eqn (1) provided a good fit. The data from our observations, along with the fit provided by Eqn (1), was also relatively close to the corrected regression obtained from the Possell *et al*. (2005) analysis at C_i above 400 ppmv. At lower C_i, the analysis of Possell *et al*. (2005) predicted continuous increases in I_s, whereas our results for aspen (Fig. 4) and sweetgum (Fig. 1) predict an asymptote as $I_{s\,max}$ is approached. The parameter values obtained from the fit of Eqn (1) to the growth CO_2 response of aspen is presented in Table 2.

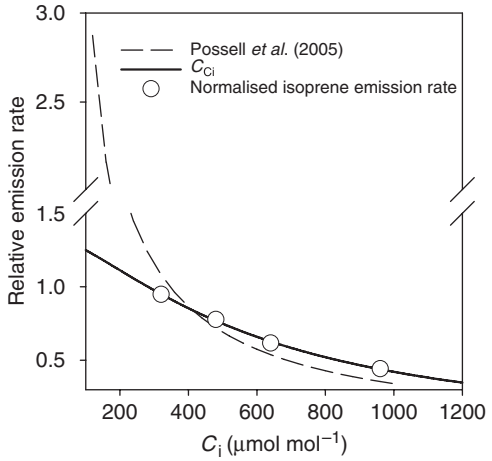

Fig. 6 Response of normalized isoprene emission rates to changes in growth CO_2 concentration ([CO_2]) estimated by Possell *et al*. (2005) (broken line) with their ambient [CO_2] corrected by the multiplier 0.7 to estimate C_i. Mean response of normalized isoprene emission rates of aspen leaves, measured at their growth [CO_2] and plotted as a function of C_i (open circles) ($n = 10$–12), and best-fit line provided by Eqn (1) (solid line).

Table 2 Empirically determined parameter values for Eqn (1) from pooled data of aspen trees grown at four different CO_2 concentrations

Parameter	'Best-fit' value
$I_{s\,max}$	1.344
h	1.4614
C*	585

Discussion

Although a small number of studies have reported that I_s from different tree species is not affected by elevated atmospheric [CO_2] (Buckley, 2001; Rapparini *et al*., 2004), a larger body of studies report a significant CO_2-induced reduction in I_s (Monson & Fall, 1989; Loreto & Sharkey, 1990; Sharkey *et al*., 1991; Loreto *et al*., 2001b; Rosenstiel *et al*., 2003; Centritto *et al*., 2004; Scholefield *et al*., 2004; Possell *et al*., 2005; Monson *et al*., 2007). (However, these past reports tend to mix together the long-term and short-term effects of [CO_2].) We found that I_s from eucalyptus, sweetgum, cottonwood, and aspen trees is reduced when grown with higher [CO_2]. These observations provide evidence for two, potentially connected, mechanisms of response to elevated CO_2: one that occurs nearly instantaneously and presumably involves adjustments to metabolite pools and enzyme activities in the existing isoprene

metabolism of a leaf, and one that occurs more slowly, over the growth period of a leaf, and presumably involves adjustments to the expression of the genes that regulate isoprene emission. It is possible that there are connections between these responses. Recent studies have shown that the activity of the cytosolic enzyme PEP carboxylase increases following instantaneous increases in leaf high C_i (the short-term response) (Rosenstiel *et al*., 2003), and during periods of high sugar loads (such as might occur during a long-term response to growth at elevated C_a) (Rontein *et al*., 2002). Common changes in the activity of this single enzyme may, therefore, link the short- and long-term responses of I_s to $[CO_2]$. We did not track the relative response kinetics for adjustments at these two time frames, nor did we track changes in the metabolite levels of enzyme activities of key enzymes; both of these types of observations would help clarify the nature of these controls and the potential for commonality between them. We note that in past studies it has been found that isoprene and monoterpene emissions respond similarly to elevated $[CO_2]$, especially in those oak species known to emit monoterpenes from a biochemical basis similar to that of isoprene (Loreto *et al*., 2001a; Rapparini *et al*., 2004). It is possible that the responses to $[CO_2]$ that we observed for I_s are explained by mechanisms that also operate to control the CO_2 response of monoterpene emissions. However, further research on the controls over monoterpene emissions are required to test this hypothesis.

In the past, models of I_s have focused on PPFD and temperature as driving variables, and have relied on empirical equations known informally as the 'Guenther algorithms' (Guenther *et al*., 1991, 1993, 1995). When deployed into regional or global models that aim to predict atmospheric chemistry, the Guenther algorithms provide no basis for describing the direct short-term or long-term effects of changes in atmospheric $[CO_2]$ (Monson *et al*., 2007). We found that the error in predicting the I_s using the Guenther algorithms, without inclusion of the growth $[CO_2]$ effect alone, is significant (Fig. 3). In order to better serve future modeling efforts, we developed a quantitative model to describe the short- and long-term responses to C_i, which could be used in combination with the traditional Guenther algorithms to predict the direct effects of $[CO_2]$ on I_s. The model was only tested with aspen leaves so, at present, it would have to be deployed with the caveat that its fit to other species has not been established. Nevertheless, given the similarities in the responses of four different species to elevated CO_2 in the short- and long-term and the results of Possell *et al*. (2005), we predict that the model will provide a relatively accurate description of the effects of future dynamics in atmospheric $[CO_2]$ on I_s.

The model that we developed is similar in its empirical formulation to that used for the Guenther algorithms. While all of these equations are derived from 'best-of-fit' functions, the observed responses are, at least for the Guenther algorithms, driven by underlying biochemical mechanisms and, therefore, there is good reason to suspect that, to some degree at least, these empirical functions also reflect biochemical mechanisms (Guenther *et al*., 1991, 1993). Now, the question arises as to whether the empirically derived CO_2 model also has mechanistic underpinnings. We have conducted an analysis to address this question and concluded that there is reason to believe that the form of Eqn (1) reflects the competition for PEP substrate between biochemical processes in the cytosol and chloroplasts of C_3 leaf cells, which in the case of varying C_i is driven by the activity of the enzyme PEP carboxylase (Appendix A). Briefly, as C_i increases, and the activity of PEP carboxylase is enhanced, reductions in the transport of PEP into the chloroplasts will reduce the level of pyruvate needed for isoprene biosynthesis in inverse, rectangular hyperbolic fashion, or sigmoidal fashion, mirroring the Michaelis–Menten-type response of PEP carboxylase to one or both of its substrates, HCO_3^- and PEP. We should note that while we have based the model on C_i, our metabolic justification would lead us to conclude that the inorganic C concentration in the cytosol of mesophyll cells is more relevant. If indeed, the activity of PEP carboxylase drives the CO_2 response, then its response to HCO_3^- might be more relevant than C_i. Basing the model on cytosolic HCO_3^-, rather than C_i will not change the overall shape of the modeled CO_2 response, but it may alter the relevant atmospheric $[CO_2]$ where sigmoidal transitions in the response occur.

We need to address the possibility that the response of I_s to $[CO_2]$ is due to artifactual effects of growing the trees in pots, with restricted rooting volume, rather than direct responses to the prevailing $[CO_2]$. In the two separate growth chamber experiments with aspen trees, the initial slope of the $A : C_i$ relationship, an indicator of photosynthetic carboxylation capacity, was lower when plants were grown at elevated $[CO_2]$ (Fig. 4a and b), suggesting that part or all of the response to the higher growth CO_2 involved downregulation of the carboxylating enzyme, Rubisco. This is a common response in experiments involving elevated $[CO_2]$, especially when potted plants are used (Thomas & Strain, 1991). The cause of the response is thought to involve a build-up photosynthate in photosynthetic cells, followed by downregulation of photosynthetic genes to bring the photosynthetic supply of sugars back into balance with photosynthetic demand (Long *et al*., 2004). It is not likely that these shifts in sugar supply and demand

directly affect isoprene emission rate, at least not from a substrate perspective, as the rate at which photosynthetic substrate used to synthesize isoprene is typically 1% or less the rate of photosynthate production (Monson & Fall, 1989). Additionally, we note that we observed the same downregulation of I_s when trees were grown at elevated [CO_2] in two different Free Air CO_2 Enrichment (FACE) experiments, one that also involved aspen trees, and neither of which involved pot-grown trees (Monson *et al*., 2007). A recent meta-analysis of FACE experiments showed that, like previous potted-plant experiments, a downregulation of Rubisco content and activity occurs, presumably due to signals received at the molecular level due to a build-up of leaf sugars when plants are grown at elevated [CO_2] (Long *et al*., 2004). Thus, these general responses of central metabolism appear to reflect a direct response to growth at elevated [CO_2], not an experimental artifact. Using cultured tomato cells grown with different supply rates of glucose, Rontein *et al*. (2002) showed that a build-up of leaf sugars also results in the upregulation of PEP carboxylase activity. Flexibility in the expression of PEP carboxylase genes in response to cellular carbohydrate supply may be a general design feature of plant metabolic controls, especially those controlling the expression of anabolic pathways (Rontein *et al*., 2002), and thus may explain the inhibition of I_s in trees grown at elevated [CO_2]. The fact that these similar responses were observed in plants from both potted-tree experiments, and FACE experiments, suggest that they are due to direct responses to the atmospheric CO_2 environment, not the soil-rooting environment.

With regard to overall CO_2 response of isoprene emissions, our results are similar to those of Possell *et al*. (2005), who compiled data from several studies of the effects long-term changes in [CO_2] on I_s. The response reported by Possell *et al*. (2005) exhibits a decrease from a maximum I_s that is approximately three times greater than that measured in present-day [CO_2] before asymptotically leveling off at I_s an order of magnitude lower than those measured at the lowest [CO_2]. We calculated a similar result for I_s at [CO_2] at the higher end of this range (Fig. 6). However, because of a reduction in the sensitivity of I_s to changes in CO_2 at low C_i values, our observations lead to the conclusion that the isoprene/CO_2 response curve of Possell *et al*. (2005) overestimates I_s at low [CO_2]. The differences in these analyses will cause uncertainties in efforts to model atmospheric chemistry during past epochs characterized by low [CO_2], and must be reconciled in order to understand biochemical controls over I_s at low C_i. If indeed the principal biochemical limitation at low C_i is the availability of G3P, then it is difficult to explain a continued increase in I_s as C_i decreases (as seen in the Possell *et al*. results) unless a mechanism is identified that would produce G3P at progressively higher rates. Alternatively, if the primary limitation to isoprene biosynthesis rate at low C_i is pyruvate, supplied from the import of PEP into the chloroplast, and the increase in PEP import at low C_i is due to the progressive limitation of PEP carboxylase in the cytosol, then a mechanism would have to be identified for the continued glycolytic production of PEP in the face of reduced triose-phosphate export from the chloroplast (to explain the results of the current study). There is a clear basis for further work on the controls over isoprene biosynthesis at low [CO_2].

It is estimated that without action to limit greenhouse gas emissions, global mean temperature will increase 2–4 °C over the next 100 years (IPCC, 2007) with a concomitant rise in background tropospheric [O_3] and an increase in the atmospheric loading of organic aerosols. Methane is also of interest to atmospheric chemists because its atmospheric lifetime is highly sensitive to perturbations in hydroxyl radical concentrations (Valdes *et al*., 2005). Within the context of these effects, any change in the atmospheric loading of isoprene could significantly influence climate change predictions. The downregulation of isoprene emission at elevated [CO_2] that we have reported must be considered within the context of predicted increases in NPP due to increased plant growth in elevated [CO_2] (Monson *et al*., 2007). However, the effects of elevated [CO_2] on NPP are complex. For example, Gielen *et al*. (2001) noted that the stimulation of biomass production relative to control plots at different FACE sites may be transient and limited only to the first few years of growth. Also, 8 years of experimental data collected at the Oak Ridge FACE site revealed that increases in NPP occurred primarily through belowground increases in biomass and not through increases in aboveground, isoprene-emitting biomass (Norby *et al*., 2004). More recently Finzi *et al*. (2007) reported a 25% increase in productivity for forest FACE experiments at Oak Ridge National Laboratory, Rhinelander (Harshaw Experimental Forest), Duke Forest, and POP-EuroFace, much of which is associated with increases in canopy biomass. Clearly, the future effects of global change on the flux of isoprene from forest systems cannot be reconciled without consideration of the direct effects of increasing atmospheric [CO_2] on both NPP and isoprene synthesis. At the time that Monson *et al*. (2007) made their case, there was no good quantitative model for use in carrying out this type of analysis. We now present a model that appears to work well in explaining the response of isoprene emissions to atmospheric [CO_2] and is consistent with the past Guenther

algorithms that are used in larger scale models of atmospheric chemistry. It is expected that this model can now be used to address some of these important questions concerning atmospheric chemistry in the face of future global change projections.

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Appendix A. The biochemical and mathematical justification for C_{Ci}

The CO_2-dependent algorithm (for calculation of C_{Ci}) that we present in Eqn (1) was derived through a Bayesian process whereby prior knowledge about the biochemistry of isoprene biosynthesis was used to infer the key relationships. Evidence obtained to date suggests that the CO_2 dependence of I_s is determined by two interacting factors: (1) at low [CO_2], I_s becomes progressively uncoupled from photosynthetic G3P production, and is sustained by the mobilization of G3P from stored carbohydrate reserves (Monson & Fall, 1989; Wolfertz *et al*., 2003); (2) at high [CO_2], I_s becomes progressively, but

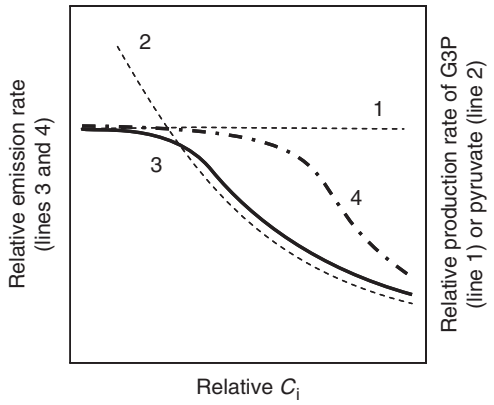

Fig. A1 Proposed relations among the supply of G3P from stored reserves (Line 1), which is assumed to be constant in the face of changing C_i; the supply of pyruvate provided by transport of PEP into the chloroplast from the cytosol (Line 2) and controlled by the Michaelis–Menten-type response of PEP carboxylase to increased C_i; a normalized response of I_s to changes in C_i similar to what was observed for trees grown at 400 ppmv CO_2 (Line 3); and a normalized response of I_s to changes in C_i similar to what was observed for trees grown at 800 ppmv CO_2 (Line 4).

indirectly, limited by increased activity of the cytosolic enzyme phosphoenolpyruvate carboxylase (PEPc) (Rosenstiel *et al*., 2003; Loreto *et al*., 2007). In order to explain the overall response of I_s to changes in C_i, we assumed the velocity of the reaction catalyzed by PEPc increases as C_i increases, and within some relatively narrow range of increasing C_i, I_s undergoes a transition from G3P limitation to pyruvate limitation (Fig. A1). Above this transition range, I_s would be controlled by the Michaelis–Menten-type response of PEPc to increases in $[CO_2]$; below this transition range, I_s would be controlled by the rate of G3P mobilization from carbohydrate reserves. According to this explanation, the sigmoidal shape of the CO_2 response represents a switch between the metabolic limitations imposed by one of two processes. Within the context of this hypothesis, the growth of plants at higher $[CO_2]$ may shift the transition range between the two limiting processes to higher C_i values, consistent with the results of Fig. 5.

Mathematically, a switch in controlling functions can be related to the same independent variable by a Heaviside function, which takes the general form:

$$H(x) = \begin{cases} f_1(x) & \text{if } c < x_1, \\ f_2(x) & \text{if } c \geq x_1, \end{cases} \tag{A1}$$

where c is a critical threshold value of x, which allows the form of the function to be toggled between two alternatives. When combined to a single function we can write:

$$f(x) = f_1(x)u_{x1} + f_2(x)u_{x2}, \tag{A2}$$

which allows f_1 to control the value of the dependent variable below the critical switch (designated as u_{x1}), and the sum of f_1 and f_2 to control its value at or above the critical switch (designated as u_{x2}). In designing a relationship to describe the CO_2-dependence of I_s, we can write:

$$C_{Ci} = I_{s\,max}u_{Ci1} - \left[\frac{I_{s\,max}C_i}{C_{i50} + C_i}\right]u_{Ci2}. \tag{A3}$$

Equation (A3) describes a switch in the CO_2 response such that below a critical $[CO_2]$ value (C_{i1}), the maximum value of I_s is limited by the rate of mobilization of G3P from carbohydrate reserves; at or above the critical C_i value (C_{i2}), the response is driven by the Michaelis–Menten-type activity of an enzyme, which we assume to be PEP carboxylase. The coefficient C_{i50} is analogous to K_m, a Michaelis constant that constrains the first-order region of a modeled, rectangular hyperbolic response of I_s to C_i.

In practical terms, it would be an improvement if we could design an analytical form of Eqn (A3) that does not depend on stepwise triggers, but rather describes a continuous dependence of I_s on C_i. We have found that the following equation, which retains the essential features of Eqn (A3), works well to describe the CO_2 dependence in continuous fashion:

$$C_{Ci} = I_{s\,max} - \left[\frac{I_{s\,max}(C_i)^h}{(C^*)^h + (C_i)^h}\right], \tag{A4}$$

where h is a tunable coefficient that permits the right-hand side of the equation to be 'penalized' exponentially at low C_i and thus allow C_{Ci} to move toward the limit defined by Term I, but to be 'amplified' exponentially at high C_i and thus allow C_{Ci} to move toward greater dependence on Term II. We have re-defined C_{i50} as C*; with this definition, C* becomes a more generalized C_i scalar, rather than a strict analogue to the Michaelis constant, K_m. By introducing C*, we can scale Eqn (A4) differentially to account for growth-CO_2 effects on the switch between the two competing metabolic controls; as growth CO_2 increases, C* should increase, shifting the penalty phase of the right-hand side of Eqn (A4) to higher C_i values.