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The effect of elevated atmospheric CO_2 and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm

EMILIANO PEGORARO*[1], LEIF ABRELL*[2], JOOST VAN HAREN*[3], GREG BARRON-GAFFORD*[4], KATHERINE ANN GRIEVE*[5], YADVINDER MALHI†, RAMESH MURTHY*[6] and GUANGHUI LIN*‡[7]

*Biosphere 2 Center, Columbia University, Oracle, AZ 85623, USA, †School of Geography and the Environment, University of Oxford, UK, ‡Key Laboratory of Quantitative Vegetation, Institute of Botany, The Chinese Academy of Science, 20 Nanxincun, Xiangshan, Beijing 100093, China

Abstract

Isoprene is the most abundant volatile hydrocarbon emitted by many tree species and has a major impact on tropospheric chemistry, leading to formation of pollutants and enhancing the lifetime of methane, a powerful greenhouse gas. Reliable estimates of global isoprene emission from different ecosystems demand a clear understanding of the processes of both production and consumption. Although the biochemistry of isoprene production has been studied extensively and environmental controls over its emission are relatively well known, the study of isoprene consumption in soil has been largely neglected.

Here, we present results on the production and consumption of isoprene studied by measuring the following different components: (1) leaf and soil and (2) at the whole ecosystem level in two distinct enclosed ultraviolet light-depleted mesocosms at the Biosphere 2 facility: a cottonwood plantation with trees grown at ambient and elevated atmospheric CO_2 concentrations and a tropical rainforest, under well watered and drought conditions. Consumption of isoprene by soil was observed in both systems. The isoprene sink capacity of litter-free soil of the agriforest stands showed no significant response to different CO_2 treatments, while isoprene production was strongly depressed by elevated atmospheric CO_2 concentrations. In both mesocosms, drought suppressed the sink capacity, but the full sink capacity of dry soil was recovered within a few hours upon rewetting. We conclude that soil uptake of atmospheric isoprene is likely to be modest but significant and needs to be taken into account for a comprehensive estimate of the global isoprene budget. More studies investigating the capacity of soils to uptake isoprene in natural conditions are clearly needed.

Keywords: biosphere, elevated CO_2, isoprene emission, microbial, *Populus*, soil sink, tropical rainforest, water stress

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Correspondence: Emiliano Pegoraro, e-mail: e.pegoraro@ed.ac.uk

[1]Present address: School of GeoSciences, University of Edinburgh, Darwin Building, Mayfield Road, Edinburgh EH9 3JU, UK.

[2]Present address: Department of Chemistry, University of Arizona, 1306 E. University Blvd., Tucson, AZ 85721, USA.

[3]Present address: USDA-ARS Southwest Watershed Research Center, 2000 E. Allen Road, Tucson, AZ 85719, USA.

[4]Present address: Department of Ecology and Environmental Biology, University of Arizona, Tucson AZ 85721, USA.

[5]Present address: Quantitative Ecology and Resource Management, University of Washington, PO Box 352182, Seattle, WA 98195, USA.

[6]Present address: 1691 W. Winchester Way, Chandler, AZ 85248, USA.

[7]Present address: Department of Global Ecology, Carnegie Institution of Washington, Stanford, CA 94305, USA.

Introduction

Isoprene (2-methyl-1,3-butadiene) is a volatile organic compound (VOC) emitted from leaves of many plant species and it has a major impact on tropospheric chemistry (Trainer *et al*., 1987; Chameides *et al*., 1988; Fehsenfeld *et al*., 1992; Fuentes *et al*., 2000; Monson & Holland, 2001). Since Went (1960) first drew attention to the importance of the emissions of terpenes from plants in desert ecosystems, appreciation of the quantitative importance of VOC emissions from leaves has grown, with estimated emissions now in excess of 10^{15} g globally per year (Guenther *et al*., 1995), an amount similar to that of the greenhouse gas methane. Isoprene dominates VOC emissions in North America (Guenther *et al*., 2000). Concerns have been expressed about how isoprene dominates atmospheric photochemical reactions in natural ecosystems and urban environments, both locally and globally (Goldstein *et al*., 1998); it is recognized as a fundamental component of biosphere–atmosphere interactions, controlling many aspects of photochemistry in the lower atmosphere (Rosenstiel *et al*., 2003). The atmospheric chemistry of isoprene is complex, leading to the production of ozone, carbon monoxide, and other toxic products in polluted air, and it plays an important role in the oxidation capacity of the atmosphere, enhancing the lifetime of methane, an important determinant of global climate. The rise in atmospheric concentrations of greenhouse gases such as CO_2 and methane is expected to have complex repercussions on the emission of isoprene by plants. Because isoprene emission is very sensitive to temperature (Monson & Fall, 1989; Singsaas & Sharkey, 2000) the result of expected future climate change may be an increased isoprene production that could result in significant perturbations of atmospheric chemistry and the global carbon cycle (Monson *et al*., 1991; Guenther, 2002). Of all terrestrial ecosystems, tropical forests are believed to be the major sources, responsible for more than 80% of annual isoprene flux (Jacob & Wofsy, 1988; Zimmerman *et al*., 1988; Guenther *et al*., 1995). Future increases in atmospheric CO_2 concentrations may partially compensate for this increase by inhibiting isoprene production while stimulating biomass production (Rosenstiel *et al*., 2003), but environmental stresses such as drought may counteract the effect of elevated CO_2 (Pegoraro *et al*., 2004; Rapparini *et al*., 2004) and lead to increased global isoprene emission under conditions of an increased global mean temperature and extended droughts suggested by some future climate scenarios (Cox *et al*., 2000).

Reliable estimates of global isoprene emission from different ecosystems require a clear understanding of the control that environmental variables such as atmospheric CO_2 concentration and soil moisture exert on both isoprene production and consumption. Some studies have been published on the effect of elevated CO_2 and water stress on isoprene emission; however, most experiments have been carried out at leaf level and on potted plants. The sources, synthesis, emission, and atmospheric chemistry of isoprene have been investigated in detail (Monson & Holland, 2001; Sharkey & Yeh, 2001). The effects of temperature and light (Harley *et al*., 1999; Fuentes *et al*., 2000) and both moderate and severe drought (Tingey *et al*., 1981; Sharkey & Loreto, 1993; Fang *et al*., 1996; Guenther *et al*., 1999; Bruggemann & Schnitzler, 2002) have been investigated at the leaf level. Effects of elevated CO_2 have involved both leaf- and stand-level studies (Monson & Fall, 1989; Guenther *et al*., 1991; Sharkey *et al*., 1991; Rosenstiel *et al*., 2003).

In contrast, there has been scant evaluation of the natural biospheric sinks for this hydrocarbon. Some soil microbes are known to use isoprene as a sole carbon supply (van Ginkel *et al*., 1987), and metabolism of isoprene in *Rhodococcus* has been explored in detail (Vlieg *et al*., 1999). Although there is evidence that soils can act as isoprene sinks in both temperate and tropical rainforest ecosystems (Cleveland & Yavitt, 1997, 1998), the significance of soil uptake in the overall isoprene budget of forest systems is still conjectural (Fall & Copley, 2000) and no specific quantification has been made so far.

As a first step in improving our understanding of the sink capacity of soil for isoprene, the Biosphere 2 Laboratory (B2L) offered an unprecedented opportunity to study environmental responses of isoprene emission and uptake in model forest ecosystems (Marino & Odum, 1999; Osmond *et al*., 2004; Walter & Lambrecht, 2004). The tightly sealed glass and steel enclosure excluded ultraviolet (UV) light (Cockell *et al*., 2000) thereby minimizing isoprene depletion by atmospheric oxidative reaction such as those involving OH. Attainment of high concentrations of isoprene from natural vegetation and observation of large fluxes in defined, temperature-regulated soil systems in response to controlled CO_2 concentrations and drought were also possible inside B2L. In an attempt to understand the environmental controls on isoprene production and consumption, we examined plant isoprene emission and soil uptake in two model ecosystems. The first was a set of three agriforest stands (3 years old) of a strong isoprene emitter, *Populus deltoides* Bartr., grown under three atmospheric CO_2 concentrations: 430, 800, and 1200 $\mu mol\,mol^{-1}$ (ppm); the second was a 12 year old synthetic model tropical rainforest with several strong isoprene emitting species. Specifically, we explored the relationship between

isoprene uptake and atmospheric CO_2 concentration and drought.

Materials and methods

Mesocosm composition

Experiments were conducted in the absence of UV light inside two UV-free glass- and stainless steel-enclosed controlled environment mesocosms of the B2L, Oracle, Arizona, USA. The design and operation of B2L are described in detail elsewhere (Lin *et al*., 1999; Zabel *et al*., 1999; Griffin *et al*., 2002).

Intensive forestry management mesocosm (IFM). The IFM comprises three agriforest cottonwood plantations (*P. deltoides* Bartr.) grown in three separated experimental bays (ca. 550 m^2, 12 000 m^3 each) operated as semiclosed systems (closed during daylight with CO_2 injection to maintain preset concentrations; open as required at night to exhaust excess CO_2) with independent control of atmospheric CO_2 concentration (430, 800, and 1200 ppm), air circulation, temperature, and precipitation (Murthy *et al*., 2003; Rosenstiel *et al*., 2003). The agriforest stands were planted from cuttings in 1998, coppiced at the end of each growing season through 2002 and exposed to controlled atmospheric CO_2 conditions during each growing season, 1999–2003. The constructed silt loam soil (1 m deep) of the agriforest has been evolving *in situ* over 12 years and has developed many of the physical and nutritional profiles of 'natural soils' (Torbert & Johnson, 2001), comparable with those used for agriforestry in SE United States. It now shows metabolic (Murthy *et al*., 2004) and microbiological properties (D. Lipson *et al*., 2004; unpublished data) 'within a reasonable range for natural soils' (Kudeyarov *et al*., 2002), with a soil organic carbon content of ca. 2–3% and a carbon : nitrogen ratio of 8.32.

Tropical rainforest mesocosm (TRF). The synthetic model tropical rainforest of the TRF (ca. 1950 m^2, 27 000 m^3) comprises ca. 130 plant species (Leigh *et al*., 1999) and was also operated as a semiclosed system, controlled growth environment. The forest is structurally and functionally representative of disturbed humid tropical rainforests in South America, but with floristically diverse pan-tropical vegetation (Leigh *et al*., 1999; G. Prance, Eden project, St Austel, Cornwall, UK, personal communication). Ringed by a shade belt of bananas and ginger, after 12 years, the upper canopy mesocosm exceeds 15 m, filling about 50% of the upper enclosure, with secondary canopy and understory plants well established. Although the TRF was exposed to a series of short-term elevated CO_2 treatments (Lin *et al*., 1999) and drought treatments since 1998, seasonal net ecosystem CO_2 exchanges (net assimilation and respiration) have remained closely comparable with those of field sites in Amazonia (Andreae *et al*., 2002; Osmond *et al*., 2004) with little evidence of marked memory effects. The constructed soil in the TRF has a subsoil layer (up to 5 m deep) and a topsoil layer (0.3 and 3.2 m in depth) (Leigh *et al*., 1999). Although soil bulk density, organic matter content, and major nutrient concentrations in this soil are similar to those of several Puerto Rican rainforests, the constructed soil is more alkaline (pH 7.5) and contains slightly higher P, K, and other nutrient elements (Scott, 1999).

Drought experiments

Two drought experiments were conducted in 2002 and 2003. Before the start of the experiments, mesocosms were watered to field capacity. In the three agriforest cottonwood plantations, water was withheld and the soil was left to dry naturally from October 21 until rewatering on November 30, 2002 (agriforest drought experiment 1) and from May 13 until rewatering on June 4, 2003 (agriforest drought experiment 2). The mesocosm temperature was maintained at 30/26 °C day/night from October until December 10, 2002 (then allowed to cool naturally to 19/15 °C until March 2003) and 30/26 °C day/night in May–June 2003. In the TRF, water was withheld from September 23 to October 28, 2002 (TRF drought experiment 1) and from April 21 to May 6, 2003 (TRF drought experiment 2), with mesocosm temperature maintained at 27/23 °C day/night.

Soil volumetric water content was continuously monitored during the experiment with Time Domain Reflectometry (TDR) probes (CS165, Campbell Scientific Instruments, Logan, UT, USA) connected to a datalogger (CR10, Campbell Scientific Instruments) inserted at four locations at two different depths, 20 and 80 cm in the soil of each agriforest bay, and in five locations (north, north-west, south, south-east, and centre at 30 and 60 cm) in the tropical rainforest. Arrays of other sensors in the mesocosms facilitated continuous monitoring of atmospheric CO_2 composition, climatic conditions (light, temperature, leaf temperature, and humidity), and trace gas fluxes in canopies.

Leaf isoprene measurements

Fully expanded leaves from the middle canopy with the same orientation (facing south) were randomly chosen for gas exchange measurements in all mesocosms. Leaf gas exchange was monitored online by clamping the cuvette of an open-path gas exchange measurement system (LI 6400; Li-Cor, Lincoln, NE, USA) onto a leaf. To avoid interference from isoprene in the atmosphere

outside the cuvette, cylinder air (Praxair Technology, San Ramon, CA, USA) (measured and confirmed to be isoprene free) was delivered to the Li-Cor measurement system. The cylinder was connected to the air inlet of the LI 6400 by a T junction allowing exhaust of excess air. Inside the cuvette, the CO_2 concentration was maintained at ca. 400 ppm and relative humidity at ca. 60% by internal controls of the LI 6400. The air flux inside the cuvette was maintained at 400 $\mu mol\,s^{-1}$. All measurements were made under the same standard conditions: leaf temperature of 32 °C and photosynthetic active radiation (PAR) of 1200 $\mu mol\,m^{-2}\,s^{-1}$. Leaves were left to equilibrate for 10 min in the cuvette to attain steady-state CO_2 and H_2O fluxes prior to isoprene measurements.

Changes in isoprene concentration were measured by proton-transfer-reaction mass spectrometry (PTR-MS). The cuvette exhaust was connected by 9 m long Teflon tubing (1.6 mm inside diameter) to a PTR-mass spectrometer (PTR-MS, Ionicon GmbH, Innsbruck, Austria; www.ptrms.com) via a T junction. Operational details of PTR-MS are described elsewhere (Lindinger *et al*., 1998; Warneke *et al*., 2001; Hayward *et al*., 2002). The air sample for isoprene concentration determination was pulled by the PTR-MS at a constant flow rate of ca. 12 $\mu mol\,s^{-1}$. Inside the PTR-MS reaction cell H_3O^+ ions produced from pure water vapor transferred a proton to compounds in the sample air that had a higher proton affinity than H_2O (PA 165.2 $kcal\,mol^{-1}$). Protonated isoprene (isoprene PA, 198.9 $kcal\,mol^{-1}$) was detected by the mass spectrometer as its molecular mass plus one (i.e. $M + H^+ = 69$) using a dwell time of 2 s (Hayward *et al*., 2002). Few compounds were analysed concurrently, allowing for high temporal resolution (ca. 7 s) between successive measurements of the same mass. The instrument was calibrated before and after experiments by a three-point calibration curve: pure certified standard (50 ppb, Praxair Technology), a dilution of the standard (25 ppb) and zero air from a compressed air cylinder. Environmental data collected inside the TRF by PTR-MS technique were also plotted against data collected by a Fast Isoprene Sensor system (FIS-02-AUTO, Hills Scientific, Boulder, CO, USA). The response of the two instruments correlated very well ($R^2 = 0.99$, Fig. 1). FIS is highly selective for isoprene (Guenther & Hills, 1998); therefore good agreement between signals detected by the two instruments indicates that any interference at M69 by other compounds in the PTR-MS is minimal, if any.

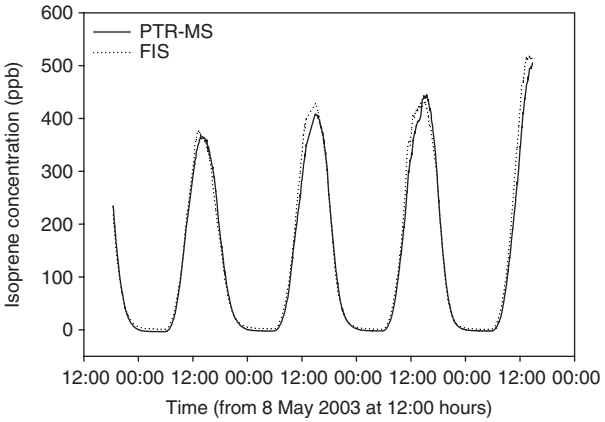

Fig. 1 Trend of isoprene concentration measured with Fast Isoprene Sensor system (FIS) and measured as M69 with proton-transfer-reaction mass spectrometry (PTR-MS) inside the tropical rainforest mesocosm over 4 days (May 8–12, 2003).

Soil isoprene measurements

Sink capacity of the mature constructed soils in the two ecosystems was also measured by PTR-MS using *in situ* soil collar techniques. Three soil collars were set up in both the 430 and 1200 ppm CO_2 bays of the agriforest, and five soil collars were set up in different locations (mainly along a north to south transect) in the tropical rainforest. Soil collars were inserted ca. 3 cm deep into the soil at least 2 weeks before the start of the experiment to allow the soil to recover from disturbance. The PTR-MS was connected by a 9 m long Teflon line (1.6 mm inside diameter) to aluminum $30 \times 30 \times 40\,cm^3$ static soil chambers equilibrated at isoprene concentrations attained in the mesocosm as a whole at the time of measurements. At the start of each measurement period the chamber was fitted onto the collar, thus preventing any gas exchange with the outside. Isoprene concentration inside the chamber was determined in real time with the PTR-MS drawing a minimum regulated air flow of ca. 9 $\mu mol\,s^{-1}$. The mixing of air inside the chamber was assured by a small fan, and small pressure changes caused by air sample collection were compensated for by a rubber balloon deflation chamber. The chambers themselves were inert with respect to isoprene uptake. To insure inertness the chambers were leak tested as follows: an exact replicate of the soil collar-chamber device, without soil and containing a plastic floor sealed to the bottom of the collar, was set up inside both the agriforest and tropical rainforest. No appreciable variation in isoprene concentration could be observed in the empty chambers by repeating the experimental protocol.

Measurements of isoprene concentration in the soil profile were made by taking soil air samples from three different depths. The PTR-MS inlet was connected by a 9 m long Teflon tube (1.6 mm inside diameter) to three stainless steel soil probes at 5, 10, and 15 cm depth, installed at one location in the centre of the TRF. To

prevent pulling in air from above the soil surface during soil profile sampling the PTR-MS air flow was regulated at its minimum, ca. 9 μmol s^{-1}, and the sampling time was minimized (ca. 2 min) to flush the tubing and collect a significant sample of air.

Mesocosm-level isoprene measurements

The glass walls of the B2L mesocosms attenuate UV radiation completely, preventing O_3 production and OH^- radical generation, and eliminating atmospheric oxidative destruction of isoprene (Cockell *et al*., 2000). Absence of isoprene destruction was tested by concurrently filling four transparent Teflon bags (of 2.5 dm^3 volume) with atmospheric air from inside the agriforest and TRF mesocosms. The bags were exposed to light inside each respective mesocosm and isoprene concentration determined every 2–3 h. Although atmospheric isoprene concentrations inside each mesocosm changed by a large amount over the course of the day, concentrations inside the bags remained constant. The east–west orientation of the three cottonwood bays means that the lowest CO_2 concentration treatment (430 ppm) (in the east) was exposed to higher light intensity earlier in the day than the other treatments, with the 1200 ppm CO_2 (in the west) treatment having higher light later in the day. Rates of ecosystem-level net isoprene emission from the agriforest in the light (1) and consumption in the dark (2) were measured with an FIS based on chemiluminescence detection. Operational details of the instrument are described in detail elsewhere (Hills & Zimmerman, 1990; Guenther & Hills, 1998). A continuous air sample collected 16 m above the ground and 2 m below the top of the mesocosm frame was continuously pumped from each of the mesocosms through a circuit of tubing (Dekoron, Goodrich Sales Inc., Naperville, IL, USA, 9.5 mm diameter, 50–90 m length) looped between the mesocosm and the FIS in an adjacent laboratory. The FIS was calibrated before and after each experiment by diluting an isoprene standard (5 ppm, Scott-Marrin, Riverside, CA, USA) over the range of 50 ppb −1 ppm isoprene. FIS instrument stability throughout the experiment was monitored by running an automated calibration cycle each mid-night using a standard (100 ppb) and zero air obtained by passing the sample stream through a scrubber before it entered the reaction cell.

FIS measurements cycled through the three agriforest mesocosms and the TRF once every 15 min. Isoprene concentration data were collected every minute at the end of the sampling period and the first data of each sampling period were automatically discarded to allow complete flushing of the short inlet line from the manifold of valves entering the FIS. In order to have similar data sets from the different mesocosms, the 1 min raw isoprene concentration data were averaged by sampling period. A spline model was then used to fill gaps smaller than 2 h and centre the data on 15 min periods.

The isoprene flux, which in our case corresponded to the *net isoprene exchange* (NIE) (the result of plant isoprene emission and soil consumption: F_{P+S}), was then calculated every 15 min for a 'closed' system (when push–pull fans were exchanging air with the outside all data were not considered) with the following equation:

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

where C_{t+1} is the concentration in the mesocosm for the following 15 min period with respect to time 't', C_{t-1} is the same for the previous 15 min period, and Δt is the length of the time period (15 min in this case). Determining the isoprene flux over the time period $2\Delta t$ has the advantage of centering the derivative on the current time period, introducing some smoothing.

Leaks in the agriforest and TRF enclosures were estimated by measuring leakage of tracer gases (sulfur hexafluoride: SF_6, freon 13B1: $CBrF_3$, or freon 12: CCl_2F_2). A known volume of the above tracer gases was routinely injected simultaneously and separately into each bay of the agriforest mesocosm and in the TRF. These gases are completely anthropogenic and do not interact with plants or soils. Leak rates were determined from the rate of decay of the gas concentration in each bay. Leak rates between bays and direction of the leaks were determined by quantifying the rate of increase in concentration of the gas in a bay where it was not injected. Although the enclosure resulted to be ca. 99% airtight, calculated leak rates were taken into account in the isoprene flux calculations by adding the leak flux to the calculated isoprene flux. Diffusion into the soil was also determined by tracer gases injections. During soil profile measurements, after SF_6 addition to the mesocosms, substantial increases of its concentration in the soil airspace were observed only up to 30 cm in depth. As the soil air volume is small (<1% in the agriforest) compared with the total volume of the bay, only ca. 0.2% of the total leak rate could be the result of diffusion into the soil.

Soil activity factor k

Isoprene consumption for the whole ecosystem in the dark and in small static soil chambers always followed an exponential decay function of the following type:

$$C = a \times e^{-kt}.$$

The constant k of the equation was calculated as $k = \mathrm{Ln}(C_2/C_1)/(t_2-t_1)$. Because it was not possible to separately quantify the physical phenomenon of isoprene diffusion into the air present in soil pores and in soil surface water, and the biological process of isoprene consumption by isoprene degrading bacteria, we called k the *'soil activity factor'*. The value of k is the measurement of the strength of the combined physical and microbial factors that are responsible for isoprene consumption by soil.

Rewetting experiment

A short rewetting experiment designed to test the dynamics of the soil isoprene sink in response to soil moisture was carried out in the cottonwood agriforest mesocosm maintained at ambient CO_2 concentrations. The experiment was carried out on May 30, towards the end of a drought experiment when soil volumetric water content was at its minimum ($<0.34\,m^3\,m^{-3}$). Three replicate static soil chambers (SC) connected to the PTR-MS were used and water was added in two steps ($100\,cm^{-3}$ at the start and $200\,cm^{-3}$ after 45 min) only to the soil surface inside the perimeter of each chamber.

Results and discussion

Absence of UV light transmission through the glass of B2L facility prevented isoprene oxidation in the atmosphere of both systems, and enclosure permitted automated estimation of ecosystem level sources and sinks of this trace gas. Isolation from rapid atmospheric oxidation caused daytime isoprene concentrations in the mesocosms to rise well above free atmospheric values, with average daytime concentration in non-stressed conditions ranging from $200\,nmol\,mol^{-1}$ (ppb) (late September) in the rainforest to 400 ppb (beginning of October) in the agriforest plantation growing at ambient CO_2 concentration. Atmospheric isoprene concentrations for natural ecosystems reported in the literature vary greatly depending on forest type, season, time of day, particular meteorological conditions at the moment of measurement, sampling height, and measurement method used. In tropical ecosystems they range typically between 3 and 7 ppb (Rasmussen & Khalil, 1988; Zimmerman *et al*., 1988; Rinne *et al*., 2002; Greenberg *et al*., 2004) with peak values of 12–30 ppb (Kesselmeier *et al*., 2002; J. P. Greenberg and A. B. Zimmerman, unpublished data; E. Pegoraro, P. R. Guenther and J. P. Greenberg, unpublished data), and in temperate ecosystems between 7 and 16 ppb (Baldocchi *et al*., 1995; Guenther *et al*., 1996; Goldstein *et al*., 1998; Fuentes & Wang, 1999; Fuentes *et al*., 1999) with peak values of as much as 140 ppb (B. Hopkins, personal communication, Washington State University, Pullman). Although concentrations obtained in the mesocosms of B2L were much higher than concentrations observed in natural ecosystems, they fell rapidly in the afternoon and night, permitting an accurate quantification of isoprene consumption by the ecosystem, an analysis that is difficult at ambient natural atmospheric concentrations.

Isoprene production

Representative diurnal courses of net isoprene production and uptake in the closed agriforest stands grown at 430, 800, and 1200 ppm CO_2, and in the tropical rainforest, before, during, and after a drought treatment, are shown in Fig. 2. The figure shows selected days in May 2003 with almost identical external incident photon fluxes. As expected from the well-characterized light-dependant diurnal pattern of isoprene emission (Harley *et al*., 1997) both experimental mesocosms were net isoprene sources during the day, the differences between daily courses predictably influenced by the earlier and later high PAR in the 430 and 1200 ppm treatments, respectively. All mesocosms were net isoprene sinks at night.

Under well-watered conditions in the agriforest stands, gross isoprene production (i.e. the total production flux minus the soil uptake) was inhibited by elevated CO_2 (Fig. 2a) and the highest emission fluxes of

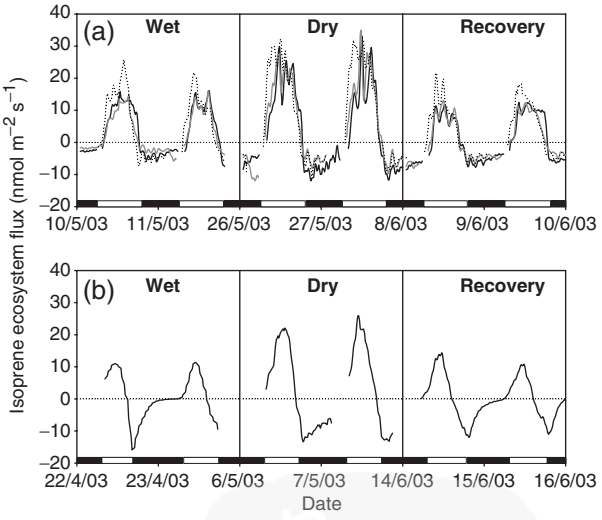

Fig. 2 Net isoprene fluxes over two wet, dry, and recovery days during a drought experiment in agriforest cottonwood plantations grown in three different atmospheric CO_2 conditions: 430 (dotted line), 800 (gray line), and 1200 (solid line) ppm (a), and in a synthetic model tropical rainforest mesocosm (b). Fluxes are given per unit area of soil surface. Daytime (white bar) and night-time (black bar) periods are indicated at the bottom of the chart.

isoprene were attained in the lowest CO_2 treatment (with an average maximum emission flux of 40.8 ± 1.6. nmol $m^{-2} s^{-1}$ compared with 21.9 ± 1.8 nmol $m^{-2} s^{-1}$ in the 1200 ppm CO_2 treatment). Drought dramatically increased net isoprene production in all forest stands mainly as a result of the drastic decline in soil uptake. However, drought also increased gross isoprene emission mainly because partial stomatal closure lowered intercellular CO_2 concentrations reducing the inhibitory effect of atmospheric CO_2 concentration (Pegoraro *et al*., 2004). Higher concentrations of isoprene accumulating in the mesocosms during drought often resulted in more rapid isoprene uptake in the system, but as shown below, at the same atmospheric isoprene concentration, drought reduced soil uptake of isoprene. Irrigation restored the production-uptake profiles to that of predrought controls within 3 days. A detailed evaluation of the effects of drought on leaf level isoprene emission from cottonwoods is given elsewhere (Pegoraro *et al*., 2004).

Experiments in the TRF maintained at 400 ppm CO_2 also revealed rapid emission and uptake of isoprene measured using the FIS method (Fig. 2b). The tropical rainforest had many isoprene-emitting species that achieved rates, based on leaf area, approaching those of the cottonwoods (Table 1). It differed from the agriforest stands by an active litter layer developed over the course of 12 years growth. The diurnal variation of isoprene fluxes differed from that in the litter-free monospecies stands of the agriforest by showing a stronger soil uptake, perhaps because the

Table 1 Average leaf isoprene emission rate (measured at leaf temperature of 32 °C and PAR of 1200 μmol $m^{-2} s^{-1}$) in nonstressed conditions for *Populus deltoides* Bartr. growing in the agriforest mesocosm at 430 μmol mol^{-1} CO_2, and for five canopy dominant species in the tropical rainforest mesocosm (± SE)

Species	Isoprene emission rate (nmol $m^{-2} s^{-1}$)
Chrysalidocarpus lutescens	19.2 ± 8.4 ($n = 9$)
Clitoria racemosa	58.3 ± 2.6 ($n = 58$)
Inga sapinoides	20.1 ± 2.0 ($n = 13$)
Pterocarpus indicus	23.0 ± 3.4 ($n = 12$)
Arenga pinnata	38.8 ± 3.3 ($n = 12$)
Populus deltoides	72.6 ± 7.1 ($n = 24$)

PAR, photosynthetic active radiation.

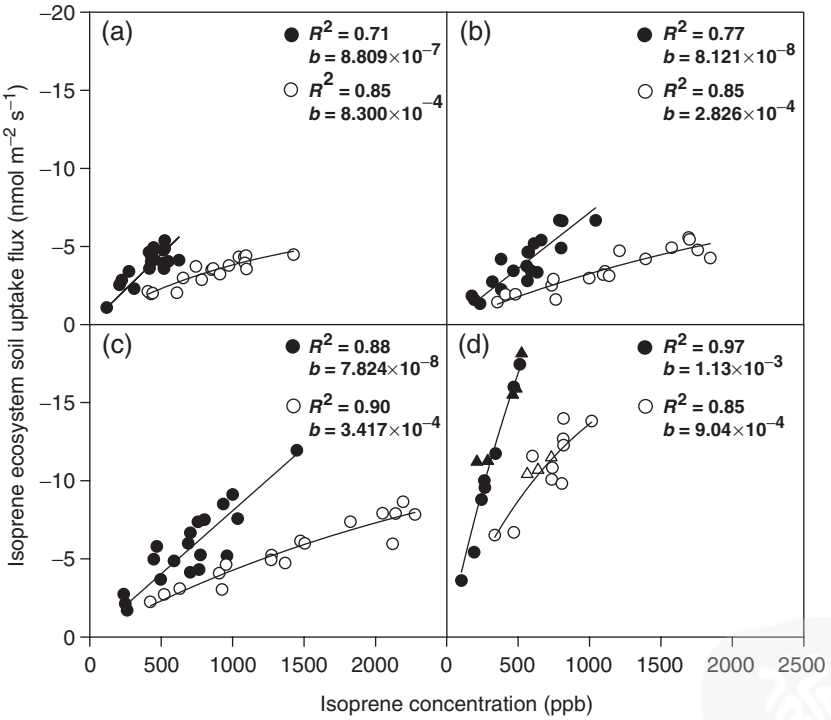

Fig. 3 Relationship between night-time mesocosm isoprene uptake flux (nmol $m^{-2} s^{-1}$) and maximum initial atmospheric isoprene concentration (ppb) in agriforest cottonwood plantations grown in three different atmospheric CO_2 treatments (430 (a), 800 (b), and 1200 ppm (c)), and in a synthetic model tropical rainforest mesocosm (d). The figure shows the relationship for wet (black symbols) and dry (white symbols) conditions during the drought experiment in the agriforest in 2003, and for two drought experiments in 2002 (circles) and 2003 (triangles) in the tropical rainforest mesocosm. All data were fitted to an exponential regression model ($F = a(1-\exp(-bC))$). Values for coefficient b and R^2 are also given.

litter layer increased the surface area of microbial occupancy thereby enhancing the uptake process. Furthermore, it showed a faster transition from production to consumption that took place earlier in the day at ca. 15:00, compared with ca. 18:00 in the agriforest system. This was probably the result of a combination of weaker isoprene emitters and stronger uptake rates in the tropical rainforest system when compared with the agriforest system.

Isoprene consumption

Isoprene concentrations in the agriforest mesocosms were adjustable between 200 and 1800 ppb by judicious use of the mesocosm exhaust system, and so we were able to explore the relationship of isoprene concentration and nocturnal uptake (Fig. 3a–c). Experiments in the tropical rainforest also showed a positive relationship between uptake rate and atmospheric isoprene concentration (Fig. 3d). It was clear that in both mesocosms, isoprene uptake in the dark increased rapidly with increasing isoprene concentration when the soil was wet, whereas it was less responsive when the soil was dry, showing that uptake was water limited. The slopes of the uptake curves in the three agriforest stands (each with ca. 550 m^3 of soil) in wet conditions were very similar, and the depression by drought was similar in all cases. The data suggest that although CO_2 concentration has a large effect on isoprene emission, it does not alter the isoprene sink capacity of the soil in the litter-free agriforest stands.

The relationships between soil moisture and soil activity factor k measured with the FIS method and with the small soil chambers for the agriforest stands is shown in Fig. 4a and b. As with previous soil respiration measurements (Murthy *et al.*, 2003), when chamber isoprene uptake rates were scaled to the surface area of the forest ecosystems, fluxes of isoprene were 1.5–3.0 times larger than actual leak-corrected system level fluxes. This discrepancy may reflect slow atmospheric transport and mixing, or measurement of isoprene metabolism in the soil beyond the confines of the soil chambers. The response of k to soil drying was very rapid, suggesting that processes in the top 3–5 cm

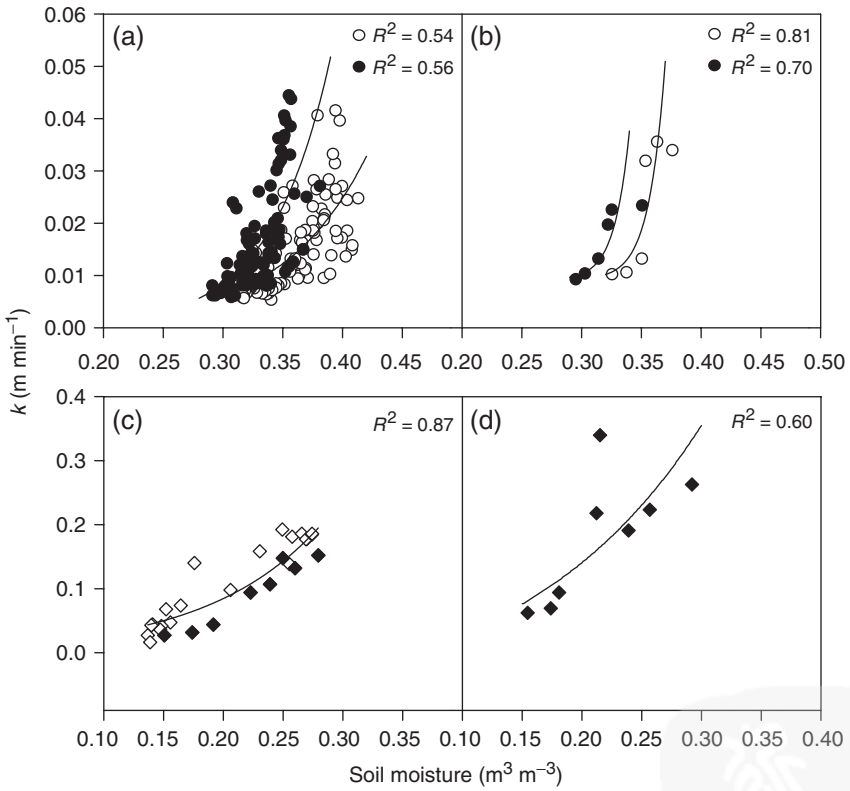

Fig. 4 Relationship between ecosystem soil activity factor k (m min^{-1}) and soil moisture (m^3 m^{-3}) in agriforest cottonwood plantations grown under two atmospheric CO_2 treatments: 430 (white circles) and 1200 (black circles) ppm (a), and in a synthetic model tropical rainforest mesocosm on selected days during two drought experiments in the years 2002 (white diamonds) and 2003 (black diamonds) (c), and relationship between the static chamber measurements of soil activity factor k (m min^{-1}) and soil moisture (m^3 m^{-3}) for the ambient (430 ppm) and elevated (1200 ppm) CO_2 treatments in agriforest cottonwood plantations (b), and for the synthetic model tropical rainforest (d), during the drought experiments of 2003.

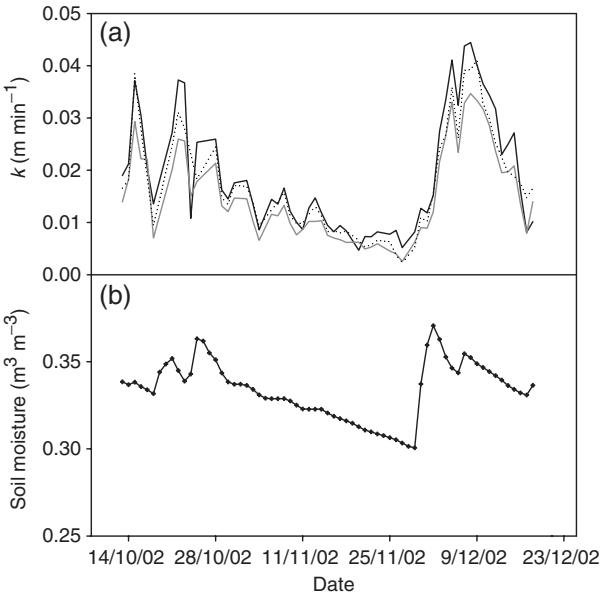

Fig. 5 Hourly average mesocosm soil activity factor k ($m\,min^{-1}$) in agriforest cottonwood plantations grown under three different atmospheric CO_2 concentrations: 430 (dotted line), 800 (gray line), and 1200 ppm (dotted line), during the 2002 drought experiment (a). Soil moisture ($m^3\,m^{-3}$) (solid line plus cross) is also shown (b).

of soil may be responsible for most of the isoprene uptake. This assumption was confirmed by measurements of isoprene concentration in the soil profile. In the agriforest stands, during the wet period only ca. 1% of the atmospheric isoprene concentration could be found at 5 cm depth, whereas during the dry period, as a result of decreasing soil isoprene uptake, as much as ca. 60% of the atmospheric isoprene reached 5 cm depth. This sensitivity to soil moisture led to strong oscillations of k during the drought period (Fig. 5). These were caused by unavoidable rewetting of the top centimeters of soil caused by condensation from mist used for controlling vapor pressure deficit (VPD) during a series of 3-day-cycles of high/low VPD treatment. Soil isoprene uptake activity for the agriforest stands showed a strong substrate limitation. Following mesocosm cooling during winter (Fig. 5) and because of leaf fall, isoprene concentrations were strongly reduced inside the agriforest stands, and although soil moisture was restored to field capacity at the end of March 2003, soil respiration and k took ca. 2 months longer to reach their optimum rates when the mesocosm was warmed in Spring 2003 (D. Lipson *et al*., 2004; unpublished data) and isoprene became available again after leaf expansion (data not shown).

The value of the soil activity factor k of ca. $0.2\,m\,min^{-1}$ found in well-watered conditions for the TRF in this study agrees remarkably well with values found by Karl *et al*. (2004) during field measurements in a tropical forest in Costa Rica. The relationship between soil activity factor k in the TRF and soil moisture (Fig. 4c and d) was similar to that in the agriforest; this soil system was evidently also very sensitive to soil water content. Similar to the agriforest, soil profile measurements showed that isoprene uptake occurred mostly in the top few centimeters of soil with only ca. 2% of the isoprene atmospheric concentration reaching 5 cm depth during the wet period. Again drought slowed down isoprene uptake and ca. 23% of the atmospheric isoprene concentration reached 5 cm depth during the dry period.

Rewetting experiment

The above interpretations were confirmed in the rewetting experiment that revealed a very rapid response (on the order of minutes) of the soil-sink strength to local changes in soil moisture content in the agriforest cottonwood mesocosm growing at ambient CO_2 concentrations (Fig. 6). At the beginning of the static soil chamber (SC) experiment, isoprene consumption by the dry soil was negligible. Within 18 min of applying $100\,cm^3$ of water to a $900\,cm^2$ dry soil surface covered by the measurement chamber, isoprene uptake increased by an order of magnitude, and further increased over the next 45 min. Addition of $200\,cm^3$ water to the same chambers further accelerated isoprene uptake, especially when measured after 120 min. In the absence of further additions of water, isoprene uptake declined to near zero within 12 h as surface soil dried out (data not shown).

In all the experiments described here we were unable to partition uptake of isoprene by the soil into diffusive and metabolic (microbial) components. However, the rapid responses to the wetting of the top centimeters of soil support the notion that soil uptake was largely microbial in origin. Diffusion into soil pores would be slowed in wet soil. In an early study, Griffiths & Birch (1961) showed that microbial populations have the ability to respond very quickly (within a few hours) to the rewetting of very dry soils. Isoprene utilization is fairly widespread among the common groups of soil bacteria, including Actinobacteria (e.g. *Arthrobacter*, *Norcardia*, *Streptomyces*, *Rhodococcus*), Firmicutes (e.g. *Bacillus*), and Proteobacteria (*Pseudomonas*). These groups are all well represented in clone libraries from B2L soil environmental DNA (D. Lipson *et al*., 2004; unpublished data). However, given the diverse physiological nature of bacteria, it is impossible to infer isoprene utilizing phenotypes based on similarity to known organisms from these data with any certainty (D. Lipson, personal communication, San Diego State

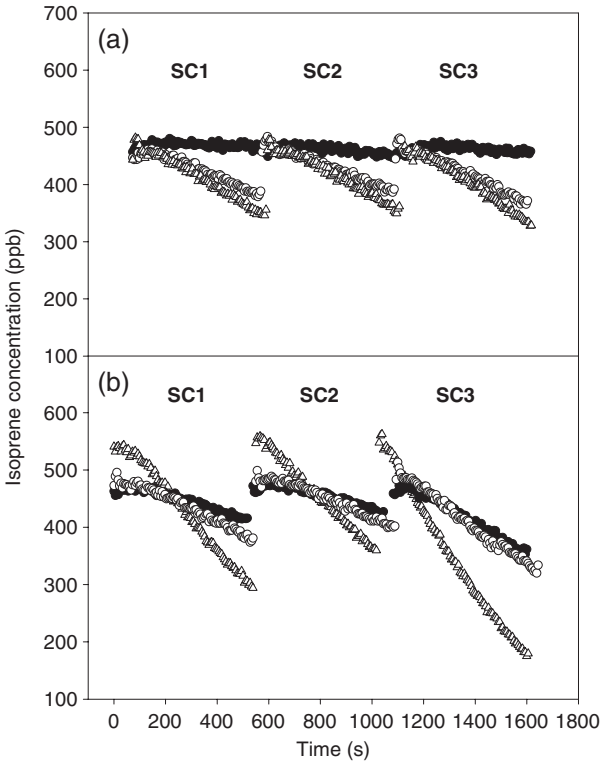

Fig. 6 Kinetics of isoprene uptake after rewetting dry soil in the agriforest cottonwood mesocosm (430 ppm CO_2). The data shown are real-time measurement by proton-transfer-reaction mass spectrometry of isoprene concentration changes inside the three replicate soil chambers (SC1, SC2, and SC3) for the different phases of the local soil rewetting experiment: dry soil (black circles) and after 18 and 45 min of applying 100 cm^{-3} of water (white circles and white triangles, respectively) (a), and after 18, 45, and 120 min of addition of another 200 cm^{-3} water (black circles, white circles, and white triangles, respectively) (b).

University, San Diego) and clearly much further research is needed.

Conclusions

Our enclosed system level experiments and soil chamber analyses demonstrate the potential magnitude of the isoprene soil sink and the effect that elevated atmospheric CO_2 concentration and drought have on this sink in the soil–plant atmosphere continuum. Concentrations of atmospheric isoprene attained in the enclosed, UV-free systems were one to two orders of magnitude higher than those reported in free atmosphere over vegetation (Rasmussen & Khalil, 1988; Zimmerman *et al*., 1988; Baldocchi *et al*., 1995; Guenther *et al*., 1996; Goldstein *et al*., 1998; Fuentes *et al*., 1999; Kesselmeier *et al*., 2002; Rinne *et al*., 2002). This study confirms the sink capacity of soils for atmospheric isoprene (Cleveland & Yavitt, 1997, 1998) and suggest that the soil bacterial metabolism of this hydrocarbon is not limited to recycling of soil-derived substrate (Fall & Copley, 2000). Furthermore, in well-watered conditions the values for k measured in the TRF in this study were similar to the results found by Karl *et al*. (2004) in Costa Rica. Although Karl *et al*. (2004) found a daytime isoprene deposition value of 0.096 m min^{-1}, the nighttime estimate was 0.12–0.18 m min^{-1}. These numbers agree remarkably well given that the uncertainties are about a factor of two. In contrast, Cleveland & Yavitt (1997) estimated a value of 0.006 m min^{-1} for tropical soils. Although there are still great uncertainties and current estimates of isoprene dry deposition might be substantially underestimated, both our Biosphere 2 measurements and the results from field measurements of Karl *et al*. (2004) would indicate that the deposition velocity may be much higher than that previously estimated (Cleveland & Yavitt, 1997).

If we assume that the relationship between soil uptake flux and ambient concentration reported in this study is still valid at natural isoprene concentrations, using the estimates for isoprene emissions from different mesocosms given by Guenther *et al*. (1995), we can estimate soil consumption of isoprene in a tropical rainforest ecosystem under free atmospheric conditions to be 0.94 Tg C yr^{-1} (assuming an average ambient concentration of 5 ppb (Rasmussen & Khalil, 1988; Zimmerman *et al*., 1988; Rinne *et al*., 2002) and 16 h a day of active soil sink) which is ca. 1% of the estimated total isoprene emission (Guenther *et al*., 1995), whereas in a temperate deciduous ecosystem the isoprene soil sink would be equal to 0.06 Tg C yr^{-1} (assuming 10 ppb (Baldocchi *et al*., 1995; Guenther *et al*., 1996; Goldstein *et al*., 1998; Fuentes & Wang, 1999; Fuentes *et al*., 1999) as an average ambient concentration and 16 h a day and 250 days a year of active soil sink). The latter is about 2% of the estimated total emission (Guenther *et al*., 1995). This indicates that soil uptake may be modest, although tests need to be made with real soils that may have developed a more mature microbial flora. Nevertheless, the soil sink needs to be taken into account for a comprehensive estimate of the global isoprene budget. It is possible that the relationship reported here (Fig. 3) does not pass through the origin, but instead isoprene fluxes reach zero at some compensation point at finite ambient isoprene concentration, in which case the fluxes estimated above may be overestimates.

Because many commercial hardwood agriforest species emit high levels of isoprene, proliferation of agriforest plantations may lead to locally elevated isoprene concentrations as high as '140 ppbv during the hottest days when winds are low' (B. Hopkins,

personal communication, Washington State University, Pullman). In these exceptional situations, the atmospheric sink for isoprene may saturate, and the soil may become an important sink for isoprene. Furthermore, our results show that, unlike soil respiration, the soil isoprene sink in the B2L agriforest is insensitive to elevated CO_2 (Murthy *et al*., 2003). Our data demonstrate that drought both stimulates emission and slows soil uptake, suggesting that in future, potentially hotter, drier environments, higher CO_2 may not mitigate isoprene emission as much as previously suggested (Rosenstiel *et al*., 2003; Pegoraro *et al*., 2004). The large-scale controlled environment experiments described here will help parameterize further model evaluations of the isoprene cycle. However, it is clear that studies in natural systems are required, and the online measurement systems deployed in B2L may be especially helpful at the lower concentrations expected in free atmosphere environments.

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