Environmental Control of Isoprene Emission: from Leaf to Canopy Scale

Emiliano Pegoraro

A thesis submitted for the degree of Doctor of Philosophy
The University of Edinburgh
2004

Declaration

This thesis has been composed by myself from the results of my own work, except where stated otherwise, and has not been submitted in any other application for a degree.

Emiliano Pegoraro

Emiliano Pegoraro
November, 2004

Per Anna,
e Ana.

Acknowledgements

Above all, I wish to thank with all my heart Dr. Ana Rey, for being always so enthusiastic and dedicated in helping me in my scientific work and thinking with endless energy and friendship, and for supporting me in the dark times with infinite love and patience. GRAZIE!!!!!

I also wish to give a very special thank to the people of the ACD division at NCAR (Boulder, USA), Dr. Alex Guenther, Jim Greenberg, Dr. Peter Harley and Pierre Prevost. They guided me through my first steps and thought me how to walk in a straight position through the thick jungle of isoprenoids. Especially Dr. Alex Guenther who has been extremely nice and showed a kind respect for my work, which helped me to get a little self-confidence and not to get so destroyed by my frequent mistakes, and Jim Greenberg which irony and sarcasm helped me to have a good laugh even in the most panicking situations.

At Biosphere 2, I would especially like to thank Prof. Barry Osmond, who has made possible most of this thesis with his support, both practical and moral. I am also very, VERY grateful to all guys at B2 for their help in my experiments and for being such nice friends: Leith, Ed, Katie, Greg, Uwe, Ralf, Aline, etc.

At Edinburgh University I wish to thank Prof. John Grace and Dr. Yadvinder Malhi for supervising my work. A special thank to Dr. Robert Clement and Dr. Jordi Martinez-Vilalta, for being always ready to help enthusiastically and for their friendship and craziness, that cheered me up in frantic moments…. I also wish to thank all the other friends and colleagues at the ex-IERM, Patrick Meir, Franz Conen, Keith Smith, etc and especially the “scot spice” Caz!!

And a great THANKS! to all the friends that have brightened and warmed up many of the grey days, dark evenings and cold nights during my stay in Edinburgh: Daniela, Chiara, Emiliano, Michele, Manuel, Christos, etc.

Abstract

Isoprene is the most abundant volatile organic compound (VOC) emitted from vegetation, mainly trees. Because it plays an important role in tropospheric chemistry leading to formation of pollutants and enhancing the lifetime of the greenhouse gas methane, concern about the response of isoprene emissions to the rise in atmospheric CO_2 concentration and global climate change has been increasing over the last few years. The consequences of predicted climate change will have complex repercussions on global isoprene emission. The increasing atmospheric CO_2 *per se* will have direct effects on terrestrial vegetation since CO_2 is the substrate of photosynthesis. Because photosynthesis is limited by CO_2 at current ambient concentrations, an increase in CO_2 is expected to increase leaf biomass (i.e. isoprene emitting surface). Predicted warmer climate, extended drought periods, the possible shift in plant species in favour of isoprene emitters and the increase in length of growing season, may cause an increase in global isoprene emissions with profound perturbations of air quality and the global carbon cycle.

The aim of this thesis was to investigate the effect of environmental variables such as light, temperature, drought and leaf-to-air vapour pressure deficit (VPD), and the short- and long-term effect of atmospheric [CO_2] on isoprene emission from temperate and tropical tree species. Both leaf and whole ecosystem level fluxes were studied. At the leaf scale, a short-term experiment with leaves of potted two-year old trees of *Quercus virginiana* was carried out, exposing plants to two drying-rewatering cycles. Leaf isoprene emission fell, but the process was considerably less sensitive to water stress than photosynthesis and stomatal conductance. In drought conditions, the large reduction in photosynthesis caused the percentage of fixed carbon lost as isoprene to increase as plants became more stressed, reaching peaks of 50% when photosynthesis was almost zero. Isoprene emissions also showed a strong negative linear relationship with pre-dawn leaf water potential (Ψ_{leaf}). In another experiment carried out at the large enclosed facility of Biosphere 2 (B2L, Arizona, USA), studying isoprene emission from leaves of three-year-old plants of *Populus deltoides* grown at three CO_2 atmospheric concentrations (430, 800 and 1200 µmol

mol^{-1} CO_2) in non-stressed conditions, instantaneous increases in atmospheric [CO_2] always resulted in a reduction of isoprene emission and a stimulation of photosynthesis. Moreover, in the long-term, the CO_2 inhibition effect for isoprene emission became a permanent feature for plants growing under elevated [CO_2]. Again, isoprene emission was less responsive to drought than photosynthesis. Both water-stress and high VPD strongly stimulated isoprene emission and depressed photosynthetic rate as a result of stomatal closure and the resulting decreases in intercellular [CO_2] (C_i). This also led to a dramatic increase in the proportion of assimilated carbon lost as isoprene. The effect of atmospheric elevated [CO_2] and its interaction with high VPD and water stress on ecosystem gross isoprene production (GIP) and net ecosystem exchange of CO_2 (NEE) in the *Populus deltoides* plantations was also studied. Although GIP and NEE showed a similar response to light and temperature, NEE was stimulated by elevated CO_2 by 72% and depressed by high VPD, while GIP was inhibited by elevated CO_2 by 58% and stimulated by high VPD. Similar to what was observed at leaf level, under water stress conditions GIP was stimulated in the short term and declined only when the stress was severe, whereas NEE started to decrease from the beginning of the experiment. This contrasting response led the percentage of assimilated carbon lost by the ecosystem as isoprene to increase as water stress progressed from 2.5% and 0.6% in well-watered conditions to 60% and 40% for the ambient and the elevated CO_2 treatments, respectively. Again, we found water limitation and high VPD off-set the inhibitory effect of elevated CO_2, leading to increased isoprene emissions. The effect of a mild water stress on GIP and gross primary production (GPP) was also observed in the model tropical rainforest mesocosm of B2L. Although GPP was reduced by 32% during drought, GIP was not affected and correlated very well with both light and temperature. The percentage of fixed C lost as isoprene tended to increase during drought because of the reduction in GPP. Consumption of isoprene by soil was observed in both systems. The isoprene sink capacity of litter-free soil of the agroforest stands showed no significant response to different CO_2 treatments, while isoprene production was strongly depressed by elevated atmospheric [CO_2]. In both mesocosms, drought suppressed the sink capacity, but the full sink capacity of dry soil was recovered within a few hours upon rewetting.

In summary, elevated CO_2 increased biomass production and photosynthesis while depressing isoprene production. However, both drought and VPD may off-set the CO_2 effect and lead to enhanced isoprene emission. We conclude that the overall effect of global climate change could be of enhancing global isoprene emissions while depressing the soil sink, and that the 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.

Table of Contents

PART I: LEAF-SCALE EXPERIMENTS

CHAPTER 3. Effect of elevated CO_2 concentration and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought....... 59

PART II: CANOPY-SCALE EXPERIMENTS

CHAPTER 4. The interacting effects of elevated atmospheric [CO_2], drought and leaf-to-air vapour pressure deficit on ecosystem isoprene fluxes 85

CHAPTER 5. Drought effects on isoprene production and consumption in the Biosphere 2 tropical rainforest mesocosm 109

CHAPTER 6. The effect of elevated atmospheric CO_2 and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm 135

List of Symbols and Abbreviations

SYMBOL	DEFINITION	UNITS
ρ_s	Bulk density of the soil	g cm^{-3}
ρ_w	Density of water	g cm^{-3}
C	Isoprene concentration	µmol mol^{-1}
C_A	Assimilated carbon	moles
C_i	Intercellular [CO_2]	µmol mol^{-1}
C_{iso}	Carbon emitted as isoprene	nmoles
F_{atm}	Flux due to change in CO_2 storage in a mesocosm	µmol m^{-2} s^{-1}
F_{in}	Flux of CO_2 entering a mesocosm	µmol m^{-2} s^{-1}
F_{leak}	Flux of CO_2 leakage across the isolation curtain	µmol m^{-2} s^{-1}
F_{out}	Flux of CO_2 leaving a mesocosm	µmol m^{-2} s^{-1}
F_{tank}	Flux of CO_2 injected inside a mesocosm	µmol m^{-2} s^{-1}
IF_{soil}	Soil isoprene uptake flux	nmol m^{-2} s^{-1}
k	Isoprene deposition velocity Soil activity factor Proportionality constant of the exponential decay function describing isoprene concentration decay	$time^{-1}$
M_d	Soil dry mass	g
M_f	Soil fresh mass	g
t	Time	Minutes
T_{air}	Air temperature	°C
T_{leaf}	Leaf temperature	°C
Ψ_{leaf}	Leaf water potential	MPa
β	Standardised parameters estimates	
θ	Soil volumetric water content	m^3 m^{-3}

ABBREVIATION	DEFINITION
B2	Biosphere 2
C	Control plants
ca.	circa
DMAPP	Dimethylallyl pyrophosphate
DOXP	1-Deoxy-D-xylulose-5-phosphate
FIS	Fast isoprene sensor
GC	Gas chromatography
GIP	Gross isoprene production
GPP	Gross primary productivity
IFM	Intensive forest management mesocosm at B2
IPCC	UN Intergovernmental Panel on Climate Change
IPP	Isopenthenyl diphosphate
LAI	Leaf area index
MEP	2-C-methyl-D-erythritol-4-P
MS	Mass spectrometry
MVA	mevalonic acid (MVA)
NEE	Net ecosystem exchange
NIE	Net isoprene exchange
NMR	Nuclear magnetic resonance
PANs	Peroxyacylnitrates
PAR	Photosynthetically active radiation
PEP	Phosphoenolpyruvate
SOC	Soil organic carbon
T	Treatment plants
TRF	Tropical rainforest
VOC	Volatile organic compound
VPD	Leaf-to-air vapour pressure deficit

List of publications

CHAPTER 2:

Pegoraro, E., Rey, A., Greenberg, J., Harley, P., Grace, J., Malhi, Y. and Guenther, A. (2004). Effect of drought on isoprene emission rates from leaves of *Quercus virginiana* Mill., *Atmospheric Environment* **38**, 6149-6156.

CHAPTER 3:

Pegoraro, E., Rey, A., Bobich, E., Barron-Gafford, G., Malhi, Y. and Murthy, R. (2004). The effect of elevated [CO_2] and VPD on isoprene emission rates from leaves of *Populus deltoides* Bartr. trees during a drought experiment, *Functional Plant Biology* **31**, 1-11.

CHAPTER 4:

Pegoraro, E., Rey, A., Barron-Gafford, G., Monson, R., Malhi, Y. and Murthy, R. The interacting effects of elevated atmospheric [CO_2], drought and leaf-to-air vapour pressure deficit on ecosystem isoprene fluxes (*Oecologia*, in press).

CHAPTER 5:

Pegoraro, E., Rey, A., Abrell, L., Vanharen, J., Guanghui, L. Drought effects on isoprene production and consumption in the Biosphere 2 tropical rainforest mesocosm (submitted to *Global Change Biology*).

CHAPTER 6:

Pegoraro, E., Abrell, L., Vanharen, J., Malhi, Y., Guanghui, L. and Murthy, R. Effects of elevated atmospheric [CO_2] and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm (*Global Change Biology*, in press).

CHAPTER 1

Introduction

1.1. Introduction

Most living systems directly or indirectly exchange chemical compounds with the atmosphere. Although in plants this exchange is clearly dominated by the emission and uptake of carbon dioxide water vapour and oxygen, they also produce and emit a class of *trace gases* denominated volatile organic compounds (VOCs). Some of these biogenic VOCs are emitted in surprisingly large amounts and have high enough chemical reactivity to represent one of the most important ways in which plant processes influence the composition and functioning of the atmosphere (Lerdau *et al.* 1997).

Isoprene (2-methyl 1,3-butadiene, C_5H_8) is the single most abundant biogenic hydrocarbon emitted from plants and exerts profound effects on atmospheric chemistry (Harley *et al.* 1999; Fuentes *et al.* 2000). It is emitted in both temperate and tropical ecosystems (Zimmerman *et al.* 1988; Guenther *et al.* 1995), with an estimated global flux of 5.1×10^{14} g C per year or about 1% of the respiratory CO_2 flux (Guenther *et al.* 1995). It is a photochemically reactive and highly volatile compound produced enzymatically in the light by chloroplasts and is emitted from leaves mainly through stomata. Isoprene production is linked to photosynthesis by the fact that *ca.* 80% of the carbon in the isoprene molecule comes from fresh photosynthate (Karl *et al.* 2002; Affek and Yakir 2003; Schnitzler *et al.* 2004). Since the ultimate atmospheric fate of isoprene is oxidation to carbon dioxide, isoprene emission also constitutes a source of atmospheric carbon and can thus play a role in global carbon cycling. For emitting species, a significant fraction of recently fixed carbon, typically 0.5-2% at 30^{o}C in the light but much higher when temperatures exceed 30^{o}C (Monson and Fall 1989; Sharkey *et al.* 1991; Baldocchi *et al.* 1995;

Harley *et al.* 1994; Harley *et al.* 1996), is immediately lost to the atmosphere as isoprene and its global emission is estimated at 0.5 Gt of carbon per year (Guenther *et al.* 1995). This can be a significant contribution to the actual carbon budget of forests, particularly from tropical ecosystems.

Because isoprene plays an important role in the oxidation capacity of the atmosphere, the earth's climate system, and the global carbon budget, interest in obtaining accurate estimates of isoprene emissions has increased over the last few years and a considerable effort has been made to improve our understanding of its biosynthesis, the environmental control on isoprene emissions, and its atmospheric chemistry.

1.1.1. The problem of climate change

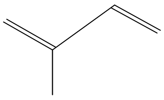
Since the industrial revolution at the end of the nineteenth century, changes in land-use, deforestation and combustion of fossil fuels have been the major contributors to the observed dramatic increase of carbon dioxide (CO_2) concentration in the atmosphere (Tans *et al.* 1989; Keeling *et al.* 1995). This increase is expected to continue through this century (Watson R.T. *et al.* 1996). The Intergovernmental Panel on Climate Change (IPCC) predicted that atmospheric CO_2 concentration ([CO_2]) will rise from the present level of *ca.* 370 μmol mol^{-1} to about 530 μmol mol^{-1} CO_2 by 2050 and to *ca.* 700 μmol mol^{-1} by the end of this century (Keeling *et al.* 1995; Watson R.T. *et al.* 1996). The concern about the increasing [CO_2] in the atmosphere arises from the important role that this gas plays in global warming and from the possible positive feedbacks involving the major carbon pools of the biosphere: vegetation and soils. Rising [CO_2] and associated feedbacks on the water cycle are expected to result in an increase of the mean temperature of the Earth by a few degrees (Ramanathan 1988; Hansen *et al.* 1988). This, in turn will have complex repercussions on the Earth's global climate with important consequences for global precipitation patterns, global evaporation, etc. (Manabe and Stouffer 1994). While global climate change itself will have major consequences on vegetation, the rise in atmospheric [CO_2] will also have important effects on vegetation. At current atmospheric [CO_2], photosynthesis is limited by CO_2 (Lawlor and Mitchell 1991), and therefore an increase in atmospheric [CO_2] is expected to increase photosynthesis. In turn, because photosynthesis is the major determinant of the growth response and productivity of the whole plant, an increase of photosynthesis is expected to enhance growth (Bowes 1991; Poorter 1993). At the same time, the increase in global temperature may also affect global carbon cycle by increasing the release of respired carbon, particularly from the large carbon pools at boreal latitudes, which will represent a positive feedback on global warming.

Many studies have been published in recent years on the responses of trees to elevated atmospheric [CO_2] and great progress has been made in the mechanistic understanding of the physiological responses of different species (e.g. Medlyn *et al.* 1999). Some model scenarios suggest that future increases in temperature and decreases in precipitation will cause the terrestrial carbon sink to become a source later this century (Cox *et al.* 2000). However, such models do not deal with the emission of volatile organic compounds by plants and the effect that this might have on the carbon cycle and on atmospheric chemistry (Fehsenfeld *et al.* 1992; Lerdau *et al.* 1997; Fuentes *et al.* 2000; Monson and Holland 2001; Guenther 2002). As we will see in detail in the following chapters, the rise in atmospheric [CO_2] will also have complex repercussions on the emissions of isoprene by plants. While on the one hand, elevated [CO_2] has the effect of inhibiting isoprene emission, on the other hand, an increase in leaf biomass means an increase in isoprene emitting surface. Furthermore, because isoprene emission is very sensitive to temperature (Monson and Fall 1989; Singsaas and Sharkey 2000), and because water stress and high water vapour deficit (VPD) may counteract the CO_2 inhibiting effect (Rapparini *et al.* 2004, Chapter 3 and 4), the overall result of expected future climate change may be an increased isoprene production that could result in significant perturbations on atmospheric chemistry and the global carbon balance cycle (Monson *et al.* 1991; Guenther 2002). Finally, the effects of climate change on global isoprene emission are further complicated by possible shifts in species composition in favour of isoprene emitters and may increase the length of the growing season (Turner *et al.* 1991; Lerdau *et al.* 1997; White *et al.* 1999).

1.1.2. Chemistry of isoprene

It was only in 1957 that Sanadze first identified isoprene (2-methyl 1,3-butadiene) as a volatile product synthesized and emitted into the atmosphere after exposure to light and under conditions of restricted CO_2, from many woody species and some grasses and ferns. Since then, phytogenous isoprene has been identified chemically by mass spectral analysis (MS), gas chromatography (GC), and nuclear magnetic resonance (NMR) studies (Sharkey *et al.* 1991). The chemical properties of isoprene are shown in Table 1.1.

Table 1.1. Isoprene: principal chemical properties.

Compound Name	isoprene
Chemical formula	C_5H_8
Molecular weight (g mol^{-1})	68.12
Boiling point (K)	307
Chemical structure	

Isoprene has a principal role in regulating the oxidation potential of the troposphere because of its high reactivity with hydroxyl radical (OH), the principal tropospheric oxidising agent (Wofsy 1976; Crutzen and Fishman 1977; Greenberg *et al.* 1985; Fehsenfeld *et al.* 1992). This has several implications for the habitability of the biosphere. In the first place, there is the relationship between the oxidation of isoprene and the generation of atmospheric pollutants such as ozone (O_3), and other oxidants such as organic peroxides (Atkinson 1997, and references therein; Chen *et al.* 1998, Atkinson and Arey 1998, Atkinson 2000). A simplified scheme is shown in Figure 1.1.

1.1.2.1. *Control over production and destruction of O_3*

Isoprene oxidation by OH radicals and O_3 produces a variety of organic peroxy radicals (RO_2), which may react with other RO_2, HO_2 or NO. In urban areas, in the presence of ultra violet (UV) radiation and high concentrations of NO_x (> 30-50 ppt), the peroxy radicals react almost exclusively with NO, leading to O_3 formation. In remote areas, in the presence of low levels of NO_x, the direct reaction of isoprene with O_3 together with the preferential reaction of RO_2 molecules with each other or with O_3, results in net O_3 destruction.

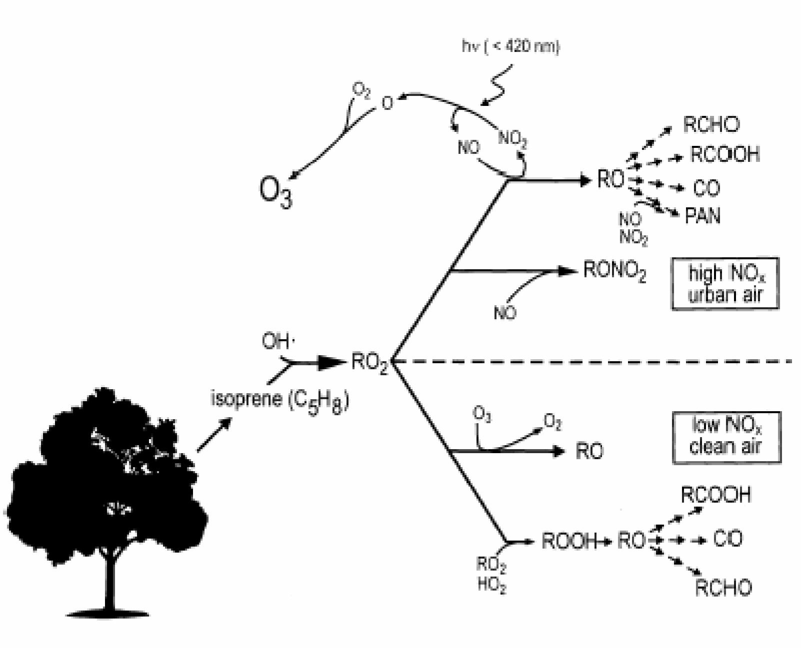


Figure 1.1. Simplified diagram illustrating the atmospheric reactions and potential products originating from oxidation of isoprene by the OH radical. The top half of the diagram shows the situation in polluted air where high concentrations of nitrogen oxides are present; the bottom half illustrates the situation in clean air (Harley *et al.* 1999).

1.1.2.2. *Control over formation of organic nitrates and PANs*

Isoprene oxidation in the presence of NO_x also leads to the formation of organic nitrates and peroxyacylnitrates (PANs). Organic nitrates are lost via wet or dry deposition, providing an effective mechanism for removal of NO_x. PANs are phytotoxic and decompose at high temperatures releasing NO_x. When formed at low temperatures they can be transported over great distances before descending in warmer regions without NO_x sources and contributing to O_3 formation.

1.1.2.3. *Control over deposition of organic acids to remote sites*

Isoprene oxidation also leads to the formation of weak organic acids (such as formic and acetic acid), which represent a large fraction of total acidity in precipitation in remote areas, where the contribution of sulphuric and nitric acid may be very low.

1.1.2.4. *Control on particulate formation*

It has been known for quite some time that ozonolysis of terpenes in the atmosphere also leads to particulate formation (Went 1960). Because atmospheric particles including organic aerosol play an important role in the radiation balance on the earth by scattering or absorbing light and acting as cloud condensation nuclei (Andreae and Crutzen 1997; Kulmala *et al.* 2004), over the last year much research has focused on the understanding of aerosol yields and associated chemical composition from photochemical reactions of isoprene and other volatile organic compounds (Hoffmann *et al.* 1997; Griffin *et al.* 1999; Di Carlo *et al.* 2004; Claeys *et al.* 2004).

1.1.2.5. *Isoprene and the global warming*

A second implication of isoprene oxidation for biospheric habitability is its influence on the global warming of the atmosphere, as it can potentially reduce the effectiveness of methane removal from the troposphere. This is because, depending on the abundance of isoprene, methane and isoprene molecules compete as alternative sinks for reaction with OH, but kinetic characteristics favour the reaction with isoprene over that with methane (Greenberg *et al.* 1985; Zimmerman *et al.* 1988).

Since isoprene emissions are highly temperature sensitive (Monson and Fall 1989; Loreto and Sharkey 1990; Monson *et al.* 1992), future increases in global temperature as a result of an increase in atmospheric concentrations of greenhouse gases such as methane could result in greater isoprene mixing ratios. This, in turn, could result in a reduced potential for removing tropospheric methane, resulting in even further global warming. Such a feedback loop (Figure 1.2) is especially relevant to future predictions of global warming, given the significantly higher potential of methane as a greenhouse gas, compared to carbon dioxide. The impact on tropospheric ozone, a greenhouse gas, is a second but equally important effect of isoprene oxidation. On a per molecule basis, these effects are further enhanced indirectly by other consequences of a global rise in temperature: a regional shift in precipitation, biomass/plant species redistribution and increased growing season length (Turner *et al.* 1991; Lerdau *et al.* 1997; White *et al.* 1999).

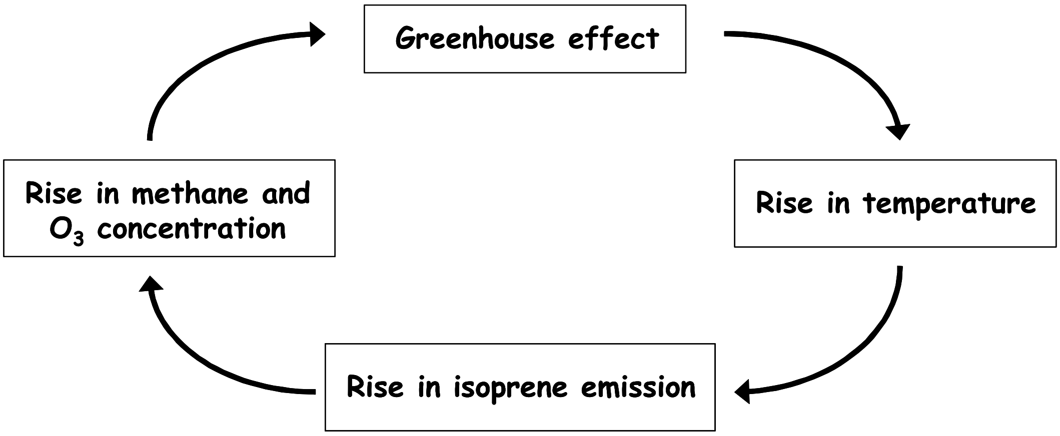


Figure 1.2. Schematic representation of the interaction of climate warming with isoprene and the possible isoprene positive feedback as a consequence of its influence on methane and O_3 concentrations in the atmosphere.

1.1.3. Control on isoprene emission

1.1.3.1. *Biosynthesis*

The universal 5-C precursor for isoprene formation is dimethylallyl pyrophosphate (DMAPP), an isomer of isopentenyl pyrophosphate (IPP), until recently thought to be formed exclusively via the mevalonic acid (MVA) pathway engaged in the cytosol (McGarvey and Croteau 1995). Latest studies demonstrate the existence of a new pathway taking place in the chloroplast and using 1-deoxy-D-xylulose-5-phosphate (DOXP) as intermediate (Lichtenthaler 1998) (Figure 1.3). This recently discovered “DOXP pathway” resolved some discrepancies that arose when it was believed that the MVA pathway was responsible for all isoprenoid biosynthesis. The most notable of these was the poor incorporation efficiency of labelled CO_2 in the isoprene molecule and the inability of inhibitors of the MVA pathway to block isoprenoid synthesis (Bach and Lichtenthaler 1983). The elimination of pyrophosphate from DMAPP leads to the formation of isoprene. This last step is catalysed by the enzyme isoprene synthase (Silver and Fall 1991; Wildermuth and Fall 1996). Isoprene synthase activity correlates with the onset of isoprene emission during leaf development (Kuzma and Fall 1993) and with seasonal variation in isoprene emission (Schnitzler *et al.* 1997). Observed light and temperature dependencies of isoprene emission are also interpretable in the context of isoprene synthase, the levels and activity of which seem to control emissions over a wide range of conditions.

Isoprene biosynthesis is clearly linked to the photosynthetic process, as shown by the light-dependence and light-saturation of the isoprene emission rate (Monson and Fall 1989). This linkage is also clearly shown by the rapid $^{13}CO_2$-labelling of the *ca.* 80% of the isoprene molecule by chloroplastic fresh assimilated carbon, and is now clear that

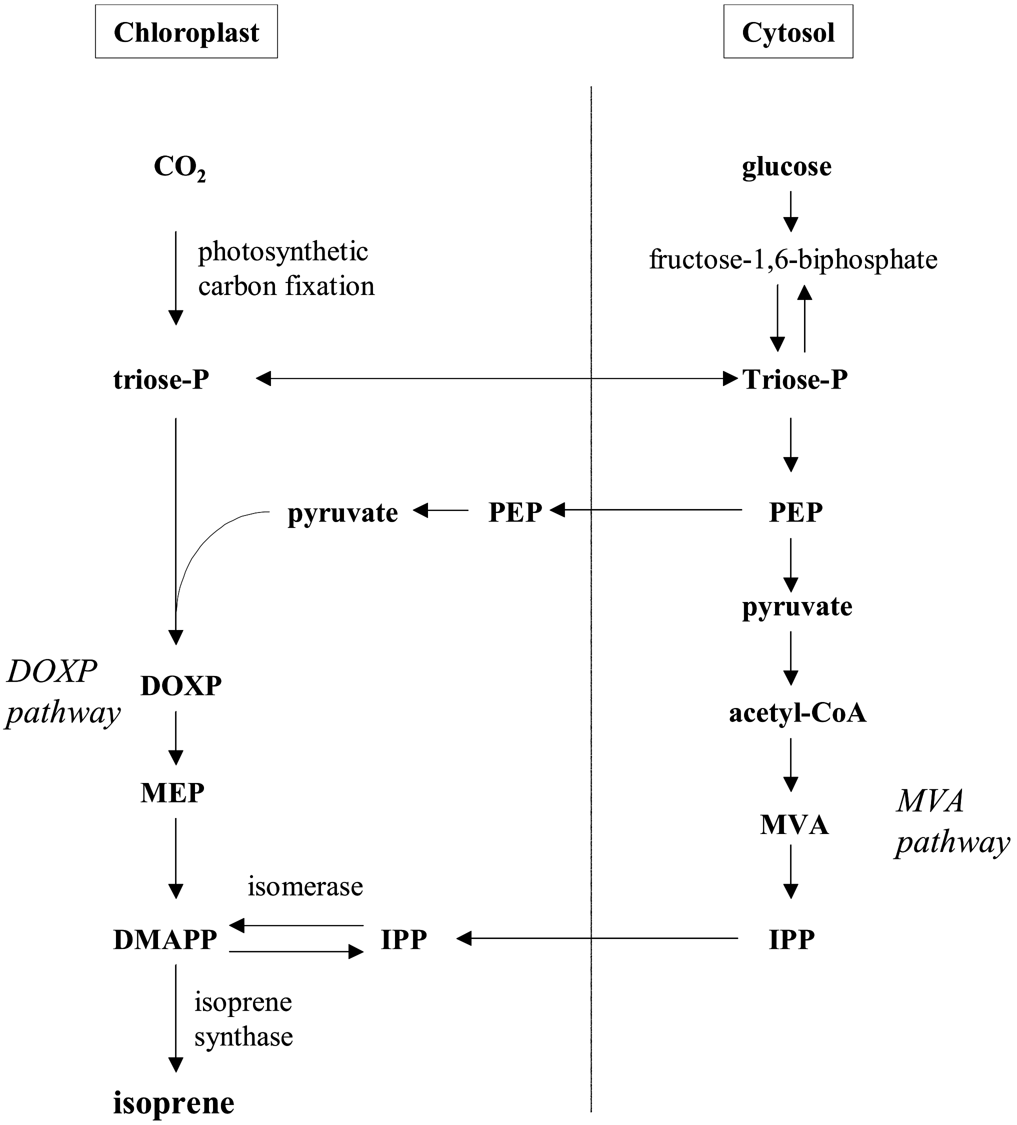


Figure 1.3. Schematic representation of the new mevalonate-independent isoprene biosynthesis pathway, and possible coupling to cytosolic glucose metabolism and IPP through the mevalonate-dependent pathway. DOXP: 1-Deoxy-D-xylulose-5-phosphate; IPP: isopenthenyl diphosphate; MEP: 2-C-methyl-D-erythritol-4-P; PEP: phosphoenolpyruvate.

ca. 20% of the carbon in isoprene comes from extra-chloroplastic sources such as cytosolic metabolite pools or breakdown of starch (Karl *et al.* 2002; Affek and Yakir 2003; Schnitzler *et al.* 2004). However, recent evidence (Loreto *et al.* 2004) suggests that these two pools of available carbon for isoprene formation are not cross-linked even when the chloroplastic pool (thus photosynthesis) is inhibited. Although the controls over DMAPP synthesis in the chloroplast are poorly understood, recent results indicate that a significant fraction of carbon entering the chloroplastic DMAPP pool is extra-chloroplastic in origin (Karl *et al.* 2002) and may arise from cytosolic phosphoenolpyruvate (PEP) through the MVA pathway. Therefore, metabolic competition for PEP could have an impact on isoprene emission. Rosenstiel et al. (2003) recently demonstrated that elevated atmospheric CO_2 might inhibit isoprene production by reducing cytosolic PEP availability because of the increased conversion of PEP to pyruvate to provide the necessary substrate for increased mitochondrial photorespiration. Photosynthesis gives, photosynthesis takes!

Understanding the mechanisms by which isoprene is biosynthesised is important for modelling isoprene emissions in situations where direct measurement of emissions is difficult or impossible. As more information becomes available about how the new pathway is regulated it will be possible to improve the mechanistic aspects of isoprene emission models.

1.1.3.2. *Short and long-term environmental control*

Response to light

In the short-term, the light dependence of isoprene emission has been observed to be similar to that of photosynthesis (Figure 1.4), but with isoprene emission presenting a higher saturation level of light (Sharkey *et al.* 1991; Harley *et al.* 1999). Its emission is negligible in the dark, but begins rapidly upon illumination, being fully induced after 30 minutes (Loreto and Sharkey 1990; Monson *et al.* 1991). Once induced, the timescale of its response to photosynthetically active radiation (PAR) is in the order of seconds.

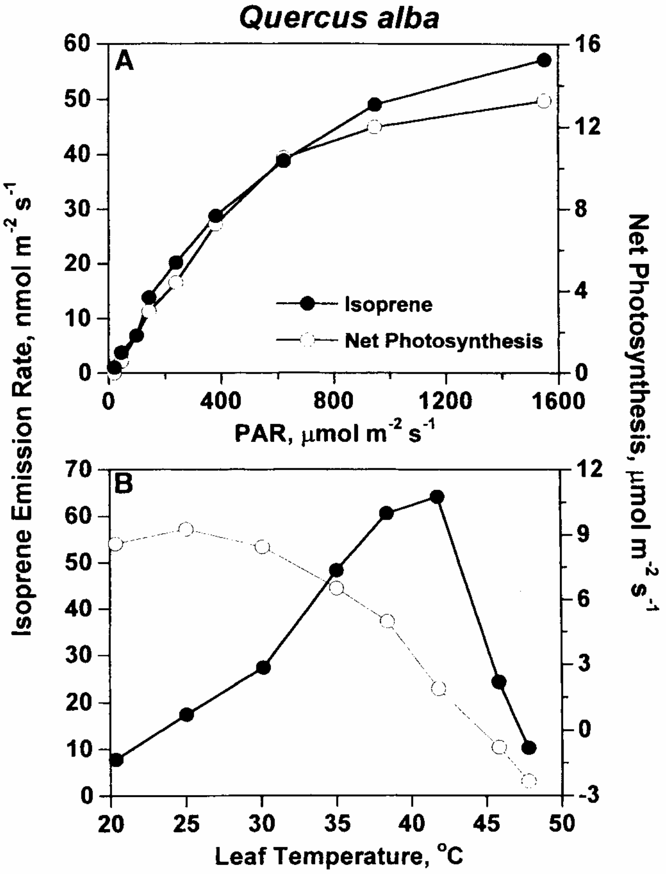


Figure 1.4. Short-term response of isoprene emission rate and net photosynthesis to varying photosynthetically active radiation (PAR) (A) and leaf temperature (B) (Harley *et al.* 1999).

In the long-term, the light environment over several days previous to measurement influences the basal rate of isoprene emission when fluxes are estimated at the plant canopy scale (Sharkey *et al.* 1999; Fuentes *et al.* 2000). The light environment affects basal rate through the impact on leaf biochemistry and on leaf-specific mass. Also, isoprene emission is enhanced by growth in a high light intensity environment (Litvak *et al.* 1996; Harley *et al.* 1996). Most studies have shown that the absolute amount of emitted isoprene is not only greater in leaves grown at high light intensity (sun leaves), but the percentage of fixed carbon lost as isoprene is also consistently higher (Harley *et al.* 1996; Lerdau *et al.* 1997).

Response to temperature

A strong short-term response of isoprene emission to temperature has been observed. Isoprene emission increases exponentially between 15°C and 35°C, with a Q_{10} (the rate of the increase in emission for 10 ^{o}C increase in temperature) generally ranging between 3 and 8, depending on species and the temperature at which it is evaluated (Monson *et al.* 1992; Sharkey and Loreto 1993; Guenther *et al.* 1993; Harley *et al.* 1997). The optimum temperature for isoprene emission, generally between 40 and 42^{o}C, is higher than that of photosynthesis, and rapidly declines above this range (Figure 1.4). The temperature response of isoprene emission is regulated by the effect of temperature on the underlying metabolism, in particular by the effect on isoprene synthase activity (Guenther *et al.* 1993; Monson *et al.* 1994). The observation that a warmer growth environment can induce isoprene emission in non-emitting leaves of plants that are potential emitters, shows that it is possible that there is an acclimation response for isoprene production (Sharkey *et al.* 1991). This can explain the general annual trend in isoprene emission, increasing through the spring, peaking in early to mid-summer and declining in the autumn (Monson *et al.* 1994). This variability in emission capacity has been found to correlate with isoprene synthase activity (Schnitzler *et al.* 1997). Also, increases in isoprene emission with temperature can continue beyond 40 ^{o}C, depending on the growth and developmental environment to which plants have adjusted.

As found for the light environment, air temperatures 2-3 days before the measurements can explain much of the variability in basal emission rate (Sharkey *et al.* 1999).

Response to water stress

The response of isoprene emissions to water stress is complex and so far no general pattern has been observed. However, several studies have focused on the effect of water limitation on isoprene emission and some general pattern can be observed. In the short-term (a few days), stress generally has little or no effect on isoprene emission, as this is not regulated by stomatal conductance. Whereas stomatal closure caused by the leaf water deficit reduces net photosynthesis, isoprene production continues, causing the internal concentration of isoprene to increase. Thus, the driving force for isoprene emission increases markedly, compensating for decreased conductance and maintaining high fluxes (Fall and Monson 1992). Furthermore it has been observed that even though isoprene emission can decline with prolonged reduced water availability (Lerdau and Keller 1997), as transpiration declines rapidly with stomata closure, the consequent leaf temperature rise may induce an increase in isoprene emission (Sharkey and Loreto 1993).

Similarly, in the medium and long term (few weeks to few months), several studies have demonstrated that isoprene emission is far less sensitive to reduced water availability than photosynthesis. Tingey *et al.* (1981) and Sharkey and Loreto (1993) conducted the first medium-term studies of the impact of drought on isoprene emission using potted plants of live oak and Kudzu (*Pueraria lobata* (Willd) Ohwi), respectively. Fang *et al.* (1996) conducted a long-term study (more than four months) exposing potted seedlings of sweetgum (*Liquidambar styraciflua* L.) to nine successive drought and recovery cycles, whereas Guenther *et al.* (1999) conducted the first study on the effects of drought on isoprene emissions from field-grown plants of *Berberis trifoliolata* and *Condalia hookeri* under natural drought conditions. Although results are variable among studies and no general pattern has been observed, the results from these studies suggest that in general isoprene emission is not affected by water limitation in the medium-term despite large reductions in photosynthesis.

Response to CO_2

The increase in [CO_2] in the atmosphere is one of the most important global-scale problems, and its consequences on isoprene emission from plants must be known in detail if we want to fully understand the role that this compound will play in a future global climate change scenario. It is now clear that increased atmospheric [CO_2] can reduce isoprene emission from plants, although contrasting responses have been observed in some cases (Monson and Fall 1989; Sharkey *et al.* 1991; Guenther *et al.* 1991; Rosenstiel *et al.* 2003). For example, Guenther *et al*. (1991) found that the isoprene emission rate from leaves of *Eucalyptus globulus* exposed to *ca.* 600 μmol mol^{-1} atmospheric [CO_2] were lower than the isoprene emission rate from leaves exposed to 100 μmol mol^{-1} CO_2, whereas Sharkey *et al*. (1991) found a contrasting responses by *Populus tremuloides* (30-40% decrease) and *Quercus rubra* (100% increase) exposed to an increase in [CO_2] from 400 to 650 μmol mol^{-1}. Recently, Rosenstiel *et al*. (2003) showed that isoprene emissions from a plantation of *Populus* *deltoides* clones grown for almost two years at 800 and 1200 µmol mol^{-1} atmospheric CO_2 were reduced by 21% and 41%, respectively, compared to plants grown at 430 µmol mol^{-1}, and went further by demonstrating that the reduced isoprene production at elevated atmospheric [CO_2] involves substrate-level regulation and is a direct result of reduced DMAPP production. They proposed that, as an increased conversion of PEP to pyruvate must occur under increased atmospheric [CO_2] to provide the necessary substrate for increased mitochondrial respiration during the day, it may reduce the cytosolic substrate availability for DMAPP synthesis.

Although it is now known that in many isoprene emitting species it is possible that isoprene emission is already inhibited at ambient atmospheric [CO_2] when compared to lower atmospheric levels of CO_2 (Monson and Fall 1989; Sharkey *et al.* 1991), and despite much progress having been made recently to further our knowledge on the biochemistry behind the mechanisms of this inhibition, the small number of experiments studying the response of mature plants grown at elevated atmospheric CO_2 in large-scale experiments, and the highly species-specific responses of isoprene emission, have prevented any generalization about a possible plant adaptation to long-term elevated CO_2.

1.1.4. Biological sinks of isoprene

Reliable estimates of global isoprene emission from different ecosystems demand a clear understanding of both production and consumption processes and the factors involved in their regulation. The sources, synthesis, and emission of isoprene have been investigated in detail (Sharkey and Yeh 2001; Monson and Holland 2001). In contrast, although the major sink for isoprene is probably chemical oxidation in the atmosphere, there has been scant evaluation of biological sinks for this hydrocarbon in the biosphere. The possibility of the existence of a biological sink for isoprene was first suggested by Rasmussen (1970), who observed that fungi on tropical foliage are able to use VOCs from vegetation as the sole carbon source for growth. More recently, it has been reported that some soil microbes use isoprene as a sole carbon supply (van Ginkel *et al.* 1987), and its metabolism in *Rhodococcus* has been explored in detail (Vlieg *et al.* 1999). Although temperate and tropical rainforest soils are recognised as a sink for isoprene (Cleveland and Yavitt 1997, Cleveland and Yavitt 1998), the significance of soil uptake in the overall isoprene budget of forest systems is still conjectural (Fall and Copley 2000) and no specific quantification has been made so far.

1.1.5. Possible physiological roles of isoprene production

One of the unresolved questions concerning isoprene emission by plants is the understanding of the physiological significance of isoprene production for plants. Without some specific physiological function, this emission would represent a substantial waste for the plant in terms of lost carbon and in terms of the energetic cost for its biosynthesis. Many aspects of isoprene production and emission (emission only after acclimation to warm growth temperatures, high Q_{10} values of emission, localization of isoprene synthase in the chloroplast) suggest that there may be a link between isoprene production and heat stress of the photosynthetic apparatus. Recently, research has been undertaken in an effort to provide evidence supporting the hypothesis that isoprene may help to protect the photosynthetic apparatus against rapid and frequent high temperature episodes. The first empirical evidence came from Sharkey and Singsaas (1995); this demonstrated that the photosynthetic apparatus of isoprene-emitting leaves of *kudzu* exhibits a greater stability at high temperatures than that of non-emitting leaves. The resistance to disruption of PSII electron transport at high temperature was increased between 3 and 10 ^{o}C when high levels of exogenous isoprene were passed over non-isoprene-emitting leaves. The authors also argued that isoprene is the only thermal protectant to be produced fast enough to protect the photosynthetic processes from the short–term heating of leaves. Although more recently several experiments claimed to demonstrate the protective role of isoprene (Singsaas *et al.* 1997; Singsaas and Sharkey 1998; Singsaas and Sharkey 2000), the precise mechanism by which isoprene would increase thermal tolerance is still unclear. A further step was made with the discovery of a thylakoid-bound form of isoprene synthase (Wildermuth and Fall 1996) that is consistent with a role in protecting membrane. Furthermore, consistent with this hypothesis is also the fact that biosynthesis of isoprene is induced by high leaf temperatures (Sharkey and Loreto 1993; Monson *et al.* 1994). However, although the thermo-protective hypothesis is attractive because it fits perfectly with many facets of the physiology of isoprene emission, it is still regarded with diffidence by many scientists after the failure of recent attempts to reproduce the protective effect and clarify its mechanism (Singsaas *et al.* 1997; Logan *et al.* 1999; Logan and Monson 1999).

Understanding the physiological role of isoprene production would also help in understanding and predicting the distribution of emitters amongst woody and grass species. Since the discovery of isoprene emission, it has been observed that not all plants emit isoprene, and it is difficult to predict which ones are emitters and which ones are not. The observation that generally shade-tolerant plant species do not emit isoprene while strong emission rates are associated with light-demanding species, suggests an adaptation by plants. Isoprene emission is common among ferns (Tingey *et al.* 1987) and is frequently found in other less evolutionary advanced plants such as mosses (Hanson *et al.* 1999). It is mostly common among trees, especially trees such as oaks with large leaves that can heat up in sunlight. On the other hand, isoprene emission is uncommon among herbaceous and crop plants, which could be explained because they use large amounts of water to keep their leaves cool. The fact that no isoprene emitter has been found among cacti despite the fact that they are subject to extreme temperatures could be explained by the fact that the temperatures are consistently high, whereas most temperate plants live in more variable conditions.

Isoprene production as a species adaptation to a particular environment could explain why it is not possible to attribute isoprene emission to particular families. It has been observed that the absence of isoprene emission in one genus examined within a large family is insufficient cause to assume the entire family contains no emitting species. Within selected families (such as *Pinaceae* and *Leguminosae*), high to non-detectable isoprene emission rates are possible (Evans *et al.* 1982). Among common plant genera, aspen, poplar and eucalyptus are high emitters (typically greater than 35 mg g^{-1} h^{-1}), sycamore, willow and spruce are medium emitters (2-35 mg g^{-1} h^{-1}), soyabeans are poor emitters and maples and many pines do not emit isoprene (Rasmussen 1970; Evans *et al.* 1982; Guenther *et al.* 1994).

1.1.6. Modelling

In order to study the impact of isoprene emission on regional and global atmospheric chemistry, the magnitude of the emission needs to be quantified. This may require the development and use of emission algorithms at the scale of leaf-level, based on the factor and mechanism governing isoprene production. Guenther *et al.* (1993) showed that leaf-level emissions from deciduous trees species is better described by relationships based on both foliage temperature and intercepted photosynthetically active radiation (PAR). The leaf-based emissions can be then integrated to the canopy scale. In general, to calculate canopy-level isoprene emissions from tall forest it is necessary to characterise the vertical variation of the canopy microclimate using measurements, or preferably models, to estimate temperature and incident PAR distribution within the canopy. Models describing canopy scale emissions differ in the manner in which atmospheric turbulence and microclimate are characterised, varying from the use of extinction rates (Lamb *et al.* 1993; Fuentes *et al.* 1995; Geron *et al.* 1997) to K theory (Gao *et al.* 1993) and random walk models (Lamb *et al.* 1996; Baldocchi *et al.* 1999).

The final integration to the landscape scale is achieved by incorporation into regional and global emission models (Guenther *et al.* 1995). As stated above, modelling systems can realistically integrate isoprene emissions to forest and regional level provided that 1) active biomass distributions both in time and space are adequately represented in the modelling systems, and 2) the environmental forcing variables driving emissions (as they vary with plant canopy depth) are reliably described.

Because tropical forest ecosystems are physiologically active all year-round, and experience wet-dry season regimes, they are probably the largest single sources of isoprene. Atmospheric measurement and modelling studies have shown that tropical ecosystems are likely to be responsible for more than 80% of the global annual isoprene flux (Jacob and Wofsy 1988; Zimmerman *et al.* 1988; Guenther *et al.* 1995). Nevertheless because of high species diversity and difficulty in access most studies on isoprene emissions have been carried out in mid- and high-latitude forest ecosystems, and there is still a substantial lack of information on the isoprene source strength from tropical systems and we still depend largely on model extrapolation

(Guenther *et al.* 1995). Although recently a considerable effort has been made in obtaining accurate estimates of isoprene emissions, there is still a need for more measurements of isoprene fluxes to quantify variations in isoprene fluxes as a result of biotic (i.e. onset of emissions and leaf senescence effects) and abiotic (i.e. drought and temperature) influences on isoprene emissions.

1.2. Aims of the study

The overall objective of this thesis is to study the emission of isoprene from plants of two contrasting forest ecosystems: a tropical rainforest and a temperate forest plantation, to improve our current understanding of environmental control over isoprene production at both leaf and ecosystem level, and to define the parameters that could be used to improve existing models to allow more precise estimates of global emissions.

The specific goals of this thesis are:

Part I: LEAF-SCALE EXPERIMENTS

(1) To investigate at the leaf scale the short (days) and medium-term (weeks) response of isoprene emission rates and photosynthesis to water stress.

(2) To find a physiological parameter that could be used in models to estimate the response of isoprene emission to water stress at leaf level.

(3) To explore the short- and long-term effect of elevated atmospheric CO_2 concentrations on isoprene emission and to determine whether, similarly to photosynthesis, isoprene emission undergoes acclimation to growth under elevated CO_2 concentrations.

(4) To determine the short-term effects of high VPD, and drought on isoprene emission rates and CO_2 uptake at the leaf level.

(5) To examine the processes behind the effect of the interaction of elevated atmospheric CO_2 concentration, high VPD and soil water limitation on isoprene emission and determine the consequences on the isoprene to carbon dioxide flux ratio.

Part II: ECOSYSTEM-SCALE EXPERIMENTS

(1) To examine the short-term response of isoprene emission and CO_2 uptake to elevated atmospheric CO_2 concentration, high VPD and water limitation conditions at the whole ecosystem scale.

(2) To determine the ecosystem acclimation to growth under elevated atmospheric CO_2 concentration in terms of both isoprene emission and CO_2 uptake.

(3) To understand the effect of the interaction between elevated atmospheric CO_2 concentration, high VPD and water stress on isoprene emission at the whole ecosystem scale.

(4) To study the relationship between isoprene emission and photosynthetic rates under normal conditions and the effect of elevated atmospheric CO_2 concentration, high VPD and water stress conditions, to determine the change in the isoprene: carbon dioxide flux ratio.

(5) To determine the effect of environmental variables such as light, temperature and soil moisture on isoprene emission from a tropical rainforest ecosystem.

(6) To explore the possible existence of a soil sink for isoprene in a temperate and tropical rainforest ecosystem, and determine the effect of soil moisture changes on its strength.

(7) To help improving *carbon balance* estimates of a tropical rainforest and a temperate forest ecosystem by quantifying the contribution of the net isoprene emission under normal conditions and in a possible climate change scenario.

1.2.1. Thesis outline

The thesis is structured in two parts. Part I covers the medium-term (few weeks) effect of environmental variables associated with climate change such as: water stress and water vapour deficit on isoprene emission from leaves of potted plants of *Quercus virginiana* Mill. and two year old plants of *Populus deltoides* Bartr.. The long-term effect of growth under elevated atmospheric CO_2 concentration on leaves of *Populus deltoides* grown for two years at three atmospheric CO_2 concentrations (430, 800 and 1200 μmol mol^{-1}) were also investigated.

Part II covers the effect of the selected environmental variables in the medium- (water stress and VPD) and long-term (elevated atmospheric CO_2 concentration) on isoprene emission from the whole ecosystem of *Populus deltoides*. The effect of water stress on ecosystem level isoprene emission was also investigated in a synthetic model tropical rainforest. Both large scale ecosystems were grown at the Biosphere 2 facility of Columbia University in Southern Arizona.

CHAPTER 1.

Introduction

This chapter introduces the background and sets the context of the thesis presenting and discussing the main issues regarding the emission of isoprene from plants, its environmental control, and its relevance for the global context climate change. It also includes the description of the experimental set-up of the different experiments that constitute this thesis. Emphasis is given to the description of the large-scale facility of Biosphere 2 Laboratory in southern Arizona, where most of the data included in this thesis were collected.

Part I: LEAF-SCALE EXPERIMENTS

CHAPTER 2.

Effect of drought on isoprene emission rates from leaves of *Quercus virginiana* Mill..

This chapter focuses on the different effect of medium-term water stress on isoprene emission and CO_2 uptake from leaves of potted plants of *Quercus virginiana* Mill., and it describes the relationship of isoprene emission with pre-dawn leaf water potential, suggesting that this parameter may be extremely useful in improving existing models by including the water limitation effect.

CHAPTER 3.

Effect of elevated CO_2 concentration and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought.

In this chapter, an experiment taking advantage of a large-scale facility aims at describing the effect of medium-term water stress and high VPD, and long-term elevated atmospheric CO_2 concentration, on leaf-level isoprene emission and CO_2

uptake from plants grown on a semi-natural soil medium. The indirect effect of VPD on intercellular CO_2 concentration and its consequences on isoprene emission are also considered and discussed.

Part II: ECOSYSTEM-SCALE EXPERIMENTS

CHAPTER 4.

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

This chapter describes the effect of medium-term water stress and high VPD, and short and long-term elevated atmospheric CO_2 concentration, on isoprene emission and CO_2 uptake of two *Populus deltoides* Bartr. plantations grown at ambient and elevated atmospheric CO_2 concentration at the Biosphere 2 facility.

CHAPTER 5.

Drought effects on isoprene production and consumption in the Biosphere 2 tropical rainforest mesocosm.

This chapter investigates the effect of medium-term water stress on ecosystem level isoprene emission from a synthetic model tropical rainforest. The leaf level basal isoprene emission rate (the rate of isoprene emission at standard conditions) of the main isoprene emitters is also reported.

CHAPTER 6.

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

This chapter investigates the strength and the response to soil water content and elevated atmospheric CO_2 concentration on isoprene uptake by the soil of *Populus deltoides* Bartr. plantations grown at three different atmospheric CO_2 concentrations. The strength of the soil sink and its response to water stress was also investigated in a synthetic model tropical rainforest.

CHAPTER 7.

Conclusions

In this chapter the most relevant conclusions of this study are summarised. A general discussion covering the objectives presented in Chapter 1 is also given.

1.3. Experimental setup

The experiments reported in this study were conducted mainly in two research facilities providing different degrees of control of the climatic variables characterising the growth environment of the plants, and characterised by different conditions of growth and developmental stage of the studied tree species: a very large growth chamber and the large-scale artificial mesocosms of Biosphere 2 Laboratory (B2L).

1.3.1. Leaf level controlled experiment

The experiment investigating the effect of water stress on isoprene emission from leaves of plants of *Quercus virginiana* Mill. was conducted in the controlled conditions of the *phytotron*, a large growth chamber facility at the National Center for Atmospheric Research (Boulder, CO, USA) (Figure 1.5). Although this facility is

Figure 1.5. Experimental set up at the NCAR laboratory glasshouse facility showing the potted seedlings of *Quercus virginiana* Mill.

similar to a glasshouse in the way that one wall is made of glass, allowing solar radiation to enter the large chamber, this is a totally sealed chamber preventing any air exchange with the outside and provided with artificial illumination, which compensates for variable outside conditions and makes it possible to set the day/night length duration, and thus to maintain perfectly controlled environmental growth conditions.

1.3.2. Large scale experiment: Biosphere 2 Laboratory

It has been recognised for over a century that the planet Earth is essentially a materially closed self-sustaining ecological system travelling in a space hostile to life. Such a notion led man's ambitions to explore and colonize space to include the idea that a closed, self-sustaining ecological system is a necessity for long-term life support at great distances from Earth. The results have been extensive efforts in both the former USSR and the USA to develop bio-regenerative life-support systems for space application. The main objectives were: 1) to create working models of the Earth's biosphere and its ecosystems, and to better understand the laws that control its life; 2) to create biospheres for human life support beyond the limits of the Earth's biosphere; 3) to create ground-based life support systems that provide a high quality of life in extreme conditions on the Earth's biosphere; 4) to develop technologies for the solution of pollution problems in our urban areas and for developing high yield sustainable agriculture.

In the USA, these objectives gave birth to the experimental project of "Biosphere 2" (named in contrast to the Earth's biosphere: Biosphere 1), the research facility for which construction was completed in 1991 in Oracle, AZ. It covers 1.27 ha and it encloses a volume of *ca.* 200000 m^3 (Figure 1.6). Biosphere 2 had no precedent because of its large scale features and internal mechanical and biological complexity: it is a unique combination of apparatus, ecology and biogeochemistry. The Biosphere 2 enclosure as a whole can be called a mesocosm. It comprises several individual model ecosystems with differing climates and management strategies, also referred to as biomes or individual mesocosms, including: a desert, a rainforest, a savannah, a

Figure 1.6. Aerial view of the Biosphere 2 Laboratory facility facing South-East showing the mesocosms: 1, tropical rainforest; 2, savannah-ocean-marsh; 3, desert; 4, intensive forestry mesocosm; 5, human habitat.

thorn-scrub (ecotone), a mangrove-marsh and a coral reef-ocean. These mesocosms as whole are generally referred to as “the wilderness area”, in contrast to the intensive agriculture biome (IAB) and the human habitat (Figure 1.7A and B).

After the end of the initial experiment (Mission 1 and 2: 1991-1994) during which Biosphere 2 was sealed from the outside to test whether it actually could serve as a self-sustaining system where human beings could survive, the management of the research facility was taken up by Columbia University (NY), and Biosphere 2 Laboratory (B2L) was reset for studies of responses of plants to possible future global environmental change. At this time the intensive agricultural biome (IAB) became the “intensive forestry mesocosm” (IFM) that was divided in three sealed bays within which the agricultural cultures where changed for three agriforest plantations constantly growing at three different atmospheric [CO_2]. With the same purpose, i.e. to be able to control the air chemical composition, humidity, temperature, etc. and to allow accurate mass balances within the single ecosystems, the mesocosms of the wilderness area were separated from one another by gas tight sealing made with plastic curtains.

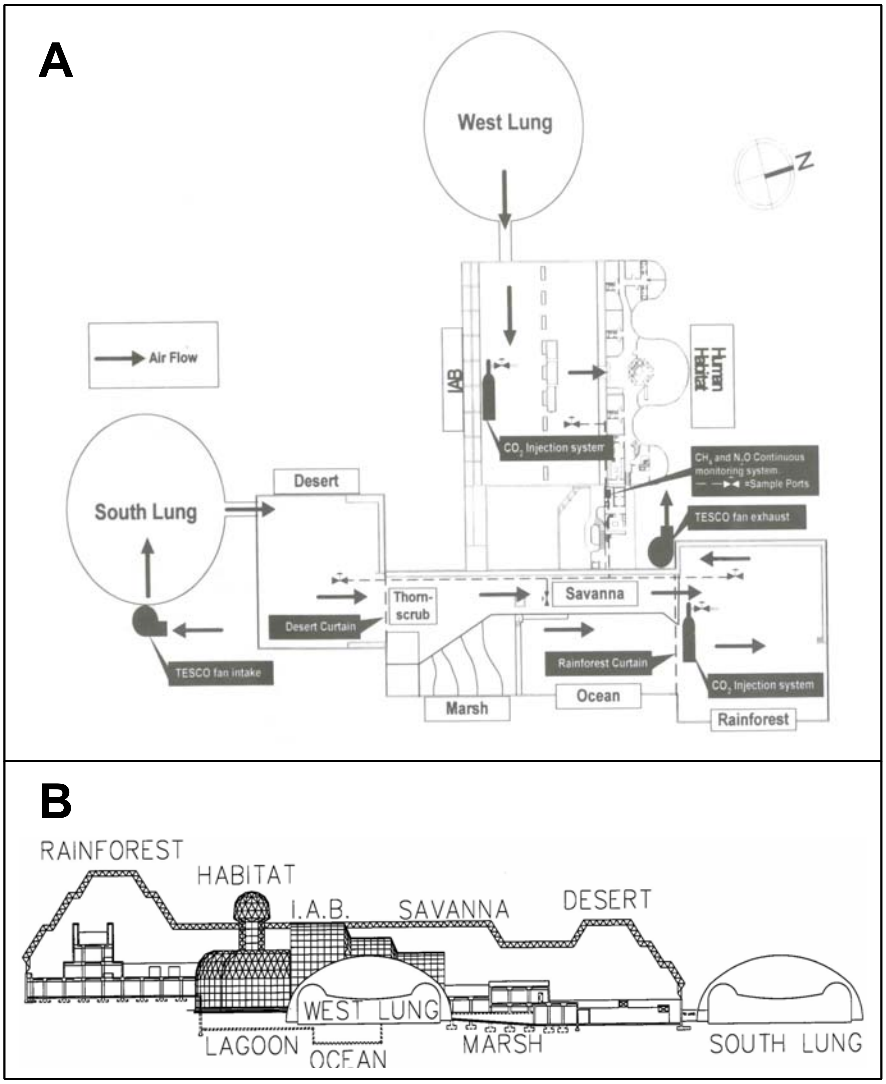


Figure 1.7. Schematic of the Biosphere 2 facility: plan view (A) in which can be seen the location of the wilderness area (desert, thorn-scrub, marsh, savannah and rainforest) and the intensive agriculture biome (IAB), and north to south cross-section (B).

While B2L is not an exact analogue of the Earth, the biomes share some of the essential biological processes and interactions that occur in nature. Thus, notwithstanding complicating issues associated with such a large facility, B2L represented a unique facility for those interested in the component processes of ecological systems because of the possibility of controlling key variables such as temperature and rainfall, which cannot be easily controlled in the field, in biomes composed of fully-grown plants on a soil medium that does not impose any limit to root expansion. Closure of the system or parts of it for brief periods allowed mass balances of total and isotopic forms of carbon, water and other substances such as trace gases to be obtained (Figure 1.8). Furthermore, for those interested in the overall balance of global processes, B2L provides insights on the behaviour of planet Earth. For example, the high soil organic matter at the start of the project caused a rise in atmospheric [CO_2] inside the enclosure of Biosphere 2 similar to that from

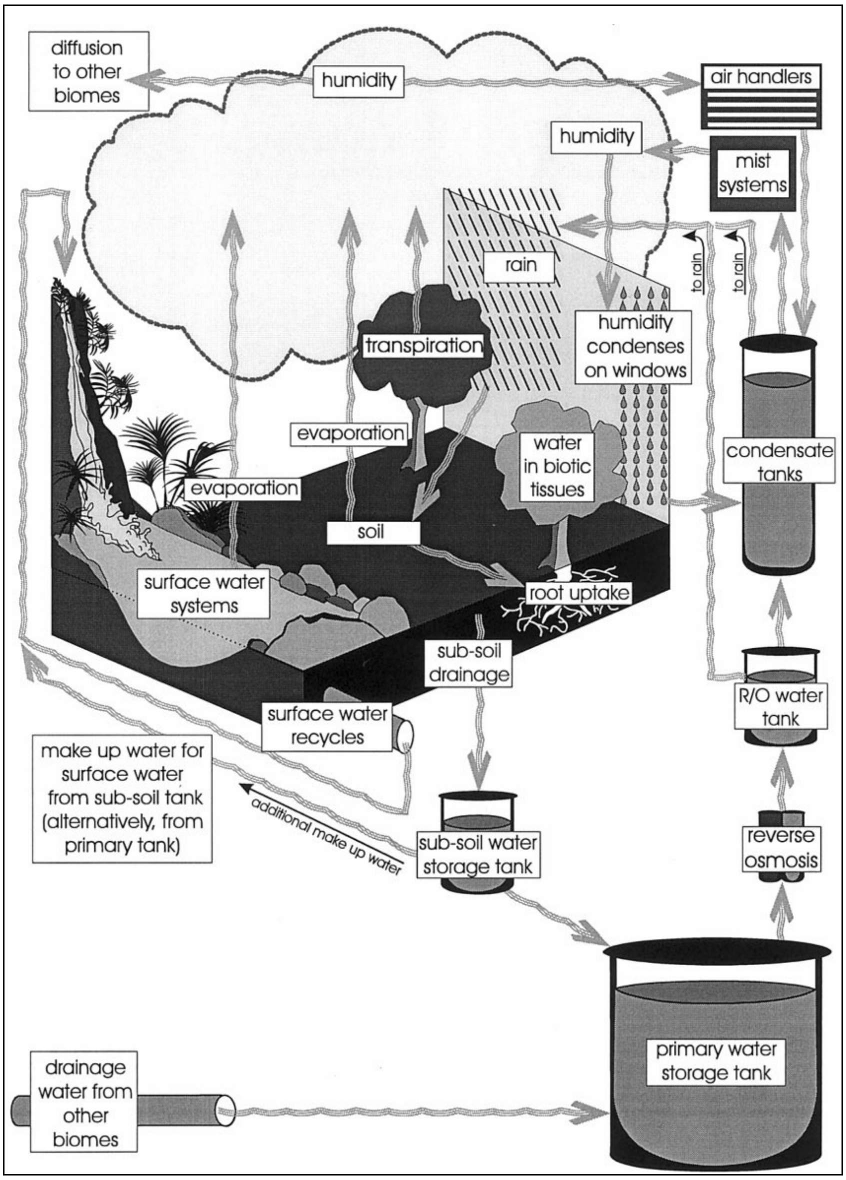


Figure 1.8. Diagram of the hydrologic cycle of the Biosphere 2 Laboratory's mesocosm.

fossil fuels in Biosphere 1, which led to effects on the vegetation and on the pH of the ocean, and in turn on the health of the coral reef, analogous to those that have occurred or might occur in Biosphere 1.

Associated with such a large scale enclosure are a number of problems, but also some remarkable advantages. One problem of B2L is that glass-enclosed structures exclude solar UV, although sunlight under glass is a whole lot more desirable than artificial illumination (Tingey *et al.* 1996). However, this can be turned to advantage, as for example in the measurement of emissions of UV-photolabile trace gases (Rosenstiel *et al.* 2003). A unique feature of B2L is that few controlled environment systems allow realistic simulation of the soil environment and mature forest systems. Although studies on potted plants may provide useful insight on the mechanisms of processes observed at the small-scale, they may lead to biased responses due to root binding and plant developmental stage that may complicate the scaling-up of observations based on these pot experiments. For example, in the course of a decade, the artificial soil in the IFM of B2L has developed a profile and biological composition characteristic of intensively managed forest soils. This soil represents a huge advance on the potting mix used for most small-scale controlled environment studies that represent at the moment the foundation for scaling up feedbacks in the terrestrial biosphere.

One of the major limits of this large facility was probably the limitation on the ability to replicate experiments. However repetition of experiments is possible and has been performed for example to assess the system variability (Lin *et al.* 1998; Rosenthal 1998; Rosenthal *et al.* 1999; Tubiello *et al.* 1999). Replication in time (in series) is routine for experimental research in the laboratory and is well appreciated in site-specific measurement systems such as flux towers. Although serial replication runs the risk of memory effects, especially in long-term experiments, these effects can and have been tested in successive years in controlled facilities such as B2L (Osmond *et al.* 2004). In large-scale field experiments, replication in space (in parallel) can be obtained to some extent in open top chambers (Norby *et al.* 1999), as in FACE experiments and flux towers. However, in these experiments it is often difficult to systematically combine and control treatments such as temperature, precipitation, and other atmospheric components with elevated atmospheric CO_2 (Isebrands *et al.* 2001; Oren *et al.* 2001).

Scaling-up through modelling is the way to combine our knowledge of separate small-scale processes in order to understand the effects of interacting environmental variables and their feedbacks on a changing climate on a global scale. Although ecosystems and climate systems are unquestionably the sum of all their parts, in the process of scaling-up, for practical purposes the whole usually has to be considered as a far more simple system than the real one. In this context, controlled large-scale experiments can be used to deduce key mechanisms at a more complex level, helping to reduce much of the detail needed for the process of scaling-up (Osmond *et al.* 2004).

In this study we took advantage of this facility to investigate both the leaf scale and the ecosystem scale isoprene emissions and soil isoprene uptake from two mesocosms: the intensive forestry management (IFM) and the tropical rainforest (TRF).

1.3.2.1. *The Intensive Forestry Management mesocosm* (IFM)

The controlled environment IFM is a UV-free glass- and stainless steel-enclosed mesocosm at the Biosphere 2 Laboratory, Oracle, Arizona, USA. It is composed of three experimental bays (approx. 550 m^2, 12000 m^3) separated by gas-tight plastic curtains and operated as semi-closed systems with independent control of atmospheric [CO_2] (430, 800 and 1200 μmol mol^{-1}), air circulation, temperature and precipitation (Murthy *et al.* 2003; Rosenstiel *et al.* 2003). Inside the three bays are grown three agriforest cottonwood plantations (day neutral clones of *Populus deltoides* Bartr.) (Figure 1.9). 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 (1m deep) of the agriforest has been evolving in place over 12 years and has developed physical and nutritional profiles of “natural soils” (Torbert and Johnson 2001), comparable to those used for agroforestry in the SE United States. It now shows metabolic and microbiological properties (Lipson et al., in review) “within a reasonable range for natural soils” (Kudeyarov *et al.* 2002), with a soil organic carbon content of *ca.* 2-3% and a carbon:nitrogen ratio of 8.3.

Figure 1.9. Top-down view of the Cottonwood plantation growing at ambient [CO_2] (430 ppm) inside the East bay of the intensive forestry mesocosm (IFM).

1.3.2.2. *The tropical rainforest mesocosm (TRF)*

Similarly to the IFM, the tropical rainforest mesocosm (1950 m^2, 27000 m^3) (Figure 1.10 and Figure 1.11) was also operated as a semi-closed system, with 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. Ringed by a shade belt of bananas and ginger, after 12 years, the top canopy mesocosm exceeds 15 m, filling about 50% of the upper enclosure, with secondary canopy and understorey plants well established. Although the tropical rainforest mesocosm has been exposed to a series of short-term elevated atmospheric CO_2 and drought treatments (Lin *et al.* 1999) 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

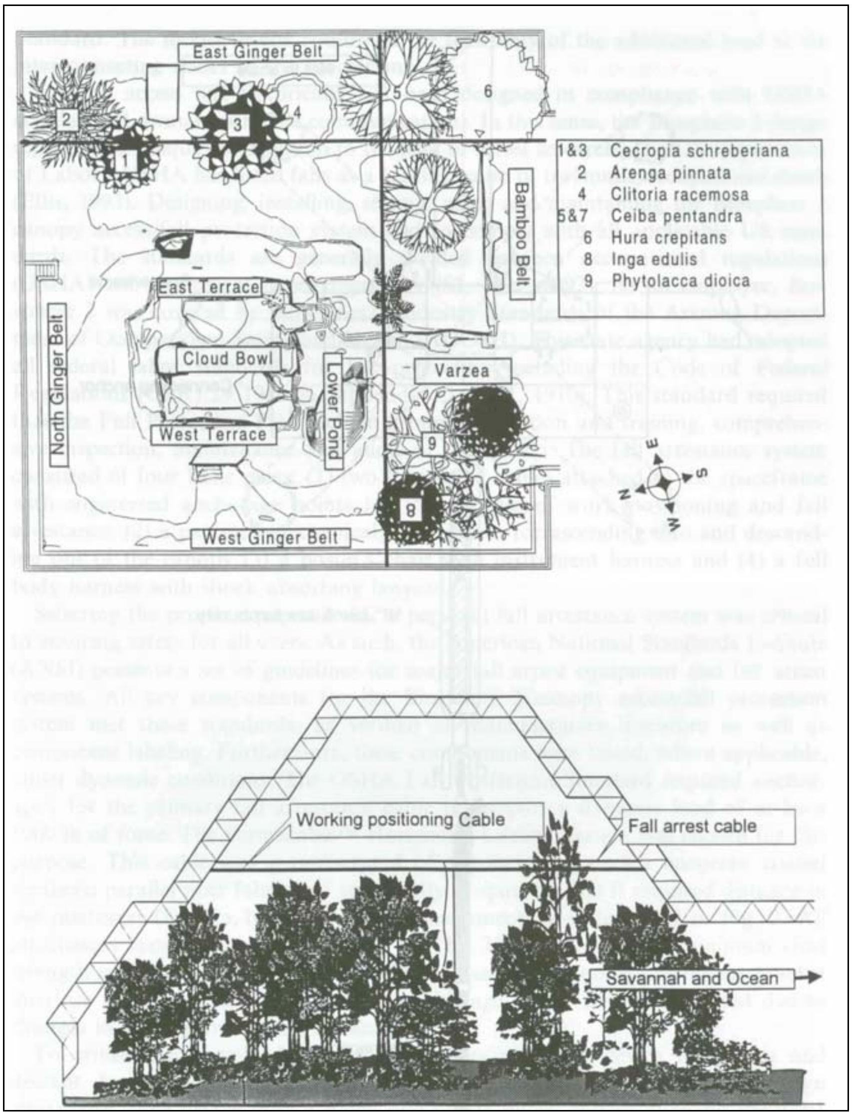


Figure 1.10. Diagram of the tropical rainforest mesocosm (TRF) showing canopy accessibility to the main large tree species.

little evidence of marked memory effects. The constructed soil in the tropical rainforest mesocosm has a subsoil layer (up to 5 m deep) and a topsoil layer (0.3-3.2 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 nutritional elements (Scott 1999)

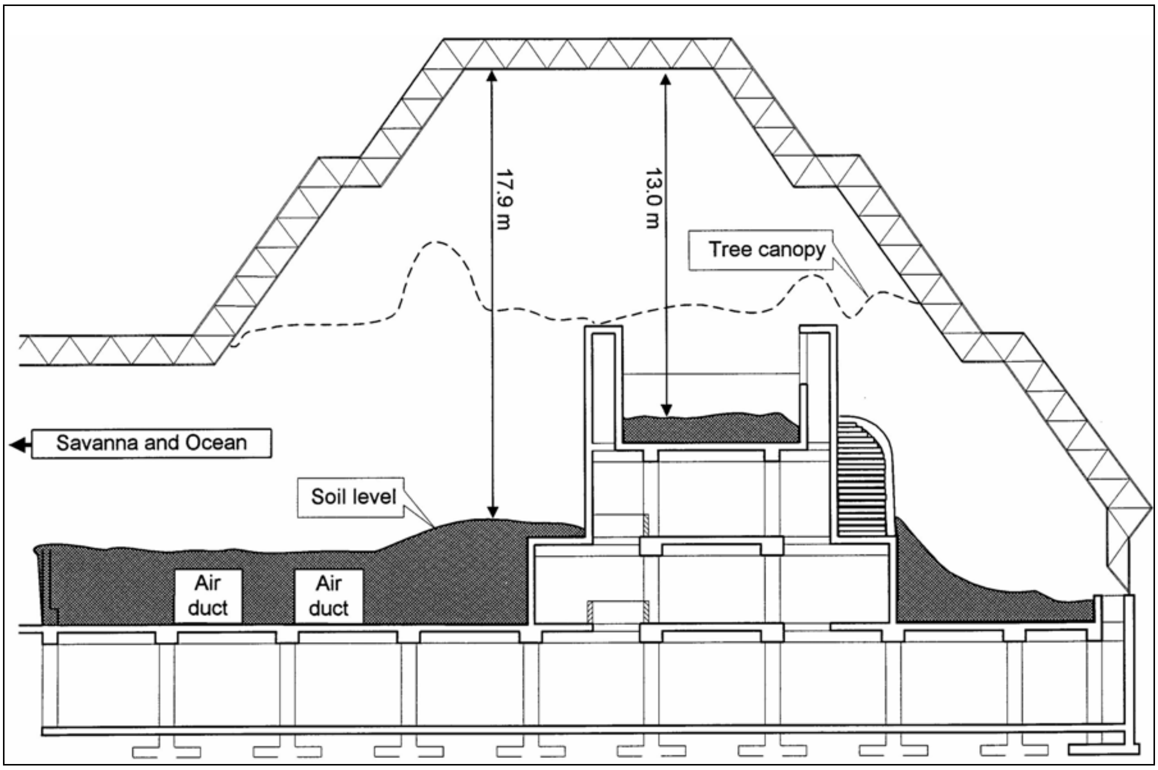


Figure 1.11. South to north cross-section of the tropical rainforest mesocosm (TRF).

The dominant canopy species include *Clitoria racemosa* Sesse & Moc., *Ceiba pentandra* (L.) Gaertn., *Cecropia schreberiana* Miq., *Arenga pinnata* (Wurmb) Merr., *Phytolacca dioica* L., *Pterocarpus indicus* Willd., *Hura crepitants* L. and *Inga feuillei* DC. among others. Most common understorey plants are: *Costus* spp., *Eppiperapium* spp., *Dieffenbochia* sp., *Ficus pumila* L., *Hedychium* spp., *Piper* spp. and *Coffea* spp. (**Figure 1.12**). To reduce light penetration into the forest floor, many edge plants were grown along the four sides of the mesocosm, including *Alpinia* spp, *Musa* spp., *Bambusa* spp. and *Zinger spectabile* Griff..

The soil profile in the rainforest mesocosm of B2L is assembled with a subsoil layer (up to 5m deep) and a topsoil layer (0.3-3.2m in depth) (Leigh *et al.* 1999). Although soil bulk density, organic matter (OM) content and major nutrient concentrations in the B2L's rainforest mesocosm are very similar to those of several Puerto Rico rainforests (Silver and Fall 1991), soils of the Biosphere 2 rainforest are more alkaline (pH around 7.5) and contain slightly higher P, K and other nutritional elements (Leigh *et al.* 1999; Lin *et al.* 1999). Temperature stratification in the upper canopy, identified by Arain *et al.* (2000) as the principal artefact of enclosure, has been minimized by high speed fans mounted in the structure to ensure adequate gas mixing in the atmosphere.

Figure 1.12. View inside the tropical rainforest mesocosm showing species heterogeneity.

PART I

LEAF-SCALE EXPERIMENTS

Effect of drought on isoprene production from leaves of *Quercus virginiana* Mill.

Pegoraro, E., Rey, A., Malhi, Y., Grace, J., Greenberg, J., Harley, P., Guenther, A.

(*Atmospheric Environment* (2004) **38**: 6149-6156)

CHAPTER 2

Effect of drought on isoprene emission rates from leaves of *Quercus virginiana* Mill.

2.1. Introduction

Isoprene is the most abundant hydrocarbon emitted by many tree species with an annual global flux estimated at 5 x 10^{14} g yr^{-1} (Guenther *et al.* 1995). Because of its high reactivity, isoprene exerts profound effects on tropospheric chemistry through the production of ozone and other oxidants, and increases the lifetime of greenhouse gases such as methane. For this reason, there is an increasing need to understand the effect of future climate change on isoprene production.

Several environmental factors are known to affect the emission of isoprene by plants (Harley *et al.* 1999; Fuentes *et al.* 2000). Some studies have focused on the effect of water-stress on isoprene emission rates (e.g. Tingey *et al.* 1981; Sharkey and Loreto 1993; Lerdau *et al.* 1997; Guenther *et al.* 1999; Bruggemann and Schnitzler 2002). Although in general isoprene emission rates are much less sensitive to drought than photosynthetic rates, results are variable among studies and no clear pattern has been observed. Some studies have observed that plants can exhibit higher isoprene emission rates upon relief of water-stress than in pre-stress conditions (Sharkey and Loreto 1993).

It is known that isoprene biosynthesis occurs within the chloroplast, and early experiments with isotopically labelled carbon dioxide ($^{13}CO_2$) have shown that *ca.* 80% of the C in isoprene is derived from newly assimilated photosynthetic C, and that carbon is incorporated into isoprene within minutes (Sanadze *et al.* 1972; Mgaloblishvili *et al.* 1979), with roughly 20% coming from alternative (not fresh photosynthate) sources (Karl *et al.* 2002; Schnitzler *et al.* 2004). Most studies have suggested that this alternative carbon may come from the slow turn-over extra-chloroplastic C sources. A recent study showed that under stress conditions such as water-stress or high temperature, this partitioning may vary in favour of alternative sources under conditions of low photosynthetic rates (Funk *et al.* 2004). However, recent evidence (Loreto *et al.* 2004) indicated that there is no cross-talking between chloroplastic and extra-chloroplastic C sources. As isoprene emission represents only a small percentage of the assimilated C and may even be formed by respiratory CO_2 recycled in leaves as demonstrated by the good relationship between these parameters (Loreto *et al.* 2004), this may suggest that the alternative C used to form isoprene when photosynthesis is depressed may also come from previously assimilated C present inside the chloroplast. Typically, in non-stressed conditions, the proportion of assimilated carbon emitted as isoprene (C_{iso}/C_A) at 30°C is around 2%, which represents a non-trivial loss of carbon to the plant (Sharkey *et al.* 1991; Baldocchi *et al.* 1995; Monson and Fall 1989; Harley *et al.* 1994; Fang *et al.* 1996). However, the carbon loss can exceed 50% during periods of stress when photosynthetic rate is depressed (Sharkey and Loreto 1993).

In order to improve our understanding of the environmental and physiological control on isoprene emission from temperate tree species, we conducted a controlled laboratory experiment. The specific objectives of the study were: (1) to investigate the effect of water-stress on isoprene emission, specifically the short (days) and medium-term (weeks) response of isoprene emission to drought stress; (2) to explore the relationship between isoprene emission and photosynthesis; (3) to calculate the isoprene:carbon dioxide flux ratio for this species, under normal and water-stress conditions; (4) to find a physiological parameter that could be used in models to estimate the isoprene emission response to water-stress. It was hypothesised that: (a) In the short-term (few days), the isoprene emission is less sensitive to water-stress than photosynthetic rate; (b) in the medium-term, the isoprene emission rates will correlate with a physiological parameter of stress.

2.2. Materials and Methods

2.2.1. Plant material

In January 2002, 16 two-year old trees of live oak (*Quercus virginiana* Mill.) were obtained from the Camellia Forest Nursery (Chapel Hill, NC, USA). The two-year old trees were transplanted to 6 dm^3 plastic pots (30 cm high and 16 cm wide) containing commercial potting soil (Miracle Grow) and placed in the National Center for Atmospheric Research (NCAR, Boulder, CO) Phytotron (a temperature-controlled greenhouse with supplemental lighting) for 70 days until the end of March when the experiment started.

2.2.2. Experimental design

Eight of the trees in the phytotron were randomly chosen as ‘control’ plants (**C**) (well-watered plants) and eight as ‘treatment’ plants (**T**) (subject to drought stress). Four of these seedlings (two treatment and two control) were used for destructive sampling and were monitored continuously for leaf temperature (T_{leaf}) and soil volumetric water content (θ) with leaf thermocouples (0.1 mm in diameter) and Delta-T probes (ML2 Theta Probe, Delta-T Devices, Cambridge, UK), respectively, connected to a datalogger (CR10, Campbell Scientific, Logan, UT, USA). These seedlings were not used for the isoprene emission measurements. In order to take into account the influence of growing conditions inside the phytotron on isoprene emission, air temperature (T_{air}), relative humidity (RH), and photosynthetic active radiation (PAR) were continuously measured and stored on an hourly basis in a CR10 datalogger.

Water-stress was imposed on eight previously non-stressed seedlings by withholding water during two successive drying-rewatering cycles. In the first cycle water was withheld from March 21 to April 2 (12 days) and in the second cycle from April 18 to April 28 (10 days). During both cycles, **T** seedlings were watered to field capacity approximately 12 hours before the start of the drought phase of each cycle. Initially, photosynthetic rates and isoprene emission rates were measured every three days and then daily when isoprene emission started to change more dramatically after treatment. The drying cycle was ended when rates of isoprene emission of the **T** seedlings were reduced to less than 50% of the values measured in the **C** seedlings. At the end of each drought period, **T** seedlings were watered daily over the following recovery period (the first from April 2 to April 18, the second from April 28 to May 8) to allow the seedlings to recover before starting the following cycle. The end of the recovery period in the first cycle was established as the time when the isoprene emission rates measured in the **T** seedlings reached the same mean emission rates as those measured in the **C** seedlings. During the first cycle, **C** seedlings were watered every three days, whereas during the second cycle they were watered every other day. The watering regime was changed to every other day because during the first cycle the **C** seedlings suffered a slight water-stress that affected mainly photosynthetic rate and stomatal conductance.

2.2.3. Water status

In order to monitor water-stress the following parameters were measured: θ, pot mass (M) and pre-dawn leaf water potential (Ψ_{leaf}). θ was continuously monitored over the duration of the entire experiment using two Delta-T soil moisture sensors. The two sensors were inserted to 10 cm depth into the pot of a **T** seedling and a **C** seedling, and hourly average signals were collected with a CR10 data logger. In addition, θ was measured with a portable sensor (ML2x Theta Probe, Delta-T Devices, Cambridge, UK), inserted into the pot at the time of each gas exchange measurement. M and θ were measured at the time of each gas exchange measurement just before putting the leaf into the leaf cuvette.

Pre-dawn Ψ_{leaf} was measured using detached leaves with a Scholander pressure chamber (Model 610 Pressure Chamber, PMS Instrument Co., Corvallis, OR, USA). Because measurements with the pressure bomb are destructive, leaves used for these measurements were collected from the two **T** and two **C** seedlings that were not used for isoprene emission measurements. However, during the drying period of the second cycle pre-dawn Ψ_{leaf} was also measured in leaves from the 12 **T** and **C** seedlings to increase the number of replicates.

2.2.4. Sampling protocol

All gas exchange, θ, pot mass, and pre-dawn Ψ_{leaf} measurements were performed during a single day using one leaf per seedling for the gas exchange measurements. Two different leaves on each seedling were used for measurements in the two cycles. At the beginning of the experiment, fully expanded leaves from the top of the canopy were randomly chosen and tagged for subsequent measurements. Prior to measurement, every seedling was taken from the greenhouse to an adjacent laboratory where a gas exchange measurement system was set up. After a 15 min. period at a PAR of 600 μmol m^{-2} s^{-1}, the gas exchange measurements were started.

2.2.5. Leaf gas exchange measurements

Photosynthetic rate, stomatal conductance and intercellular [CO_2] (C_i) were measured using a LI-6400 gas exchange measurement system (Li-Cor, Lincoln, NE, USA). To avoid large oscillations in the CO_2 level of the air supply (374 μmol mol^{-1} ± 4%), the LI-6400 inlet drew air from outside the laboratory and an empty canister of 2 dm^3 volume was placed in line before the instrument to buffer against short-term variations. The air supply was humidified using an in-line bubbler because of the very low RH of the external air. RH inside the cuvette was maintained at 60% (± 10%).

For measurements of *Isoprene emission rates*, an air sample of 300 ml was pulled from the outlet of the leaf cuvette through a "T" junction and 20 cm of Teflon® PFA tubing into a custom-made inlet system capable of vacuum sample collection and isoprene cryofocusing. The isoprene sample was then transferred to the 0.25 mm ID x 30 m MXT-624 capillary column (RESTEK Corporation, Bellefonte, PA, USA) of a portable gas chromatograph (SRI 310, Buck Scientific, East Norwalk, CT, USA) by a flow of high purity He that was used as carrier gas. Isoprene was separated with a carrier (He) flow of 3 cm^3 min^{-1} and with a temperature program from 40 to 200 °C at 10 °C min^{-1}. Isoprene eluting from the column was measured using a flame ionisation detector (FID), and the peak was integrated using a PeakSimple-32 integrator (SRI, Buck Scientific, East Norwalk, CT, USA). Additional details of the analytical system can be found in Greenberg *et al.* (2003). Before the start of the experiment the FID-gas chromatograph was tested for a few days for linear response and detection stability by diluting a known isoprene standard (50 ppb in N_2) at the concentrations of: 2, 5, 10, 20, 40 and 80 ppb. The linearity and the good stability (*ca.* 2%) of the results suggested that a single injection of a known isoprene standard (25.5 ppb in N_2) on each measurement day was sufficient to calibrate the system.

All measurements were made between 10:00 and 15.00, and measurements on each leaf were made at the same time each day (± one hour). All measurements were made under the same standard conditions: T_{leaf} of 28 $^{\circ}$C, PAR of 800 μmol m^{-2} s^{-1} and air flow of 400 μmol s^{-1}. After a leaf was placed in the cuvette, a minimum of 10 min. was allowed for equilibration, and all measurements were made after steady-state conditions were realised, as indicated by continuous monitoring of CO_2 and H_2O fluxes.

2.2.6. Statistical analyses

In order to analyse the data, the experiment was considered as two cycles: *cycle I* (21 March-2 April) and *cycle II* (18 April-8 May), each comprising a drought and a recovery phase. All data analyses were done with SAS software (SAS Institute Inc., Cary, NC, 1995). Results over the whole experiment were analysed using a multivariate analysis of variance (MANOVA). For photosynthetic rate and stomatal conductance, data were transformed into logarithms to satisfy the assumption of homogeneity of variance. To take into account the overall effect during the course of the experiment, data were first analysed using a two-way ANOVA with repeated measures with drought as a factor (proc GLM) where treatment, time and their interaction were considered. When this test was significant at a 5% level of probability, a single ANOVA was used to test specific differences on each date. Linear and non-linear regressions (Proc NLIN) were used to determine the relationships between different physiological variables. Values in the text are reported as means ± 1 standard error (SE).

2.3. Results

2.3.1. Growth conditions

Inside the phytotron, seedlings were grown under a 12 hour day (from 7:00 am to 7:00 pm) and a PAR of 333 ± 4 µmol m^{-2} s^{-1} (n = 48) with average maximum of 511 ± 17 µmol m^{-2} s^{-1} (n = 48) (Figure 2.1A). T_{leaf} for **T** and **C** seedlings was on average

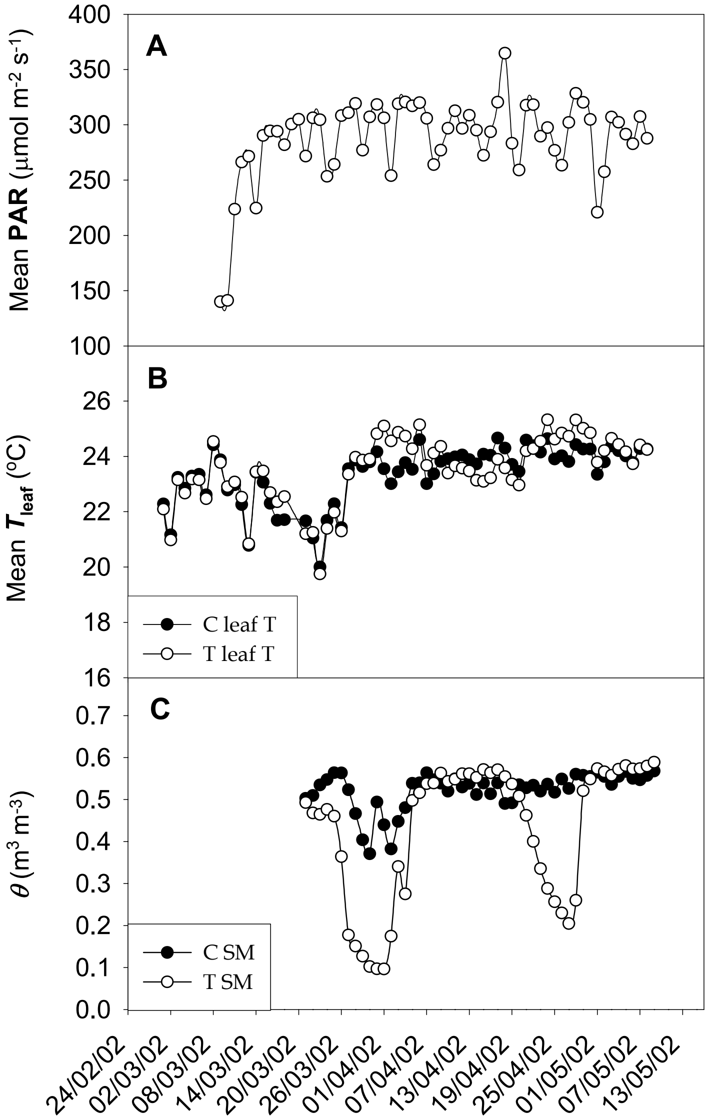


Figure 2.1. Growth conditions inside the phytotron during the experiment: daylight average photosynthetic active radiation (PAR) (A) and leaf temperature (T_{leaf}) (B), and 24 hours average of soil volumetric water content (θ) (C) for **T** and **C** seedlings.

22.9 ± 0.9 °C (n = 48). During the two periods of drought stress, T_{leaf} of the **T** seedlings tended to be higher than T_{leaf} of the **C** seedlings as a consequence of the reduced cooling effect of transpiration (Figure 2.1B). During March, there were two strong temperature declines caused by the external extreme weather (snowstorms). These temperature drops affected the phytotron internal temperature and clearly influenced T_{leaf} as shown in Figure 1B. Towards the end of March, temperatures increased and remained stable in the phytotron during the whole period of the experiment. The θ measured for the **C** seedlings was on average 0.5 ± 0.005 m^3 m^{-3} (n = 38). During the two drought periods, θ decreased to less than 0.1 m^3 m^{-3} in the first cycle, and to *ca.* 0.2 m^3 m^{-3} in the second cycle in the **T** seedlings (Figure 2.1C).

2.3.2. Drought effect on leaf isoprene emission and gas-exchange parameters

At the beginning of the experiment (March 21, day 0), there were no significant differences (all $P > 0.05$) in θ (*ca.* 0.45 m^3 m^{-3}), isoprene emission rate (*ca.* 17 nmol m^{-2} s^{-1}) and gas exchange parameters (photosynthetic rate: *ca.* 5.6 µmol m^{-2} s^{-1}; stomatal conductance: *ca.* 0.048 mol m^{-2} s^{-1}) between the **T** and **C** seedlings (Figure 2.2). In the **T** seedlings, after six days of treatment, θ was reduced to *ca.* 0.1 m^3 m^{-3} (Figure 2.2A). Drought strongly decreased photosynthetic rate and stomatal conductance to 1.94 ± 0.55 µmol m^{-2} s^{-1} and 0.013 ± 0.002 mol m^{-2} s^{-1}, respectively on day 6 (Figure 2.2B and C), while isoprene emission remained essentially constant until day 6 at approximately 16.5 ± 2.1 nmol m^{-2} s^{-1} (Figure 2.2D). Isoprene emission rates started to decline daily from day 8 of the experiment when θ was less than 0.1 m^3 m^{-3} and pre-dawn Ψ_{leaf} was *ca.*–1 MPa. The isoprene emission rate decreased to 6.2 ± 1.1 nmol m^{-2} s^{-1} at the end of the first drying cycle on day 12, with a pre-dawn Ψ_{leaf} of *ca.*–2 MPa. In contrast to the slow decline in the isoprene emission over time, photosynthetic rate and stomatal conductance decreased very rapidly from day 2 to almost zero on day 8, and remained essentially constant until day 14. Both photosynthetic rate and stomatal conductance tracked the decline in θ (although with a one day lag period).

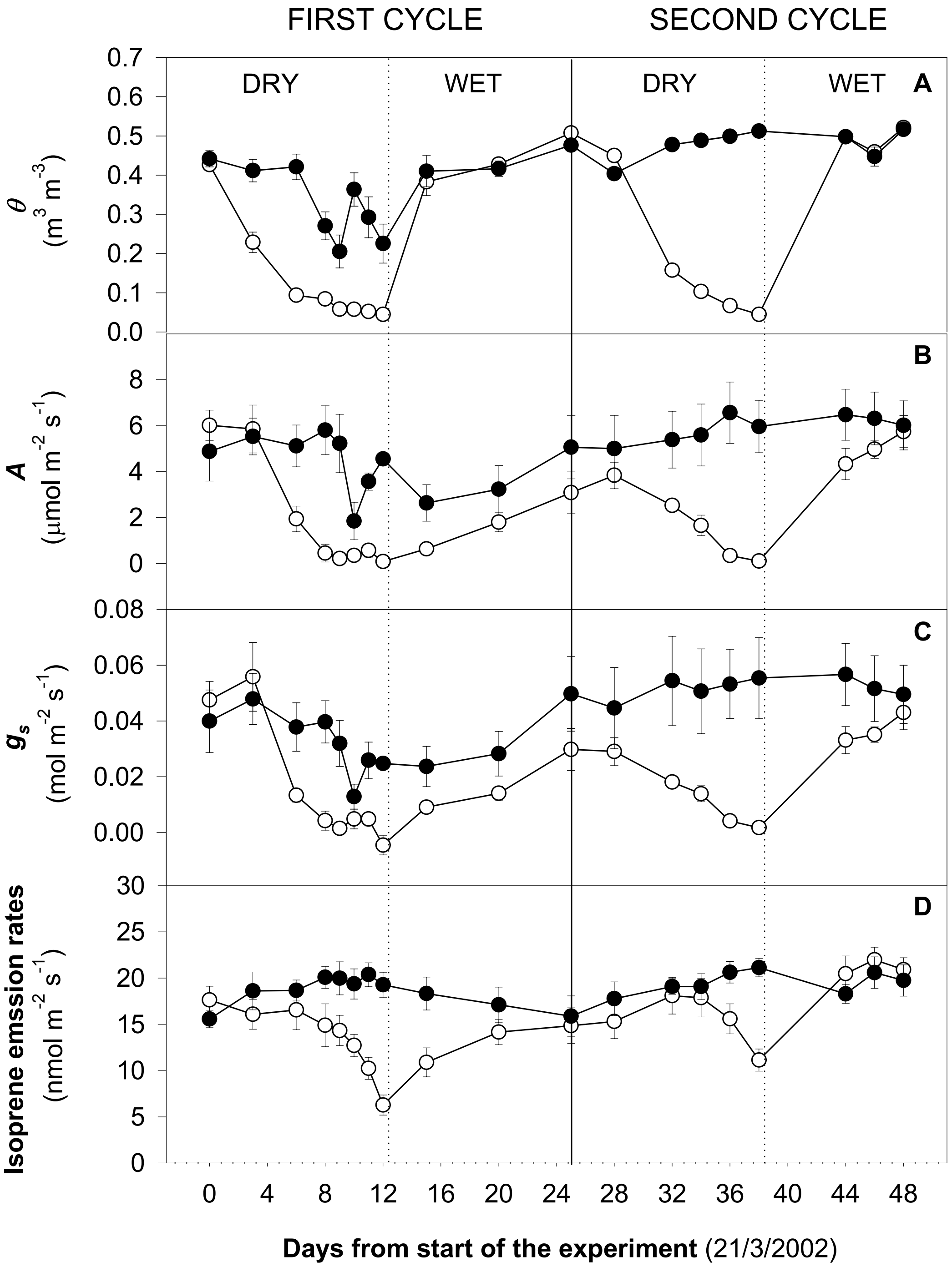


Figure 2.2. Time course of soil volumetric water content θ (A), photosynthetic rates (*A*) (B), stomatal conductance (g_s) (C) and isoprene emission rates (D) for the **T** (white circles) and **C** (black circles) seedlings during the first and second drying-rewatering cycles. Each point is the mean ± SE (n = 6).

In the **C** seedlings, leaf isoprene emission rate remained essentially constant at *ca.* 18 nmol m^{-2} s^{-1}. Photosynthetic rate and stomatal conductance dropped from 5.8 ± 1.0

μmol m^{-2} s^{-1} and 0.040 ± 0.007 mol m^{-2} s^{-1}, respectively on day 8 to 1.8 ± 0.8 μmol m^{-2} s^{-1} and 0.013 ± 0.004 mol m^{-2} s^{-1} on day 10, as a consequence of a small drought stress. θ decreased from 0.42 ± 0.03 m^3 m^{-3} to 0.20 ± 0.04 m^3 m^{-3} during the three day interval between day 6 and day 9 during which the **C** seedlings were not watered. During the 16-days recovery period (from day 12 to day 28), isoprene emission recovered quickly upon re-watering, reaching the same values measured in the **C** seedlings (*ca.* 15 nmol m^{-2} s^{-1}) on day 13. Photosynthetic rate and stomatal conductance still showed a slight sign of water-stress at the start of the second cycle, but the differences between rates measured in the **T** and **C** seedlings on day 16 were not statistically significant ($P > 0.05$).

During the second drought period, from day 28 to day 38, θ decreased from 0.45 ± 0.01 to 0.045 ± 0.007 m^3 m^{-3} (Figure 2A). Similarly to the first cycle, in the second cycle photosynthetic rate and stomatal conductance decreased at a faster rate than isoprene emission, which began to decline later, reaching values of 0.10 ± 0.01 μmol m^{-2} s^{-1}, 0.0020 ± 0.0002 mol m^{-2} s^{-1} and 11.1 ± 1.2 nmol m^{-2} s^{-1}, respectively by day 38 (Figure 2.2B, C and D).

In the second recovery period, isoprene emission measured in the **T** seedlings reached the rates measured in the **C** seedlings on day 44 at *ca.* 20.5 nmol m^{-2} s^{-1}, with θ having recovered to *ca.* 0.5 m^3 m^{-3}. Photosynthetic rate and stomatal conductance from the **T** seedlings recovered to values measured in the **C** treatment by day 48 at *ca.* 5.74 μmol m^{-2} s^{-1} and *ca.* 0.043 mol m^{-2} s^{-1}, respectively. In the **C** seedlings, photosynthetic rate, stomatal conductance and isoprene emission remained essentially stable at *ca.* 5.9 μmol m^{-2} s^{-1}, 0.05 mol m^{-2} s^{-1} and 19.5 nmol m^{-2} s^{-1}, respectively. θ remained essentially constant at *ca.* 0.48 m^3 m^{-3}.

At the beginning and the end of the experiment and during the recovery period, the C_{iso}/C_A was only around 2%. During the two severe water-stress periods when photosynthetic rate was less than 0.5 μmol m^{-2} s^{-1}, the C_{iso}/C_A reached values as high as 50%. Because these values were obtained when photosynthetic rate was close to zero, calculations may have been somewhat misleading. However, for values of photosynthetic rate larger than 0.5 μmol m^{-2} s^{-1}, the C_{iso}/C_A still increased significantly with decreasing θ (Figure 2.3). In the **C** treatment, the C_{iso}/C_A remained

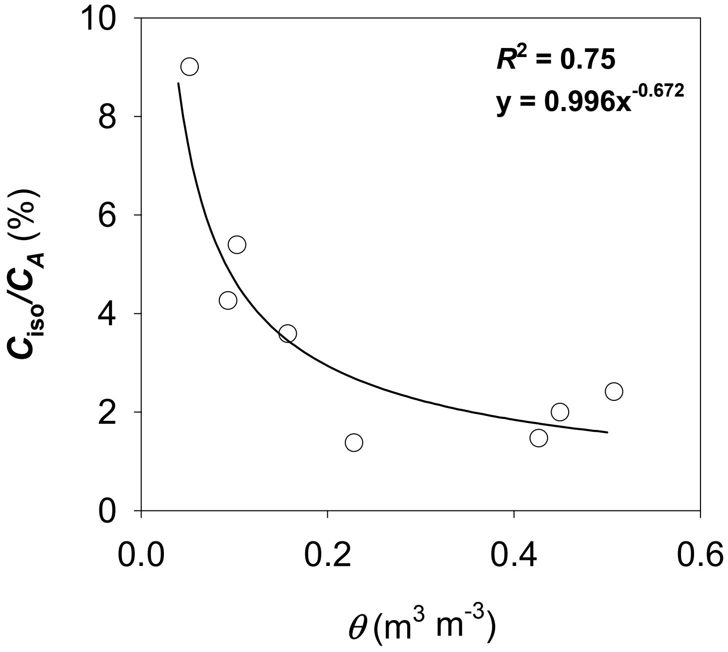


Figure 2.3. Relationship between the percentage of assimilated carbon emitted as isoprene (C_{iso}/C_A) and soil volumetric water content θ for the **T** seedlings during the dry periods. C_{iso}/C_A is shown only for days in which photosynthetic rate was larger than 0.5. Each point represents the mean of six replicates.

essentially constant at *ca.* 2%, with the exception of a rapid increase to values of *ca.* 5% reached in response to the slight water-stress that the **C** seedlings suffered during the first cycle.

During the two consecutive cycles, there was a significant difference between the response of isoprene emission and that of photosynthetic rate to changes in θ, stomatal conductance and pre-dawn Ψ_{leaf} ($P > 0.01$ in all cases for the repeated measures analysis). Although isoprene emission responded to water-stress later and more slowly than photosynthetic rate, both showed a distinctive response to θ during the drought period and the recovery period (Figure 2.4A and B). For equivalent θ, both isoprene and assimilation showed higher rates during the drought phase than during the recovery phase. Isoprene emission did not show any relationship to stomatal conductance for stomatal conductance values higher than 0.02 mol m^{-2} s^{-1}. Only when stomatal conductance decreased below 0.02 mol m^{-2} s^{-1} in the **T** seedlings isoprene emission dropped dramatically (Figure 2.4C). As expected, and opposite to isoprene emission, photosynthetic rate showed a strong linear correlation with stomatal conductance ($R^2 = 0.93$) for both **T** and C seedlings (Figure 2.4D). Isoprene emission only started to decline when photosynthetic rate was already reduced by as much as 90% which corresponded to a C_{iso}/C_A of *ca.* 10% (Figure 2.4E and F). Isoprene emission dropped significantly when the carbon cost to the seedling for isoprene emission was very high, i.e. during the most severe period of the drought when photosynthetic rate was reduced almost to zero.

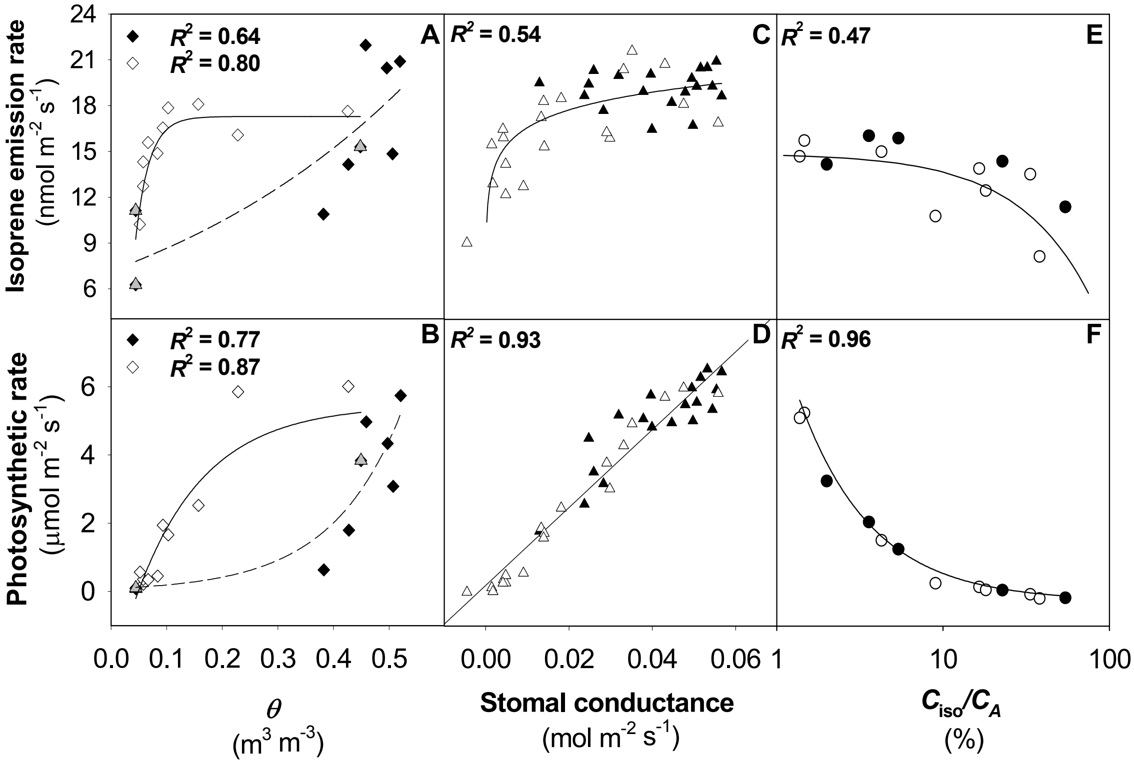


Figure 2.4. Relationship of isoprene emission rates and photosynthetic rates with soil volumetric water content (θ) (A and B), stomatal conductance (C and D) and the percentage of assimilated carbon emitted as isoprene (C_{iso}/C_A) (E and F). Each point represents the mean of six replicates. In (A) and (B) symbols represent the **T** seedlings during the dry (white diamonds) and the wet (well-watered and recovery) (black diamonds) periods over the whole experiment (two cycles). The "Common" points (grey triangles) correspond to the transition between one phase and the next one. Curves have been drawn only for a better illustration of the trend. In (C) and (D) symbols represent the **T** (white triangles) and **C** (black triangles) seedlings over the whole experiment (two cycles). In (E) and (F) symbols represents the **T** seedlings during the drought phase of the first (white dots) and the second (black dots) cycle. Note that the abscissa scale is logarithmic.

The effect of decreasing pre-dawn Ψ_{leaf} on photosynthetic rate was very strong (Figure 2.5A), with photosynthetic rate immediately reduced to zero when pre-dawn Ψ_{leaf} reached *ca.* –1 MPa. isoprene emission and pre-dawn Ψ_{leaf} showed a well correlated ($R^2 = 0.64$) linear negative trend (Figure 2.5B).

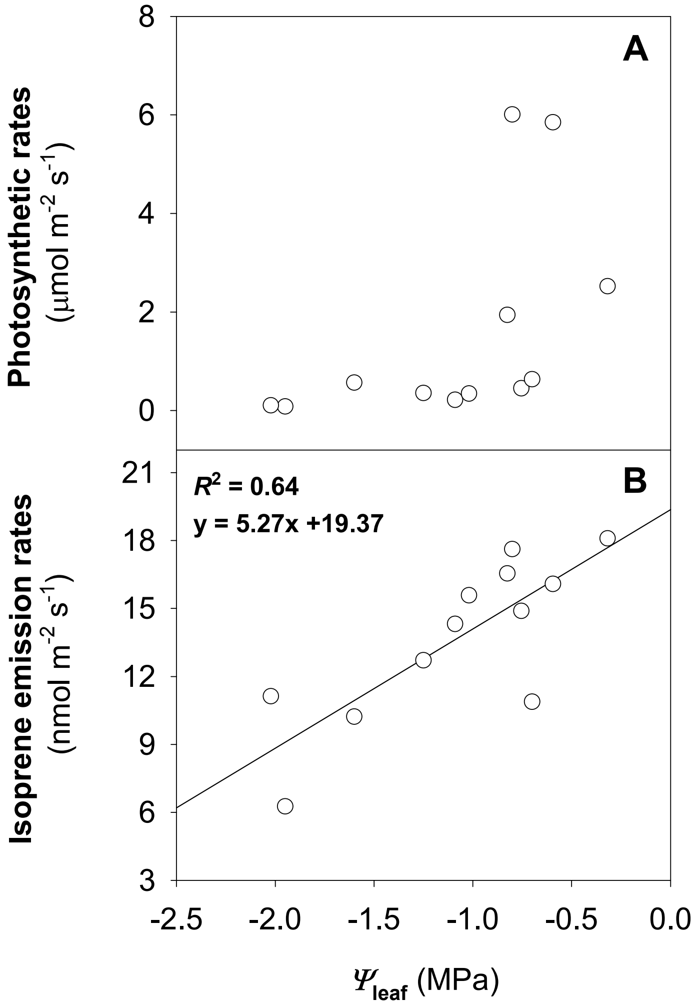


Figure 2.5. Relationship between photosynthetic rates (A) and isoprene emission rates (B), and pre-dawn leaf water potential (Ψ_{leaf}) for the **T** seedlings during the whole experiment (two cycles).

The negative effect was not as pronounced as for photosynthetic rate, probably as a result of the little effect stomata have in controlling isoprene emission from leaves (Fall and Monson 1992). The relationship between isoprene emission and photosynthetic rate for the **T** seedlings over the duration of the experiment, divided into drought period and watered period, is shown in Figure 2.6. Isoprene emission during drought did not decline much until photosynthetic rates were very low and was characterised by a faster recovery during the re-watering period.

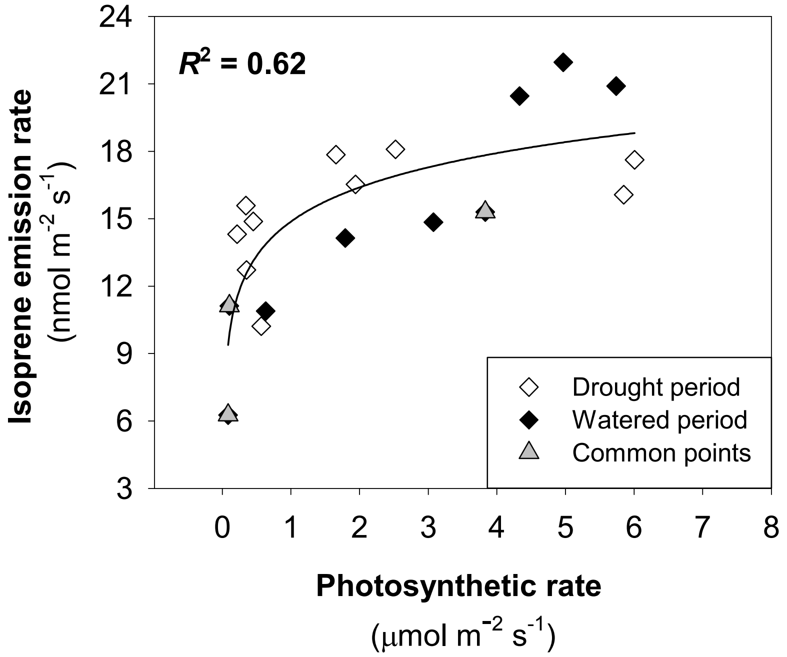


Figure 2.6. Relationship between isoprene emission rates and photosynthetic rates for the T plants during the dry (white diamonds) and wet (well-watered and recovery) (black diamonds) periods, over the whole experiment (two cycles). The "Common points" (grey triangles) represent the transition days between one phase and the next one. Each point represents the average of six replicates.

2.4. Discussion

Our results indicate that although isoprene emission decreased when the water-stress was severe, it was considerably less sensitive to drought than photosynthetic rate and stomatal conductance (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996; Lerdau *et al.* 1997; Guenther *et al.* 1999; Bruggemann and Schnitzler 2002). It was also apparent that isoprene emission could recover from water-stress much more quickly than photosynthetic rate and stomatal conductance. Furthermore, we observed for the first time that for equivalent θ both isoprene emission and assimilation appear to have higher rates during the drying phase than during the recovery phase. Unlike photosynthesis, the response of isoprene emission to drought appears to be independent of stomatal dynamics (Tingey *et al.* 1981; Fall and Monson 1992; Fang *et al.* 1996). In the short-term, during the drought phase, even though stomatal conductance was drastically reduced over an eight day period, isoprene emission remained essentially constant. Only when stomatal conductance decreased below *ca.* 0.01 mol m^{-2} s^{-1} were isoprene emissions drastically reduced. This finding suggests that stomatal conductance did not control isoprene emission and that an internal factor controlled the decrease in the emission rate when the water-stress was severe. In the short-term, isoprene emission also appeared not to be directly dependent on photosynthetic rate. Although most of the carbon in isoprene (between 72 and 91%) is derived from recent photosynthate, it has been observed that plants also use extra-chloroplastic alternative, slow turn-over, sources (Sharkey *et al.* 1991; Affek and Yakir 2003; Delwiche and Sharkey 1993; Karl *et al.* 2002; Funk *et al.* 2004; Schnitzler *et al.* 2004). Moreover, it has been found that under stress conditions plants may increase the relative contribution of this alternative carbon to over 30% to maintain high isoprene emission when photosynthetic rates are inhibited (Schnitzler *et al.* 2004; Funk *et al.* 2004). As suggested by recent evidence (Loreto *et al.* 2004), because in non-stressed conditions isoprene represents only a small fraction of the fresh photosynthate and may even be formed by respiratory CO_2 recycled in leaves, it is possible that isoprene may also use chloroplastic C pools as an alternative source when photosynthesis is inhibited. Although in the current study it was not possible to quantify the relative contribution of fresh photosynthate and slow turn-over and chloroplastic alternative sources, our results are consistent with these findings. It is likely that isoprene emission started to decline when the C_{iso}/C_A was only 10% because the slow turn-over alternative carbon sources did not provide enough C. The drop of isoprene emission after a period of severe water-stress may be the result of the depletion of these alternative sources.

Furthermore, drought stress appeared to have a profound influence on the percentage of C_{iso}/C_A. Previous studies showed that in non-water-stressed plants *ca.* 1-2% of the photosynthetically fixed carbon is emitted as isoprene (Sharkey *et al.* 1991; Baldocchi *et al.* 1995; Monson and Fall 1989; Harley *et al.* 1994), whereas under stress conditions (water-stress, high temperature, etc.) C_{iso}/C_A may exceed 30% (Sharkey and Loreto 1993; Fang *et al.* 1996). During the current study, we found a C_{iso}/C_A for the **C** seedlings of *ca.* 2%. In the **T** seedlings, the proportion of carbon lost as isoprene increased during water-stress periods, with peak values that exceeded 50%. These peaks were the result of a major reduction in photosynthetic rate (close to zero) while isoprene emission remained high. However, the percentage of carbon lost as isoprene clearly increased from the beginning through the whole drought period showing a good relationship with θ. Because of the strong dependence of isoprene emission on leaf temperature, this carbon loss may become even larger in areas with warm climate where drought-induced stomatal closure may have a large indirect effect on isoprene emission by increasing leaf temperature.

In a climate change scenario with higher temperatures and prolonged droughts, the ratio of isoprene emission to photosynthetic rate could dramatically increase with significant impact on the global terrestrial carbon balance, especially in regions such as the tropics which are estimated to contribute more than 80% of the annual isoprene flux (Jacob and Wofsy 1988; Zimmerman *et al.* 1988; Guenther *et al.* 1995). Although many studies have been published over the past ten years on isoprene emission by plants, additional research focusing on water-stress using a range of species is needed in order to enable us to model plant isoprene emission response to drought in different climate scenarios. It is desirable to relate biogenic emissions to measurable physiological parameters that control emission variations. Pre-dawn Ψ_{leaf} is the most likely candidate for describing the role of water limitations on biogenic emissions from leaves and there are several vegetation models that can be used to predict it. This parameter also may be needed for estimating accurate T_{leaf} during drought conditions. As our results suggest, there may exist a tight relationship between isoprene emission and pre-dawn Ψ_{leaf} during water-stress episodes. Although it is likely that the slope of the relationship between pre-dawn Ψ_{leaf} and isoprene emission varies widely between species, we believe that pre-dawn Ψ_{leaf} could be a useful parameter to include in isoprene emission models to account for effects of drought stress. Although measured under controlled conditions, the observed values of pre-dawn Ψ_{leaf} in this study are similar to those observed for oak species in the field during summer natural drought conditions (Martinez-Vilalta *et al.* 2003; Bombelli and Gratani 2003; Owens and Schreiber 1992). The coefficient of the relationship between isoprene emission and pre-dawn Ψ_{leaf} reported in this study could therefore be used by modellers to set up a first model parameterization that could be improved upon in the future.

Finally, investigations using potted plants and laboratory measurements provide valuable information from which to build hypotheses, but these studies often yield results that differ from those observed using field-grown adult plants in their natural environment. There is therefore a strong need for further field studies to test any definitive model for the isoprene emission response to water-stress in natural ecosystems.

Effect of elevated CO_2 concentration and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought

Pegoraro, E., Rey, A., Bobich, E.G., Barron-Gafford, G., Grieve, K.A., Malhi, Y., Murthy, R.

(*Functional Plant Biology* (2004) **31**: 1-11)

CHAPTER 3

Effect of elevated [CO_2] and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought

3.1. Introduction

Many studies have been published in recent years on the response of trees to elevated atmospheric [CO_2] and great progress has been made in the mechanistic understanding of the physiological responses of different species (e.g. Medlyn *et al.* 1999). Models suggest that future increases in temperature and decreases in precipitation will cause the terrestrial carbon sink to become a source later this century (Cox *et al.* 2000). However, such models do not deal with the emission of volatile organic compounds (VOCs) by plants and the effect that this might have on the carbon cycle and on atmospheric chemistry (Fehsenfeld *et al.* 1992; Lerdau *et al.* 1997; Fuentes *et al.* 2000; Monson and Holland 2001; Guenther 2002). Whereas isoprene emissions have profound effects on atmospheric chemistry because of the high reactivity of isoprene, many environmental variables, such as light and temperature, exert strong control on its biosynthesis and emission (Harley *et al.* 1999; Fuentes *et al.* 2000; Niinemets *et al.* 2004).

It is known that atmospheric CO_2 concentration can affect isoprene emission from plants (Monson and Fall 1989; Sharkey *et al.* 1991; Guenther *et al.* 1991; Rosenstiel *et al.* 2003; Scholefield *et al.* 2004). For example, Guenther *et al.* (1991) found that isoprene emission rates from leaves of *Eucalyptus globulus* exposed to *ca.* 600 µmol mol^{-1} atmospheric [CO_2] were lower than isoprene emission rate*s* from leaves exposed to 100 µmol mol^{-1} CO_2. Sharkey *et al.* (1991) found contrasting responses by *Populus tremuloides* (30-40% decrease) and *Quercus rubra* (100% increase) exposed to an increase in atmospheric [CO_2] from 400 to 650 µmol mol^{-1}. Rosenstiel *et al*. (2003) showed that isoprene emission rates from *Populus deltoides* clones grown for almost two years at 800 and 1200 μmol mol^{-1} atmospheric [CO_2] were reduced by 21% and 41%, respectively, compared to plants grown at 430 μmol mol^{-1} CO_2. Moreover, it has been observed that isoprene emission is already inhibited at ambient [CO_2] when compared to lower atmospheric levels of CO_2 (Monson and Fall 1989; Sharkey *et al.* 1991).

The results from previous studies on the effect of water stress on isoprene emission (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996; Guenther *et al.* 1999; Bruggemann and Schnitzler 2002; Pegoraro *et al.* 2004a) suggest that isoprene emission is much less sensitive to drought than is photosynthesis. Although isoprene emission seems to be independent of stomatal dynamics (Fall and Monson 1992), stomatal closure caused by water stress or high atmospheric water vapor pressure deficit (VPD) may lead to decreases in intercellular [CO_2] (C_i), leading to increases in isoprene production. Furthermore, the reduced transpiration may increase leaf temperature, which also induces higher isoprene emission rates (Fang *et al.* 1996; Harley *et al.* 1999). Despite great progress, our understanding of the effect of water stress on isoprene emission is still limited. Results are variable among studies and no general pattern has been observed. Moreover, most studies have been done on potted plants (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996; Bruggemann and Schnitzler 2002; Pegoraro *et al.* 2004a) and very few have been done on field-grown plants in natural drought conditions (e.g. Guenther *et al.* 1999). Although experiments on potted seedlings provide useful mechanistic information about processes, they do not always yield results that are applicable to forest ecosystems (e.g. Medlyn *et al.* 1999).

To study the effect of atmospheric [CO_2] and VPD, and their interaction with drought on isoprene emission in an agriforest plantation, an experiment was set up inside the controlled environment research facility of the Intensive Forestry Mesocosm (IFM) of Columbia University's Biosphere 2 Laboratory (B2L, Oracle, AZ, USA). The specific objectives of this study were: (1) to investigate the short-term solitary and interactive effects of elevated [CO_2], VPD, and drought on isoprene emission; (2) to study the long-term (permanent) effects of plants' acclimation to elevated CO_2 on isoprene emission; and (3) to calculate the isoprene:carbon dioxide flux ratio for this species under different CO_2, VPD and soil moisture conditions. It was hypothesised that: (I) plants growing in elevated atmospheric $[CO_2]$ undergo an acclimation of the metabolic processes linked to photosynthesis and respiration, so that the inhibition of isoprene production caused by elevated $[CO_2]$ becomes a long-term feature, and (II) high VPD and water stress, by decreasing leaf stomatal conductance and consequently decreasing C_i levels, induce higher isoprene emission rates as a consequence of the reduced CO_2 inhibitory effect. Compared with studies carried out on potted plants, the ability to perform this experiment in the IFM allowed us the opportunity to study the response of fully-grown plants to variations of selected and fully controlled environmental variables, which, on the other hand, would have never been possible in the outside world. Thus, this large-scale facility allowed us to undertake an unprecedented manipulation study over large forest stands.

3.2. Material and Methods

3.2.1. Plant material

The study was carried out in the Intensive Forestry Mesocosm (IFM) facility at the Biosphere 2 Laboratory (B2L) between October 21 and November 30, 2002. This facility consists of three experimental bays at three atmospheric [CO_2]: 430, 800 and 1200 µmol mol^{-1}. Cottonwood clones (*Populus deltoides* Bartr.) were grown from cuttings planted at the start of the 1998 growing season. Thereafter trees were coppiced at the end of each growing season, through the winter of 2001. At the time of this experiment (growing season 2002) the aboveground shoots were six-months old and *ca.* 6 m tall. The soil was 1 m deep; when installed the soil was a mixture of bare soil (60%) and organic matter (40%). Presently, the textural classification of the soil is a silt-loam (Torbert and Johnson 2001). When the soil was analysed in 2004, it contained 2-3% soil organic carbon (SOC) and a C:N ratio of 8.3, similar to that of an agricultural system.

3.2.2. Growth conditions

Plants inside the three bays were grown under the following environmental conditions: average total daily photosynthetically active radiation (PAR) of 15.1 ± 3.5 mol m^{-2} day^{-1} at the top of the canopy; day length of approximately 11 hours, day/night air temperatures of 32 ^{o}C/26 ^{o}C, and a VPD of either 1 or 3 kPa for the LOW and HIGH VPD settings, respectively. In each bay, PAR was measured at different heights (3, 6 and 9 m above ground level) in the canopy using 12 sensors (Apogee Instruments, Logan, UT, USA) installed at four locations (NE, NW, SE and SW). Air temperature and relative humidity were measured using a weather station with thermocouples and a hydrometer (HT205W, Rotronics, La Roche sur Foron, Haute-Savoie, France) mounted at *ca.* 9 m above the soil surface in each bay and shielded from solar heating. VPD was calculated from these data as the difference between the partial pressure of water vapour of saturated air at ambient temperature and the measured partial pressure of water vapour. All data were measured every 15 seconds, averaged and stored every 15 minutes using dataloggers (Campbell-CR10x, Campbell Scientific Inc., Logan, UT, USA). Soil volumetric water content (θ) was determined at a depth of 10 cm in four locations in each bay. Previous studies on the below ground biomass of the stands found that most of the fine root biomass was concentrated in the top 10 cm of soil (Greg Barron-Gafford, pers. comm.). Soil samples of approximately 20 g were taken at depths of 10 cm using a soil auger. The soil samples were placed in pre-weighed tins, which were then sealed and transported to the laboratory where they were weighed. Samples were then placed in a forced-draft oven at 100 ^{o}C for five days, removed, and weighed again. The bulk density of the top 10 cm of the soil was measured and θ (m^3 m^{-3}) was determined as:

$$\theta = [(M_\mathrm{f} - M_\mathrm{d})]/M_\mathrm{d} * \rho_\mathrm{s} * 1/\rho_\mathrm{w}$$

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

3.2.3. Experimental design

The three bays were closed all day with no exchange with the outside air with the exception of a 2 h period at dawn to facilitate the expulsion of night-time respired CO_2. This was necessary only if the CO_2 inside the bay increased to such high concentrations that the trees were unable to bring down the [CO_2] to the desired target concentration on the next day. From October 23 (day 0) to November 29 (day 37) water was withheld in the three bays. The three bays were subjected to two VPD level, which were imposed in three alternate cycles of six days during the drought: *first cycle*, day 2 to day 7; *second cycle*, day 8 to day 13; and *third cycle*, day 20 to day 25. Each cycle consisted of one LOW VPD level set at *ca.* 1 kPa and one HIGH VPD level set at *ca.* 3 kPa (see Table 3.1). At the start of the experiment (day 0), VPD was set to LOW, and it was changed to HIGH on day 5. Starting from day 5 each level was maintained for a period of three days until day 25 when the VPD level was left on HIGH for the rest of the experiment to accentuate the drought stress on the plants.

3.2.4. Sampling protocol

Before the beginning of the gas exchange measurements, five trees were randomly chosen in each bay and followed over the whole experiment. From each tree, one fully expanded leaf was randomly selected from the same position (middle canopy) and orientation (south facing) in the canopy and tagged for subsequent measurements. To avoid large diurnal sampling biases, all gas exchange measurements and collection of air samples for the determination of isoprene emission were made between 10:30 and 14:30.

3.2.5. Leaf gas exchange measurements

Photosynthetic rate, stomatal conductance and intercellular [CO_2] (C_i) were measured using an open-path LI-6400 gas exchange measurement system (Li-Cor, Lincoln, NE, USA). All measurements were made using the same standard cuvette conditions: leaf temperature of 32 ^{o}C and PAR of 1500 μmol m^{-2} s^{-1} and VPD similar to that in the ambient growing conditions. Leaf temperature and light conditions were chosen to match as closely as possible the average outside conditions at the time of measurements to reduce the equilibration time to the cuvette conditions. After a leaf was placed in the cuvette, a minimum of 10 min was allowed for equilibration, and all measurements were made after steady rates of exchange of CO_2 and H_2O were obtained. To avoid interference of 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.

For measurements of isoprene emission rates, the air exiting the cuvette was collected inside a Teflon® bag (2.5 dm^3 volume) connected to the exhaust of the leaf cuvette. Isoprene concentration inside the bags was determined in the laboratory using a gas chromatograph (GC) (SRI 310, Buck Scientific, East Norwalk, CT, USA) with a custom-made inlet system capable of vacuum sample collection and isoprene cryofocusing. A 300 cm^3 sample of the air contained in the bag was trapped in a first loop packed with 60/80 mesh Tenax TA (Sigma Aldrich, Dorset, UK) kept at 4^{o}C to reduce the sample volume and at the same time to eliminate excess amounts of water that could skew the isoprene detection. The sample was then cryogenically concentrated in a second loop kept under liquid nitrogen and transferred to the GC column (0.25 mm ID x 30 m MXT-624 capillary column, RESTEK Corporation, Bellefonte, PA, USA) in a flow of ultra high purity helium. Eluted isoprene from the column was measured using a flame ionisation detector (FID). Additional details of the analytical system are given by Greenberg *et al*. (2003).

A summary of the different experiments and measurements carried out is presented in Table 3.1. In order to study the effect of elevated [CO_2] on isoprene emission and its interaction with drought, leaf gas exchange measurements were made on seven occasions over 31 days between day 6 and day 37 for the three CO_2 treatments.

Table 3.1. Diagram summarising the experiments carried out at Biosphere 2 Laboratory facility in the Intensive Forestry Management biomes of *Populus deltoides*. Growth and measurement [CO_2] (µmol mol-1), VPD (HIGH: 3kPa, LOW: 1kPa), soil moisture (m^3 m^{-3}) and dates (given as days from the beginning of the experiment) are given.

EXPERIMENT	**Growth [CO_2]**	**Measurement [CO_2]**	**VPD**		**Soil moisture**	**Days**
I. **CO_2 and drought** (37 days)	430 800 1200	430 800 1200	HIGH		Decrease gradually over the course of the experiment	6 12 24 29 33 35 37
II. **CO_2, VPD and drought**	430 800 1200	430 800 1200	CYCLE (LOW/HIGH)	**I**	**WET** ($\theta > 0.2$)	0 6
				II	**DRY** ($0.14 < \theta < 0.2$)	9 12
				III	**VERY DRY** ($\theta < 0.14$)	22 24
III. **CO_2 acclimation**	430 1200	430/1200 1200/430	LOW HIGH		$0.16 < \theta < 0.18$	9-10 12-13
			HIGH		$\theta < 0.14$	33-34

All measurements were made in days with conditions of HIGH VPD to accentuate the drought effects. The first measurements were made in conditions of non-limiting soil water content ($\theta > 0.2$ m^3 m^{-3}).

In order to understand the interactive effects of elevated [CO$_2$], VPD and water stress on isoprene emission, leaf gas exchange measurements were made during three cycles of alternating conditions of LOW and HIGH VPD. During the first and second cycles, measurements were made over nine days between day 4 and day 13 ($\theta > 0.14$ m^3 m^{-3}). To observe the effect of drought on isoprene emission and its interaction with elevated [CO$_2$] and VPD, two additional measurements during another cycle were taken on day 22 and 25, when the trees were assumed to be water stressed ($\theta <$ 0.14 m^3 m^{-3}).

In order to understand whether plants had acclimated to elevated [CO$_2$] and the effects of [CO$_2$] on isoprene emission had become long-term (permanent) effects, leaf gas exchange measurements were made at both ambient (430 µmol mol^{-1}) and elevated (1200 µmol mol^{-1}) [CO$_2$] in the lowest (430 µmol mol^{-1}) and highest (1200 µmol mol^{-1}) CO$_2$ treatment mesocosms. Leaves were placed in the cuvette and always measured first at the growth [CO$_2$]. To determine the interaction of CO$_2$ and VPD, gas exchange measurements were made on two different days: on day 10, the third day of the second LOW VPD cycle, and on day 13, the third day of the second HIGH VPD cycle. Moreover, to observe the effect of drought, a third set of measurements were made on day 34, when the plants were assumed to be water stressed ($\theta < 0.14$ m^3 m^{-3}).

3.2.6. Statistical analysis

All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, 1995). When looking at the effect of elevated [CO$_2$] on isoprene emission during the drought period, the overall mean comparison of photosynthetic rate, stomatal conductance, isoprene emission rate and θ between CO$_2$ treatments were analysed with a multivariate analysis of variance (MANOVA). The experimental unit was the individual tree (n = 5). To take into account the overall effect during the course of the experiment, data were first analysed using a two-way ANOVA with repeated measures with drought, treatment, and time and their interactions as factors. When this test was significant for treatment at a 5% level of probability, a single ANOVA was used to test differences on each date to understand how and when the CO_2 treatment affected the specific variables under study. When analysing the interactions of VPD and elevated [CO_2] on isoprene emission rates during the drought, the three factors: cycles, VPD and CO_2 treatments, were first tested for significance using a three-way ANOVA. For each cycle, photosynthetic rate, isoprene emission rate, stomatal conductance, and C_i were first analysed using a two-way ANOVA in which CO_2 and VPD treatment, and their interaction, were considered. Within each cycle, significance for HIGH and LOW VPD of photosynthetic rate, isoprene emission rate, stomatal conductance, and C_i in the three CO_2 treatments was tested using a two-way ANOVA. When looking at the effect of acclimation to [CO_2] on isoprene emission and its interaction with VPD and θ, linear and non-linear regressions were used to determine the relationships between the different physiological variables. Variation around the mean is reported as 1 standard error (SE).

3.3. Results

3.3.1. Effect of elevated [CO_2] on CO_2 uptake and isoprene emission

At the beginning of the experiment (day 0), θ was 0.27 ± 0.01 $m^3\ m^{-3}$, and decreased gradually, until it reached a value of 0.10 ± 0.01 $m^3\ m^{-3}$ at the end of the experiment (day 37) (Figure 3.1A). Water limitation reduced photosynthetic rate when θ was < 0.2 $m^3\ m^{-3}$, whereas the decline in isoprene emission began only when θ was < 0.14 $m^3\ m^{-3}$. On day 6 of the experiment, photosynthetic rate of leaves grown at 430 μmol mol^{-1} of CO_2 were significantly lower ($P < 0.01$) than those grown in the 800 (*ca.* 72% higher) and in the 1200 (*ca.* 123% higher) μmol mol^{-1} CO_2 treatments (Figure 3.1B). During the course of the drought, photosynthetic rate decreased by *ca.* 84%, 65% and 89%, for the 430, 800, and 1200 μmol mol^{-1} CO_2 treatments, respectively. Stomatal conductance showed a similar trend but there were no statistical differences between CO_2 treatments over the course of the experiment ($P > 0.05$ in all cases; Figure 3.1C). In all CO_2 treatments, stomatal conductance declined steadily from day 12 to day 29, with a reduction of *ca.* 89%, 75% and 94% for the 430, 800 and 1200, μmol mol^{-1} CO_2 treatments, respectively, and, from that point remained essentially constant to the end of the experiment. On day 6, isoprene emission rates were significantly greater ($P < 0.01$) in the 430 μmol mol^{-1} CO_2 treatment than in the 800 and in the 1200 μmol mol^{-1} CO_2 treatments (*ca.* 19% and 28% lower, respectively) (Figure 3.1D). Isoprene emission rates changed little for leaves grown in the 800 and 1200 μmol mol^{-1} CO_2 treatments from day 6 to day 29 whereas in the 430 μmol mol^{-1} CO_2 treatment they decreased remarkably until rates were similar to emission rates of the two elevated CO_2 treatments by day 24. From day 33 to the end of the experiment, isoprene emission rates continued to decline slowly reaching minima of 38.3, 36.4 and 27.5 nmol $m^{-2}\ s^{-1}$ in the 430, 800 and 1200 μmol mol^{-1} CO_2 treatments, respectively.

Over the course of the drought, the ratio of the carbon lost as isoprene to carbon assimilated, i.e. the percentage of carbon used in isoprene production compared to the amount of assimilated carbon (C_{iso}/C_A), changed significantly, particularly in the

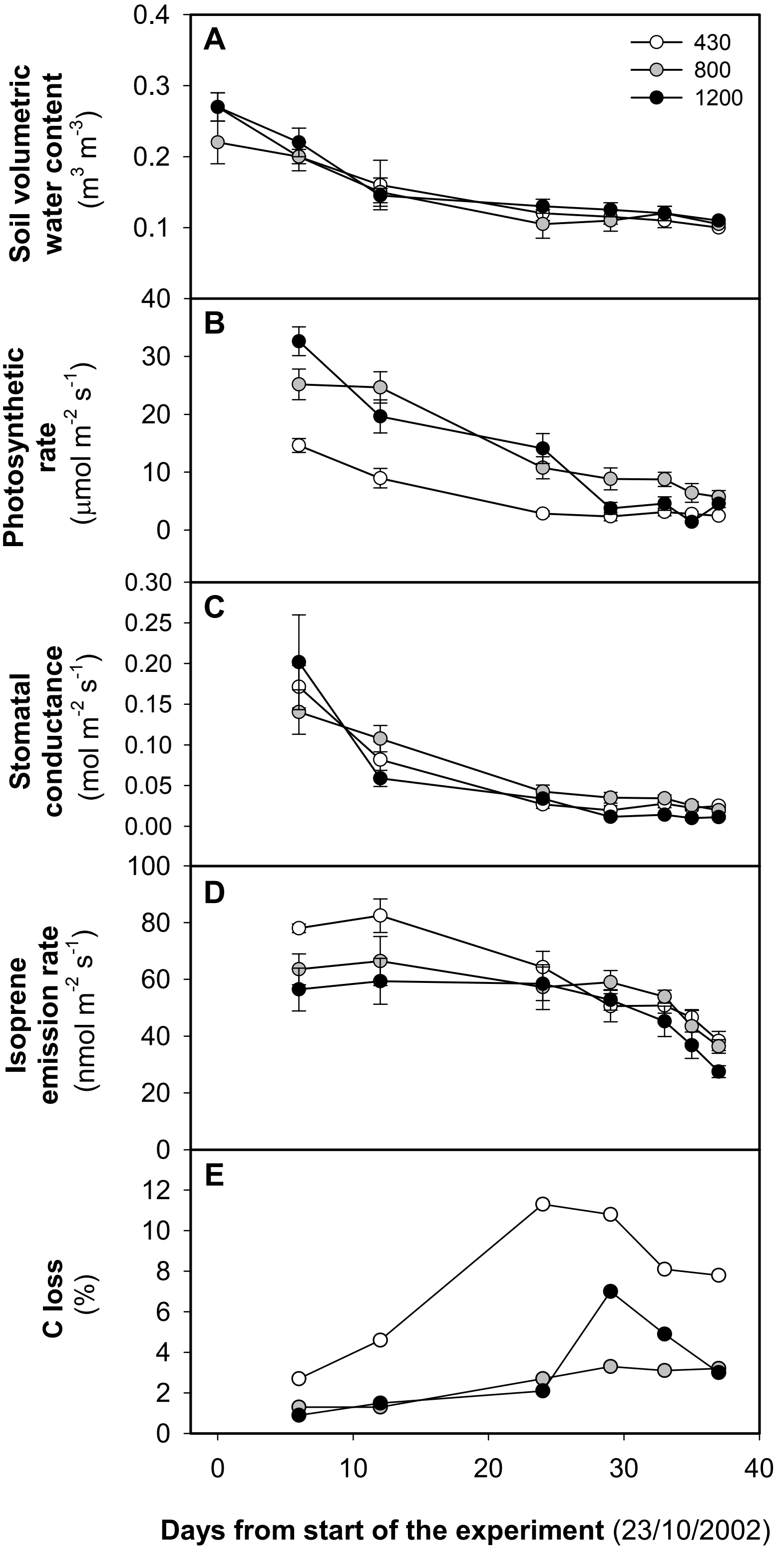


Figure 3.1. Time course of θ (A), photosynthetic rate (B), stomatal conductance (C), isoprene emission rates (D) and C_{iso}/C_A (E) for the three [CO_2] growth treatments (430, 800 and 1200 µmol mol^{-1}, white, grey and black circles, respectively) measured in HIGH VPD (*ca.* 3 kPa) during the course of the drought experiment. Values are means ± 1 SE (n = 5).

430 μmol mol^{-1} CO_2 treatment (Figure 3.1E). At the beginning of the experiment, when soil moisture was not limiting ($\theta > 0.2$ m^3 m^{-3}) C_{iso}/C_A was small (*ca.* 1-3%) and differed slightly among the CO_2 treatments, with the 430 μmol mol^{-1} being the highest. As the drought progressed, in the 430 μmol mol^{-1} CO_2 treatment, the combination of water limitation and HIGH VPD conditions strongly depressed photosynthetic rate leading to an increase of as much as four times in the C_{iso}/C_A by day 24. From day 33, because isoprene emission rates started to decline steadily in all CO_2 treatments whereas photosynthetic rate remained essentially constant, C_{iso}/C_A decreased through the end of the experiment. In the 800 and 1200 μmol mol^{-1} CO_2 treatments, because of higher photosynthetic rate and smaller isoprene emission rates, C_{iso}/C_A increased less and more slowly than in the 430 μmol mol^{-1} CO_2 treatment.

3.3.2. Effect of elevated [CO_2] and VPD on CO_2 uptake and isoprene emission

In the first VPD cycle, in WET conditions ($\theta > 0.2$ m^3 m^{-3}) and during the LOW VPD phase, photosynthetic rates were similar in the two elevated CO_2 treatments and significantly greater (*ca.* 75%) than in the ambient CO_2 treatment ($P < 0.001$; Figure 3.2A). In HIGH VPD, photosynthetic rates for the three CO_2 treatments were lower compared to the LOW VPD phase and significantly different from each other ($P < 0.05$).

During the second cycle, although θ had decreased to 0.17 m^3 m^{-3} (DRY conditions, Figure 3.2B), in the LOW VPD phase photosynthetic rate showed similar values to those measured during the first cycle. During the HIGH VPD phase, photosynthetic rates measured in the 430 and 1200 μmol mol^{-1} treatments were strongly reduced (*ca.* 40%) compared to the WET phase (Figure 3.2A), whereas rates in the 800 μmol mol^{-1} CO_2 treatment did not change.

During the third cycle, in very dry conditions ($\theta < 0.14$ m^3 m^{-3}), photosynthetic rate showed again a similar pattern to the previous cycles, with photosynthetic rates measured in the elevated CO_2 treatment being significantly greater than those measured in the ambient CO_2 treatment ($P < 0.05$) (Figure 3.2C).

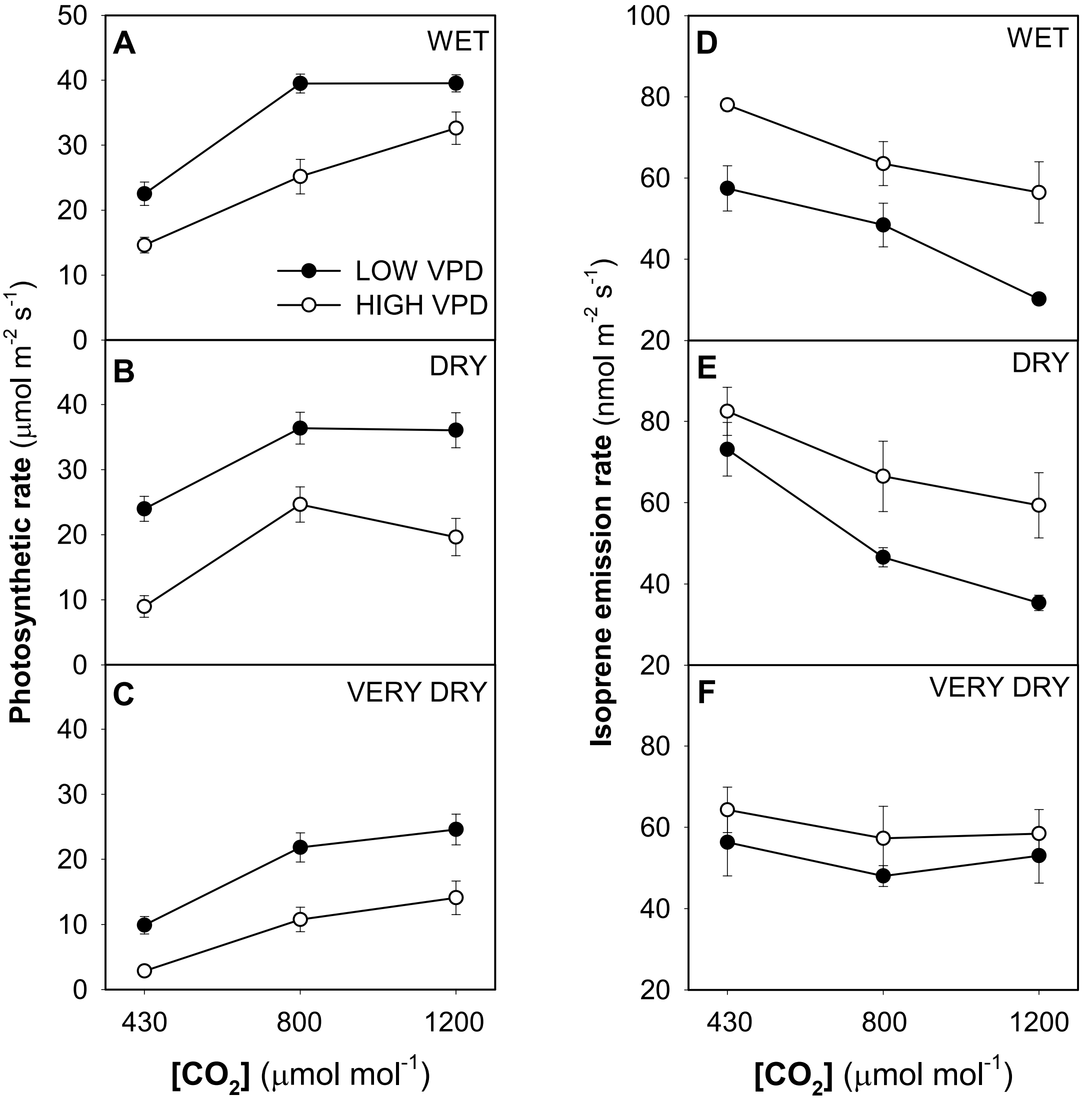


Figure 3.2. Effect of [CO_2] growth treatment (430, 800 and 1200 μmol mol^{-1}, white, grey and black bars, respectively) on leaf isoprene emission rate and photosynthetic rate measured in HIGH (*ca.* 3 kPa) and LOW (*ca.* 1 kPa) VPD during three cycles corresponding to a soil water content of 0.22, 0.17 and 0.13 m^3 m^{-3}: WET, DRY and VERY DRY, respectively. Symbols represent means ± 1SE (n = 5).

However, compared to the second cycle, photosynthetic rates were drastically depressed, particularly in the elevated CO_2 treatments and in LOW VPD, when they were reduced by 24% in the 430 μmol mol^{-1} CO_2 and by 36% in the 800 and 1200 μmol mol^{-1} CO_2, whereas in the HIGH VPD they were reduced by 9% in the 430 μmol mol^{-1} CO_2 and by 25% and 20% in the 800 and 1200 μmol mol^{-1} CO_2, respectively.

In the first VPD cycle, isoprene emission rates measured at both LOW and HIGH VPD were significantly different between the 430 and the 1200 μmol mol^{-1} CO_2 treatments ($P < 0.001$) and always decreased with increasing [CO_2] (Figure 3.2D). In LOW VPD, isoprene emission rates measured in leaves grown in the 1200 μmol mol^{-1} treatment were 47% lower than leaf isoprene emissions measured in the 430 μmol mol^{-1} CO_2 treatment. In HIGH VPD, isoprene emission rates for each CO_2 treatment were significantly higher ($P < 0.001$) than during the LOW VPD treatment. At this time, emissions from the 1200 μmol mol^{-1} CO_2 treatment were only 28% lower than emissions from the 430 μmol mol^{-1} CO_2 treatment.

During the second VPD cycle, in dry conditions ($0.2 > \theta > 0.14$ m^3 m^{-3}), leaf isoprene emission rates remained essentially the same in the 800 and 1200 μmol mol^{-1} CO_2 treatments, at both the HIGH and LOW VPD treatments, whereas isoprene emission rates measured in the 430 μmol mol^{-1} CO_2 treatment increased drastically (27.3%) in LOW VPD conditions, reaching the values measured in HIGH VPD conditions (Figure 3.2E).

By the third cycle, in very dry conditions ($\theta < 0.14$ m^3 m^{-3}), isoprene emission were not significantly different between CO_2 treatments in both the HIGH and LOW VPD treatments and between VPD treatments ($P > 0.05$ in all cases). Drought appeared to have strong contrasting effect on isoprene emission mainly in the 430 and 1200 μmol mol^{-1} treatments. During the LOW VPD setting, isoprene emission rates decreased by 23% in the 430 μmol mol^{-1} CO_2 treatment whereas they increased by 50% in the 1200 μmol mol^{-1} CO_2 treatment. During the HIGH VPD setting they both decreased: 22% in the 430 μmol mol^{-1} CO_2 treatment and only 1.5% in the 1200 μmol mol^{-1} CO_2 treatment.

During all measurements over the period of the three VPD cycles, within each CO_2 treatment the higher values of isoprene emission obtained during the HIGH VPD phases were associated with the lower values of C_i (Figure 3.3).

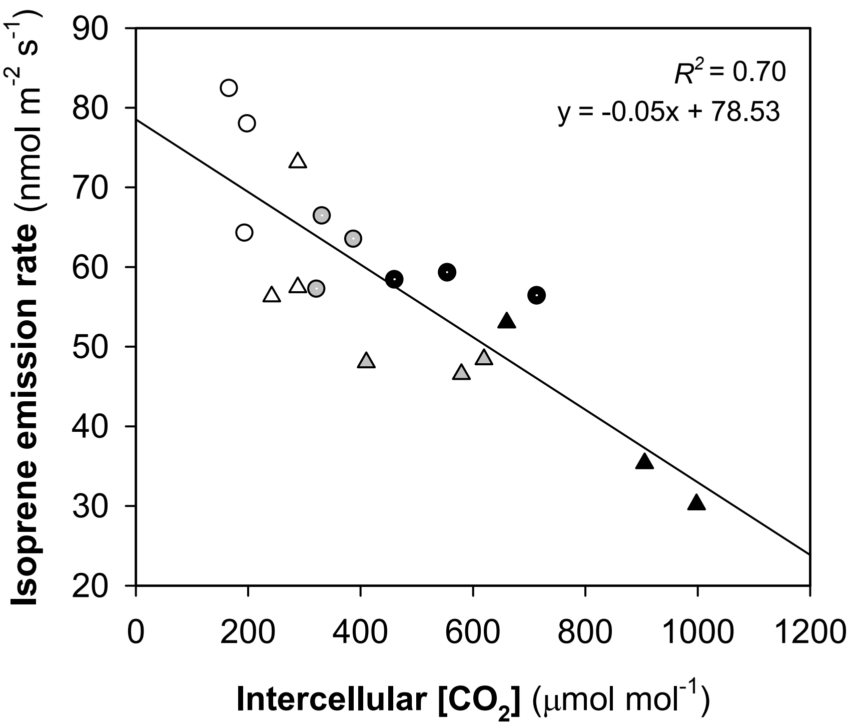


Figure 3.3. Relationship between intercellular [CO_2] (C_i) and leaf isoprene emission rate measured at LOW (*ca.* 1 kPa) (triangles) and HIGH (*ca.* 3 kPa) (circles) VPD during the course of the drought experiment. Each symbol represents the mean (n = 5) of trees grown at three different atmospheric [CO_2]: 430, 800 and 1200 µmol mol^{-1}, white, grey and black circles, respectively.

In wet conditions and during the LOW VPD phase, isoprene emission represented a carbon loss of 1 to 2% of the assimilated carbon in photosynthesis (C_{iso}/C_A), with the largest loss in the 430 µmol mol^{-1} CO_2 and the smallest in the 1200 µmol mol^{-1} CO_2 (Figure 3.4A). At this time the loss was only slightly larger in HIGH VPD than in LOW VPD conditions (Figure 3.4B). Over the three VPD cycles, in LOW VPD, C_{iso}/C_A increased with decreasing θ in all CO_2 treatments and the carbon loss doubled when water stress was most severe. In HIGH VPD, C_{iso}/C_A for all CO_2 treatments showed the same pattern although it dramatically increased particularly in the 430 µmol mol^{-1} CO_2 treatment, where at the end of the water-stress period the C_{iso}/C_A was approximately four times higher than in wet conditions.

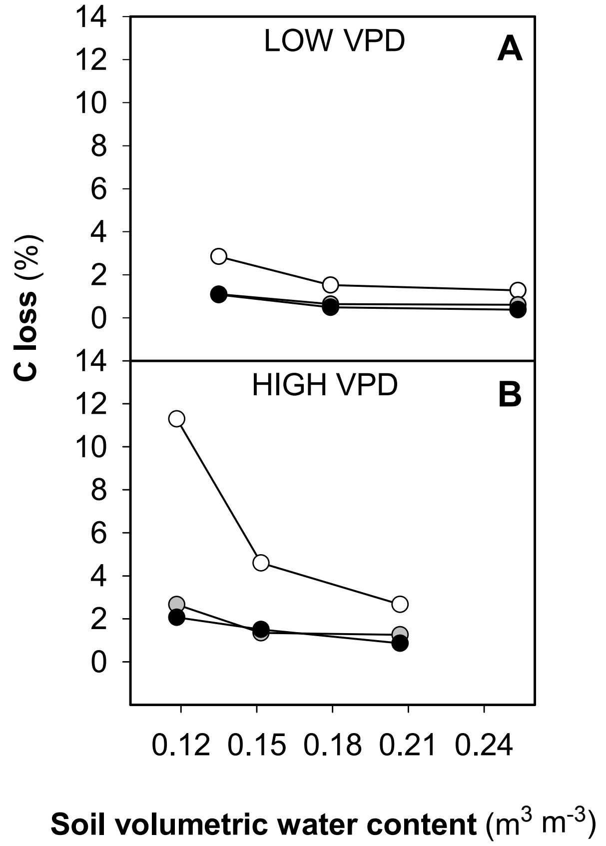


Figure 3.4. Effect of LOW (*ca.* 1 kPa) (A) and HIGH (*ca.* 3 kPa) (B) VPD on the percentage of carbon lost as isoprene emission (C_{iso}/C_A), and the interaction with soil volumetric water content (θ) during the course of the drought experiment. Each C_{iso}/C_A was calculated using the mean (n = 5) of trees grown at three different atmospheric [CO_2]: 430, 800 and 1200 µmol mol-1, white, grey and black circles, respectively.

3.3.3. Effect of CO_2 acclimation and VPD CO_2 uptake and isoprene emission

Photosynthesis: At the time of the second VPD cycle, with values of $\theta > 0.14$ m^3 m^{-3}, when leaves were measured at their growth [CO_2], photosynthetic rates of leaves grown at 1200 µmol mol^{-1} CO_2 were significantly higher ($P < 0.01$) than those of trees grown at 430 µmol mol^{-1} CO_2, both at HIGH and LOW VPD (Figure 3.5A). In both CO_2 treatments, elevated [CO_2] measurement concentration always stimulated photosynthetic rates compared to rates measured at ambient [CO_2] measurement concentration. However, when compared at the same [CO_2] ("*measurement concentration*") in LOW VPD, leaves grown at 430 µmol mol^{-1} CO_2 always exhibited higher photosynthetic rates than leaves grown at 1200 µmol mol^{-1} CO_2. HIGH VPD caused a strong decrease in photosynthetic rates in both CO_2 treatments, particularly that of the ambient CO_2 treatment (*ca.* 63%). Consequently, differences between photosynthetic rates of leaves grown at ambient and elevated [CO_2] treatments disappeared when measured at the same [CO_2] ($P > 0.05$).

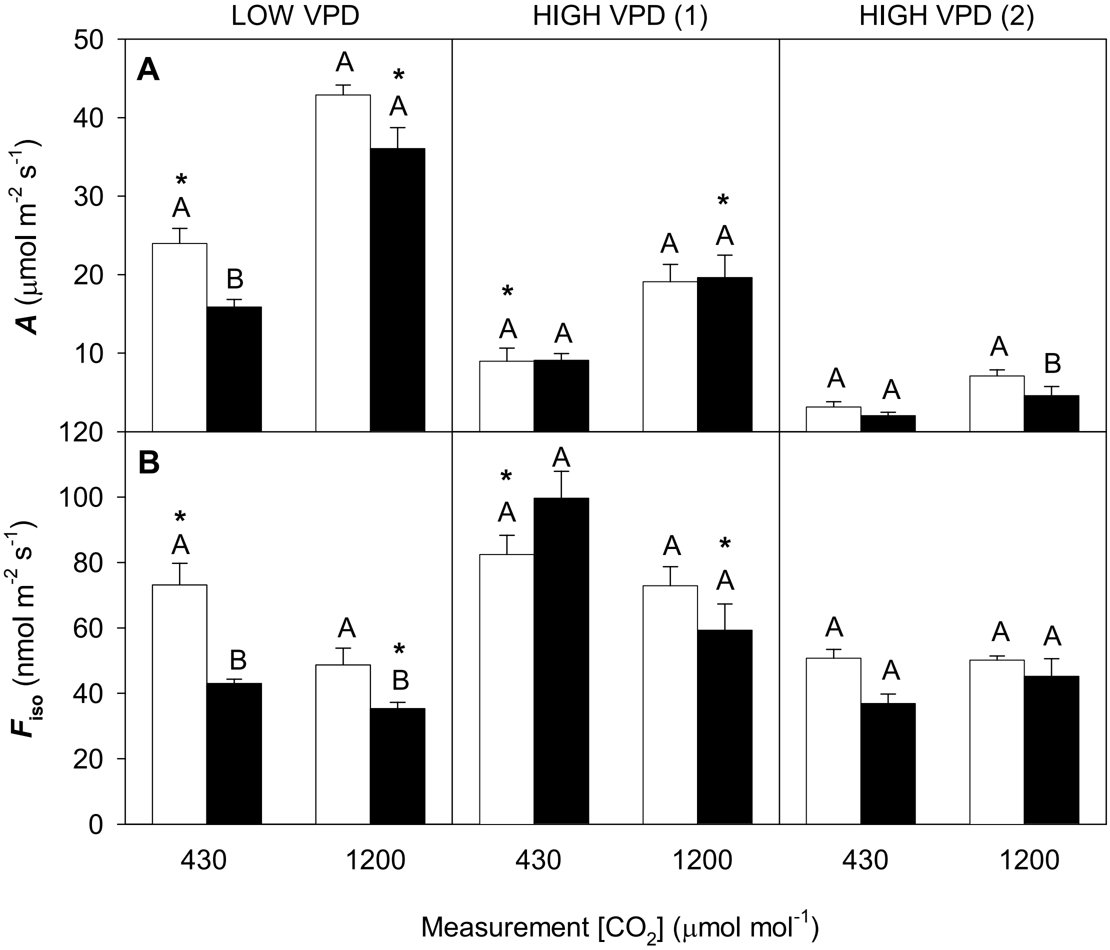


Figure 3.5. Effect of CO_2 growth concentration (430 and 1200 µmol mol^{-1}, white and black bars, respectively) on leaf photosynthetic rates (A) and isoprene emission rates (B) measured at 430 and 1200 µmol mol^{-1} [CO_2]. Measurements were made in LOW VPD (*ca.* 1 kPa), HIGH VPD (*ca.* 3 kPa) with soil volumetric water content $\theta = 0.15$ m^3 m^{-3} (1) and HIGH VPD with $\theta = 0.11$ m3 m-3 (2). Bars represent means ± 1 SE (n = 5). Bars at the same measurement [CO_2] with different letters are significantly different at 5% (LSD test). In each panel, bars of the ambient and elevated CO_2 treatment with asterisk are significantly different at 5% (LSD test).

Towards the end of the experiment when values of θ were < 0.14 m^3 m^{-3}, i.e. when the effect of drought was most severe, photosynthetic rates were measured again at HIGH VPD conditions and rates were further reduced by *ca.* 65% and *ca.* 77% in the ambient and elevated CO_2 treatments, respectively (Figure 3.5A).

Isoprene emission: As seen in Figure 3.1, in wet conditions, isoprene emission rates of leaves grown and measured at elevated [CO_2] were always significantly lower than those of leaves grown and measured at ambient [CO_2] ($P > 0.05$). When leaves of the ambient and elevated CO_2 treatments were measured in LOW VPD conditions and at both ambient and elevated [CO_2], isoprene emission rates were significantly lower in the elevated CO_2 treatment than in the ambient CO_2 treatment ($P < 0.001$) (Figure 3.5B). When measured at HIGH VPD, isoprene emission rates were stimulated in both CO_2 treatments at both ambient and elevated CO_2 measurement concentrations compared to those measured in LOW VPD conditions. The stimulation effect was particularly strong in the elevated CO_2 treatment where emissions doubled when measured at 430 μmol mol^{-1} CO_2 and increased by 68% when measured at 1200 μmol mol^{-1} CO_2, while in the ambient [CO_2] treatment, leaf isoprene emissions increased only by 13% when measured at 430 μmol mol^{-1} CO_2 and by 50% when measured at 1200 μmol mol^{-1} CO_2. Similar to the effect on *photosynthetic rates,* at HIGH VPD there were no statistical significant differences in isoprene emission rates between CO_2 treatments measured at both ambient and elevated [CO_2] ($P > 0.05$ in all cases). When measured again at HIGH VPD but in limiting soil moisture conditions ($\theta = 0.11$ m^3 m^{-3}), compared to the previous measurement isoprene emission rates were reduced by *ca.* 35% in the ambient CO_2 treatment at both 430 and 1200 μmol mol^{-1} CO_2 measurement concentrations, and by *ca.* 57% and *ca.* 24% in the elevated CO_2 treatment at 430 and 1200 μmol mol^{-1} CO_2 measurement concentrations, respectively.

3.4. Discussion

Isoprene emission rates are generally believed to decrease with increases in atmospheric [CO_2] (e.g. Monson and Fall 1989; Sharkey *et al.* 1991; Guenther *et al.* 1991; Rosenstiel *et al.* 2003). Our experiment with enclosed model cottonwood plantations confirmed this negative response, and went further demonstrating that in plants growing in a CO_2–rich atmosphere this inhibition may become a permanent feature. Rosenstiel *et al*. (2003) found that although isoprene emission is generally considered closely linked to photosynthesis, an increase in atmospheric [CO_2] alters plant metabolism by stimulating CO_2 fixation and reducing the availability of cytosolic phosphoenolpyruvate (PEP), necessary for the synthesis in the chloroplast of dimethylallyl-diphosphate (DMAPP), the immediate precursor in isoprene biosynthesis. This may help explaining the contrasting response of assimilation and isoprene emission in plants acclimated to elevated CO_2. As documented in previous studies carried out in the field (Rey and Jarvis 1998), our results show that although photosynthetic rate was stimulated in elevated CO_2 atmospheric concentration, there was a permanent metabolic change as a result of plant acclimation leading to a reduced photosynthetic efficiency: plants grown at elevated [CO_2] always showed lower photosynthetic rate than plants growing at ambient [CO_2] when measured at the same [CO_2]. This metabolic acclimation may include an alteration of the PEP partitioning in favor of the photosynthetic process, resulting in a permanent reduction in the necessary substrate for isoprene production. As our results indicate, isoprene emission also showed a permanent change in its response to elevated CO_2. When measured at the same [CO_2], the leaves of trees grown at 1200 μmol mol^{-1} CO_2 always displayed reduced isoprene emission rates compared to leaves of trees grown at 430 μmol mol^{-1} CO_2. This result supports our first hypothesis that when plants acclimate to elevated [CO_2], inhibition of isoprene emission becomes a long-term feature.

However, for the first time it was demonstrated that high VPD may counteract the CO_2 effect by enhancing isoprene emission in ambient and elevated [CO_2]. It appears that the inhibitory effect of elevated CO_2 on isoprene emission was compensated for by the stomata closure as a result of the higher water vapour concentration gradient between the leaves and the atmosphere. The observed stimulation of isoprene emission was related to stomatal closure in the sense that this resulted in a decrease in C_i, which in turn would decrease the inhibitory effect of elevated CO_2 on isoprene emission (Monson and Fall 1989; Rosenstiel *et al.* 2003). Therefore, these results generally support our second hypothesis that under high VPD or water stress conditions isoprene emission from trees may be stimulated as a consequence of the reduced CO_2 inhibition effect.

Although isoprene emission rates from leaves of large trees of *Populus deltoides* in Biosphere 2 Laboratory decreased when water stress was severe, they appeared to be considerably less sensitive to drought than photosynthetic rates and stomatal conductance. These results are similar to those obtained from a previous study (Pegoraro *et al.* 2004a) on potted plants of *Quercus virginiana* Mill.. For relatively wet soil conditions, elevated [CO_2] were associated with lower isoprene emission, but this difference tended to disappear with the progression of drought. There have been many studies on the response of trees to high CO_2, soil moisture stress and high VPD demonstrating that high VPD and low soil moisture tend to reduce stomatal conductance diminishing the effect of elevated CO_2 and decreasing photosynthetic rates (e.g. Field *et al.* 1995; Curtis 1996; Will and Teskey 1997). In our experiment the depression of photosynthetic rates and the increase in isoprene emission caused by water stress and HIGH VPD resulted in a major increase in the carbon loss from plants. At the beginning of the experiment, when soil water contents were not limiting, C_{iso}/C_A was similar to that observed in other studies (*ca.* 2%) (Sharkey *et al.* 1991; Baldocchi *et al.* 1995; Monson and Fall 1989; Harley *et al.* 1994; Fang *et al.* 1996). As soil water decreased, during the HIGH VPD periods C_{iso}/C_A reached values that exceeded 10%. These values where similar to what observed for other species during drought, although carbon losses exceeding 50% have been observed when photosynthetic rate is reduced to almost zero (Tingey *et al.* 1981; Sharkey and Loreto 1993; Harley *et al.* 1996; Fang *et al.* 1996; Pegoraro *et al.* 2004a). C_{iso}/C_A values were higher for the ambient CO_2 treatment than for the elevated CO_2 treatment because of the higher isoprene emission rates and because the increase in the percentage of the carbon loss was mainly caused by major reductions of photosynthetic rates.

Towards the end of the experiment, the declines in rates of isoprene emission observed in all three treatments were probably the result of the long period of depression of the photosynthetic process. Although most of the carbon in the isoprene molecule comes from the recent photosynthate (Sharkey *et al.* 1991; Delwiche and Sharkey 1993; Karl *et al.* 2002), under stress conditions plants may use extra-chloroplastic slow-turnover alternative carbon sources which increase their carbon contribution with increasing decline in photosynthetic rates (Funk *et al.* 2004). Evidence from a recent study (Loreto *et al.* 2004) suggests that the two pools do not cross-talk even when the fresh chloroplastic pool is inhibited, but because in non-stressed conditions isoprene represent only a small fraction of the fresh photosynthate and may even be formed by respiratory CO_2 recycled in leaves, it is possible that isoprene may also use chloroplastic C pools as an alternative source when photosynthesis is inhibited. However, when the water stress is severe and cause prolonged depression of photosynthetic rates, it is possible that the decline in isoprene emission reflects the depletion of both these alternative carbon pools.

Populus deltoides, like almost all fast-growing tree species, emits large quantities of isoprene. Following the resolutions of the Kyoto protocol, afforestation on large spatial scales with such fast growing species has been encouraged as a way to fight the increase in atmospheric [CO_2] with carbon sequestration in the form of forests and wood products. However, continued expansion of plantations of high isoprene emitting species can result in a significant influence on regional atmospheric chemistry, increasing ozone pollution, perturbing biogeochemical cycles, and further contributing to the global warming by enhancing the lifetime of methane, a powerful greenhouse gas (Rosenstiel *et al.* 2003). Although increased atmospheric [CO_2] enhances biomass production and reduce ecosystem isoprene emissions (Rosenstiel *et al.* 2003), more specific studies are needed to understand the exact contribution of the different environmental and metabolic control variables on isoprene emissions from different species. The results from the present study showed that drought and high VPD have the opposite effect of elevated [CO_2], increasing isoprene emission and decreasing the photosynthetic process. Future climate scenarios suggest global increases in mean temperature and localised reduction in precipitation in many regions of the world (Houghton *et al.* 2001). As a result, future increases in global temperature, VPD and drought may shift this balance strongly in favour of isoprene emission, meanwhile depressing biomass production.

PART II

CANOPY-SCALE EXPERIMENTS

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

Pegoraro, E., Rey, A., Barron-Gafford, G., Monson, R., Malhi, Y., Murthy, R.

(*Oecologia*, in press)

CHAPTER 4

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

4.1. Introduction

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

Eastern cottonwood (*Populus deltoides* Bartr.) is a common species grown in commercial agriforest plantations, mostly in temperate climates. The increased establishment of short-rotation agriforests has been promoted as a means of satisfying the increasing demand for wood and paper products (Fenning and

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

In order to improve our understanding of the control of environmental parameters on isoprene emission from a cottonwood agriforestry plantation, we set up an experiment inside the controlled environment research facility of the intensive forestry mesocosm (IFM) of Columbia University's Biosphere 2 Laboratory (B2L). The overall objective of the study was to improve our understanding of the interacting effects of elevated atmospheric [CO_2], soil water deficit and leaf-to-air water vapour pressure deficit (VPD) on isoprene emission. Specifically, we aimed to test the hypothesis that high VPD and reduced soil water availability may override the suppression of isoprene emission at high [CO_2]. The specific objectives of this study were: (1) to investigate the effect of elevated atmospheric [CO_2] on ecosystem isoprene emission; (2) to examine the interaction between elevated atmospheric

[CO_2] with VPD and drought stress; (3) to study the relationship between isoprene emission and photosynthesis during water stress; and (4) to analyse the fundamental canopy-level relationships between ecosystem isoprene emission and environmental variables such as light, temperature, soil moisture and VPD.

4.2. Material and Methods

4.2.1. Plant material

Experiments were carried out during the autumn of 2002 in the Intensive Forestry Mesocosm (IFM) facility at the Biosphere 2 Laboratory (B2L) Oracle, AZ, USA (1130 m elevation, 32° 35′ N latitude, 110° 51′ W longitude), in three agriforest cottonwood plantations (day-neutral clones of *Populus deltoides* Bartr.) grown in three separate experimental bays (of approx. 12000 m^3 volume and 550 m^2 soil surface) with independent daytime control of atmospheric [CO_2]: 430, 800 and 1200 μmol mol^{-1}, air circulation, temperature and precipitation (Rosenstiel *et al.* 2003; Osmond *et al.* 2004). The three mesocosms were operated as semi-closed systems with a set of push-pull fans working only for a 2 hour period at dawn to facilitate the expulsion of night time respired CO_2, exchanging the air inside the mesocosms with outside air (reaching an exchange rate of up to 110 m^3 min^{-1} for each mesocosm, a turnover time of 100 min). Air handlers and three additional fans keep each mesocosm well mixed (SF_6 is well mixed within 12 minutes after injection).

The cottonwoods were planted from cuttings in 1998, coppiced at the end of each growing season through 2002 and exposed to controlled atmospheric [CO_2] during each growing season in 1999-2003. Because of CO_2 fertilization effect, in October 2002 (the time of the experiment) the trees grown in the elevated [CO_2] had accumulated more biomass than the trees grown at ambient [CO_2]: trees were 6.5 and 6.8 m tall with a leaf area index (LAI) of 1.9 and 3, in the 430 and 1200 μmol mol^{-1} CO_2, concentration bays, respectively. The constructed silt loam soil (1 m deep) of the agriforest had been evolving *in situ* over 12 years and had developed the physical and nutritional profiles of "natural soils" (Torbert and Johnson 2001), comparable to those used for agriforestry in the SE United States. It now shows metabolic (Murthy *et al.* 2004) and microbiological properties (Lipson *et al.*, in review) "within a reasonable range for natural soils" (Kudeyarov *et al.* 2002), with a soil organic carbon content of *ca.* 2-3% and a carbon:nitrogen ratio of 8.3.

4.2.2. Growth conditions

Although the glass structure of the Biosphere transmits 72% of incoming photosynthetically active radiation (PAR), the low latitude of the site allowed maximum PAR values to reach 2000 μmol m^{-2} s^{-1}. Average daily total PAR at the canopy level was 15.62 ± 3.40, 13.98 ± 3.13 and 15.71 ± 3.74 mol m^{-2} day^{-1} for the 1200, 800 and 430 μmol mol^{-1} bay, respectively. Day length was 11.5 hours at the start of the experiments and 11.0 hours at the end, air temperature (T_{air}) was set at $34^{o}C/22^{o}C$ day/night, and relative humidity (RH) was *ca.* 75% and 30% for the low (1kPa) and high (3 kPa) VPD settings, respectively.

Arrays of sensors facilitated continuous monitoring of atmospheric [CO_2], environmental conditions (light, air temperature, leaf temperature, and relative humidity) and trace gas fluxes. PAR was measured in each bay with 12 sensors (Apogee Instruments, Logan, UT, USA) installed at four evenly distributed locations in each bay (NE, NW, SE and SW) and mounted at three different heights (3, 6 and 9 m above the surface) for each location. VPD was calculated from T_{air} and RH data which were measured using a weather station (HT205W Rotronics Hydrometer, La Roche sur Foron, Haute-Savoie, France) mounted at *ca.* 9 m height in each bay and shielded from solar heating. Soil moisture (θ) was monitored with Time Domain Reflectometry probes (TDR CS165, Campbell Scientific Instruments, Logan, UT, USA) inserted at four locations in each bay at two depths: 20 and 80 cm. All data were collected every 15 seconds, averaged and stored every 15 minutes using data-loggers (Campbell-CR10x, Campbell Scientific Inc., Logan, UT, USA).

4.2.3. Experimental design

Because the mesocosms are arranged east to west (430, 800 and 1200 μmol mol^{-1} CO_2, respectively), this study focuses on results from the 430 and 1200 μmol mol^{-1} mesocosms since these two mesocosms received similar integrated light and are most directly comparable. The experiment was carried out towards the end of the 2002 growing season: from October 23 (day 0) to November 29 (day 37) water was withheld from the trees in the bays and the soil was left to dry out naturally. The two CO_2 treatments were combined in temporal sequence with two levels of VPD, imposed in four alternate cycles during the drought. Each cycle consisted of one low VPD period set at a midday value of *ca.* 1 kPa and one high VPD period set at a midday value of *ca.* 3 kPa. Each VPD phase was maintained for three days, starting on day 3 with the low VPD, until day 23 when the VPD level was left on high for the remainder of the experiment to accelerate drying out of the soil and to accentuate the water stress on the trees. The two phases (low and high VPD) of the first cycle occurred when θ was maximal, whereas the following phases were associated with decreasing θ. However, only the last cycle showed a clear interaction with water stress in the low VPD phase.

4.2.4. Ecosystem CO_2 exchange

Net ecosystem exchange of CO_2 (NEE) was calculated for each bay from changes in [CO_2] measured at 15 minute intervals (for a more detailed description see Murthy *et al.* 2004). Data for NEE calculation for each sampling period were collected at the end of the sampling period.

4.2.5. Ecosystem isoprene flux measurements

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

4.2.6. Gross isoprene production (GIP)

The isoprene ecosystem flux, which in our case corresponds to the *net isoprene exchange* (NIE) (isoprene emission minus isoprene consumption), was then calculated every 15 minutes for a “closed” system with the following equation:

$$NIE = \frac{\Delta C}{\Delta t} = \frac{(C_{t+1} + (C_{t+1} * L) - (C_{t-1} + (C_{t-1} * L))}{2 \times \Delta t}$$

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 L is the leak rate. Δt is the length of the time period (15 minutes). This had the effect of centring the derivative on the current time period, and simultaneously introduced some smoothing.

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

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

$$\text{GIP}=\text{NIE}-F_{\text{soil}}$$

4.2.7. Data analysis

To study the response of GIP and NEE to controlled and uncontrolled environmental variables, we used midday averages (10:45 to 15:45). This allowed us to exclude the morning periods in which the push-pull fans were flushing the bays and thus we may consider the system as a perfectly closed system.

Data were analysed using both bivariate correlation and multiple regression between midday averages of GIP as the dependent variable and PAR, T_{air}, θ and VPD (used as dummy variable with coding: 0 for LOW VPD and 1 for HIGH VPD) as independent variables. Analyses were done for the ambient and the elevated CO_2 treatment independently. All assumptions were checked using SAS software and it was necessary to perform a logarithmic transformation on the dependent variable to ensure homogeneity of variance. In addition, this transformation allowed us to perform a simpler analysis since the relationships between the predictor variables and

GIP were linear. The bivariate correlations revealed which predictor variables were significantly related to GIP. Then, we applied a linear multiple regression model using all significant variables as predictors. Beta weights (standardised multiple regression coefficients) were also calculated.

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

4.3. Results

Over the course of the experiment, GIP showed a rapid response to short-term (day to day) changes in all environmental parameters (i.e. [CO_2], PAR, T_{air}, VPD and θ), whereas, in the short term, NEE was sensitive mainly to the variation of light and θ (Figure 4.1A and B). In well-watered conditions, elevated [CO_2] caused a decrease in the GIP of *ca.* 30% compared to the isoprene emissions in ambient [CO_2] (Figure 4.1A), whereas NEE was stimulated, being *ca.* 153% higher in elevated [CO_2] than in ambient (Figure 4.1B). This was partly the result of a larger LAI developed in elevated [CO_2] than in ambient [CO_2]. Therefore, when considering fluxes per unit of leaf area, the decrease of GIP (nnmol m_{leaf}^{-2} s^{-1}) caused by elevated [CO_2] increased to *ca.* 58%, whereas the stimulation of NEE (μmol m_{leaf}^{-2} s^{-1}) was only *ca.* 72%. GIP showed a very rapid response to changes in the [CO_2] (Figure 4.1C), with rates increasing almost coincidentally with decreasing [CO_2]. Again, when corrected for the difference in LAI between the two CO_2 treatments (data not shown), GIP from the ambient CO_2 treatment was significantly higher ($P < 0.001$, the two regression curves were tested with F-tests) than GIP from the elevated CO_2 treatment over the whole duration of the CO_2 experiment (Figure 4.2A). As expected, NEE rates increased with increasing [CO_2] (Figure 4.2B). However, the ambient and elevated CO_2 growth treatments caused a difference in the instantaneous response at ambient and elevated atmospheric [CO_2] (i.e., when tested with the F-test the two regression curves were not significantly different). At ambient atmospheric [CO_2], the trees in the elevated CO_2 treatment showed higher NEE rates than the trees grown in the ambient CO_2 treatment, and at elevated atmospheric [CO_2], the NEE fluxes in the elevated CO_2 growth treatment were lower than fluxes in the ambient CO_2 treatment. Over the three month period of the experiment, midday average PAR progressively decreased from *ca.* 900 (mid September) to *ca.* 600 (mid December) μmol m^{-2} s^{-1} (Figure 4.1D), with the exception of a few days during which rainstorms and heavy cloudiness caused a significant drop in the available light. These occasional drops in radiation also caused the air temperature inside the bays to deviate from the set point (Figure 4.1E).

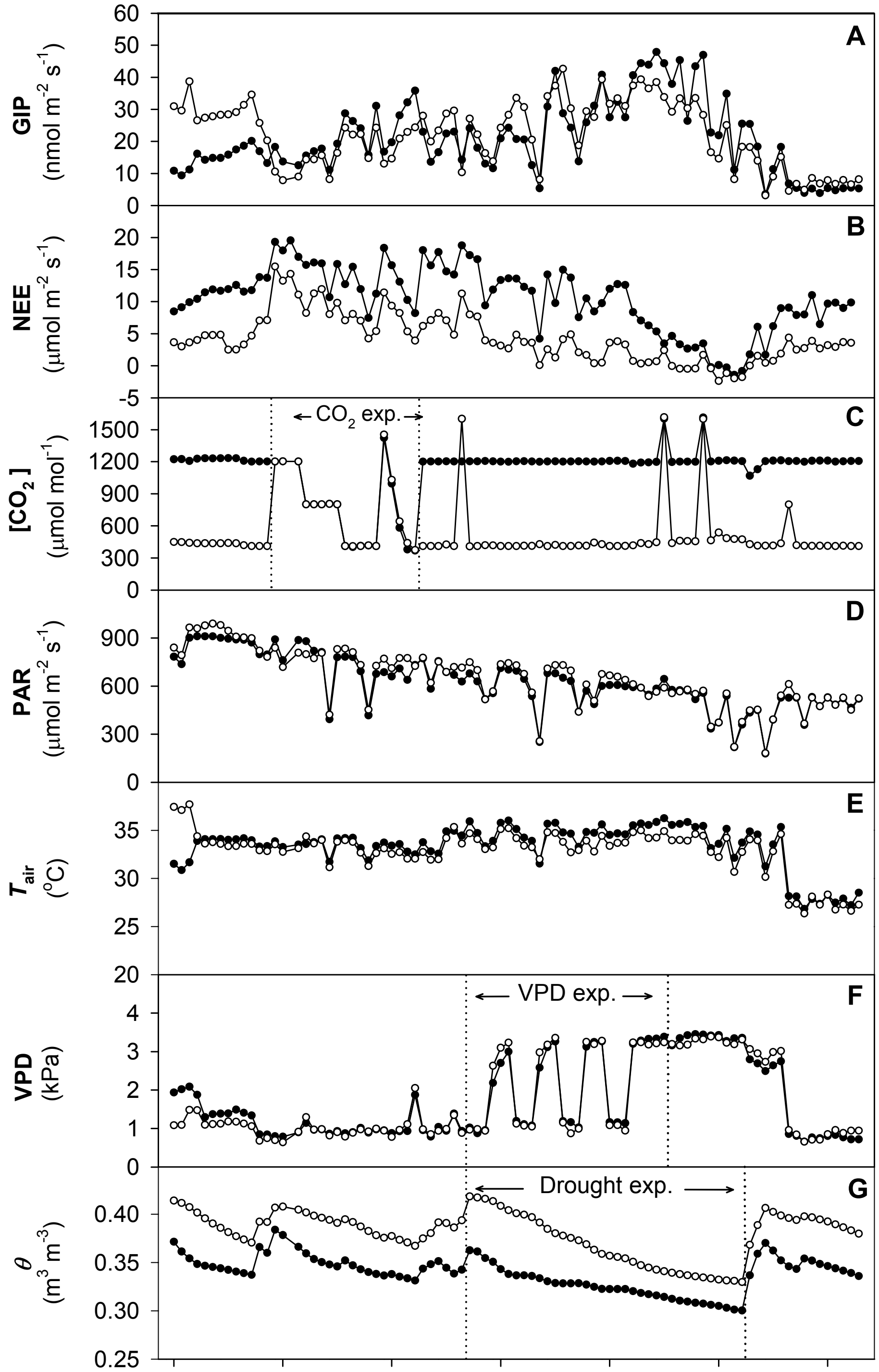


Figure 4.1. Central daytime averages (10:45 to 15:45) of GIP (A) and NEE (B), and environmental variables: [CO_2] (C), PAR (D), *T*air (E), VPD (F) and θ (G), for the 430 and 1200 µmol mol^{-1} CO_2 treatments (white and black circles, respectively) over the experimental period (17/9 to 14/12/2002). The vertical dotted lines correspond to the boundaries of the CO_2, VPD and drought experiments.

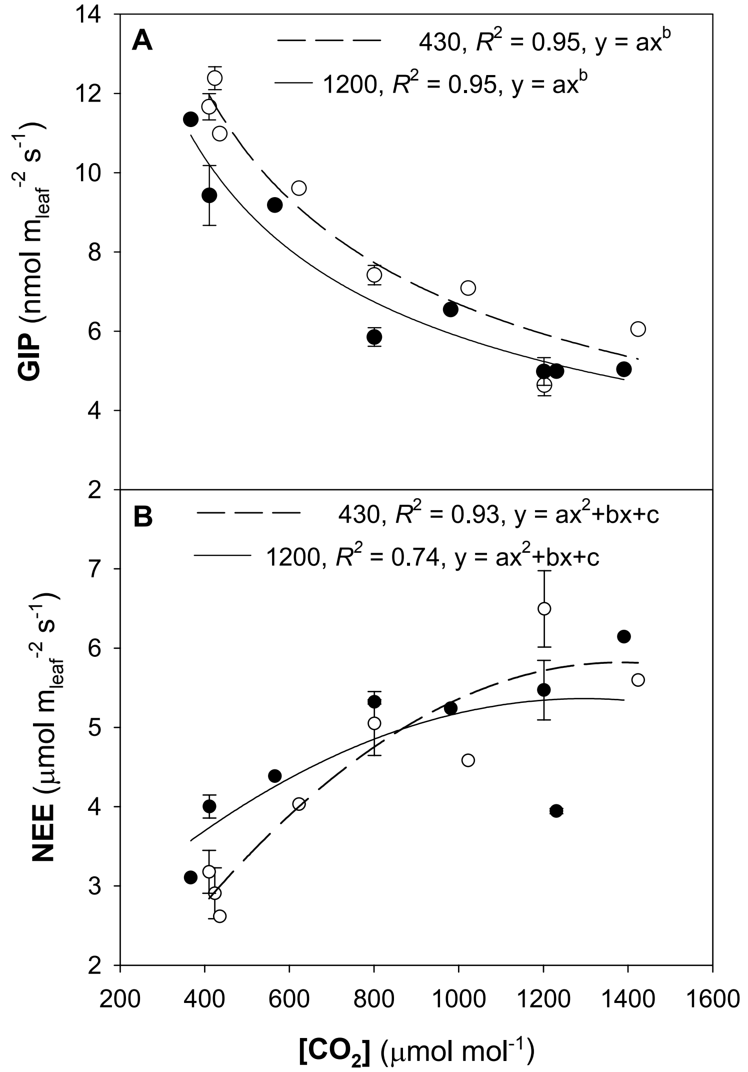


Figure 4.2. Relationship between central daytime (10:45 to 15:45) average of GIP (A) and NEE (B) expressed on a leaf area basis, and [CO_2], for the ambient (white dots) and the elevated (black dots) CO_2 treatments. Symbols represent means ± SE (n = 3-6).

With the exception of these days and of the first three and last ten days, air temperatures remained essentially constant around the set point, rising only slightly during the drought period.

Although the daily onset of isoprene emission from leaves was triggered by the increase in PAR in the early morning (at around 07.30 with the onset of a PAR of *ca.* 100 μmol m^{-2} s^{-1}, data not shown), GIP showed a stronger response to temperature than to light (Figure 4.3). Furthermore, the response of GIP to both light and temperature was significantly (*P* <.005) more pronounced in high than in low VPD conditions in both the ambient and elevated CO_2 treatments. However, the light response curve for the elevated CO_2 growth treatment showed a clear saturation at high instantaneous CO_2 treatment at PAR of only 750 µmol m^{-2} s^{-1} under high VPD conditions and 850 µmol m^{-2} s^{-1} under low VPD conditions; there was less evidence of GIP saturation with light in the trees from the ambient CO_2 growth treatment.

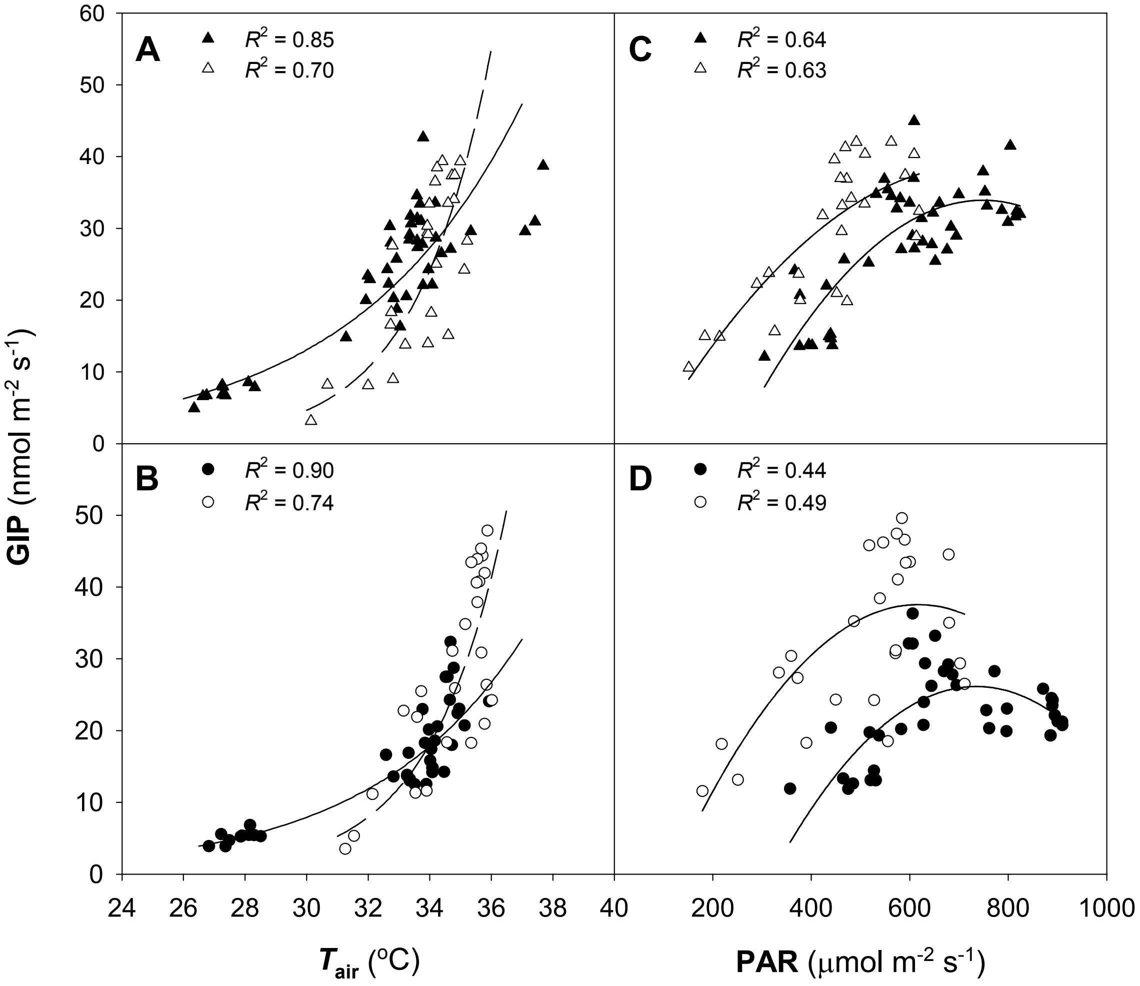


Figure 4.3. Relationship between central daytime (10:45 to 15:45) average of GIP and the two non-controlled environmental variables: T_{air} and PAR, for the ambient (A and C) and the elevated (B and D) CO_2 treatments. Data are grouped for the low (*ca.* 1 kPa) (black dots) and the high (*ca.* 3 kPa) (white dots) VPD phases.

During the high VPD (*ca.* 3 kPa) phases in well watered conditions, isoprene emission fluxes were always stimulated in both CO_2 treatments (Figure 4.4). Although during the first VPD cycle GIP was always lower in the elevated CO_2 treatment than in the ambient CO_2 treatment, the stimulating effect of high VPD on the isoprene emission in this treatment was stronger (*ca.* 47%) than for the ambient CO_2 treatment (*ca.* 29%). In the following cycles, the combination of high VPD with water stress accentuated the stimulation of GIP in the elevated CO_2 treatment, and the GIP tended to become higher than fluxes from the ambient CO_2 treatment. In the last cycle, when θ was significantly reduced and water stress was affecting NEE, the stronger stimulation on the GIP from the elevated CO_2 treatment was observed also at low VPD (Figure 4.4).

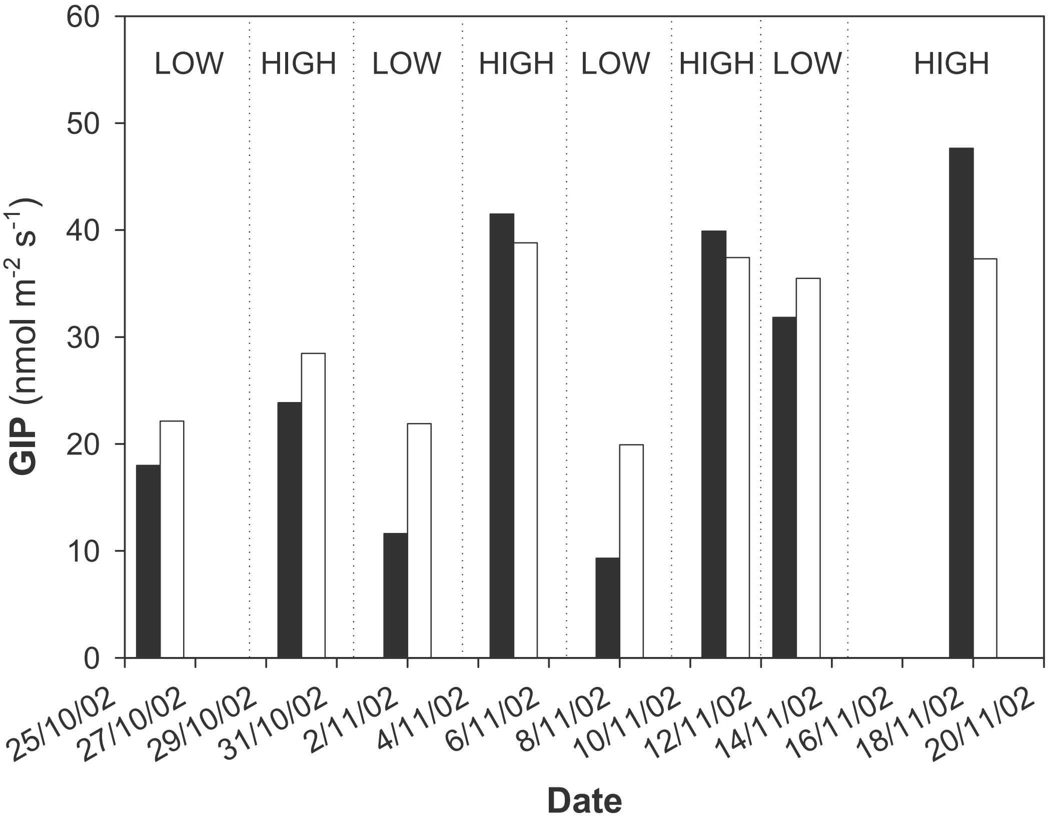


Figure 4.4. Central daytime (10:45 to 15:45) average of GIP in the ambient (white bars) and elevated (black bars) CO_2 treatments during the four cycles of low-high (*ca.* 1-3 kPa) VPD.

Although θ was always *ca.* 10% lower in the elevated CO_2 treatment than in the ambient CO_2 treatment (Figure 4.1D), it did not seem to have an effect on isoprene emission. Under low VPD conditions the increase in GIP due to water limitation was evident in both CO_2 treatments only at the time of the last low VPD phase, when the water stress was severe (Figure 4.4). In contrast, under high VPD conditions GIP started to increase from the first high VPD phase of the drought period on October

28, when θ was still high, 0.41 and 0.35 $m^3\ m^{-3}$ for the ambient and the elevated CO_2 treatment, respectively (Figure 4.5).

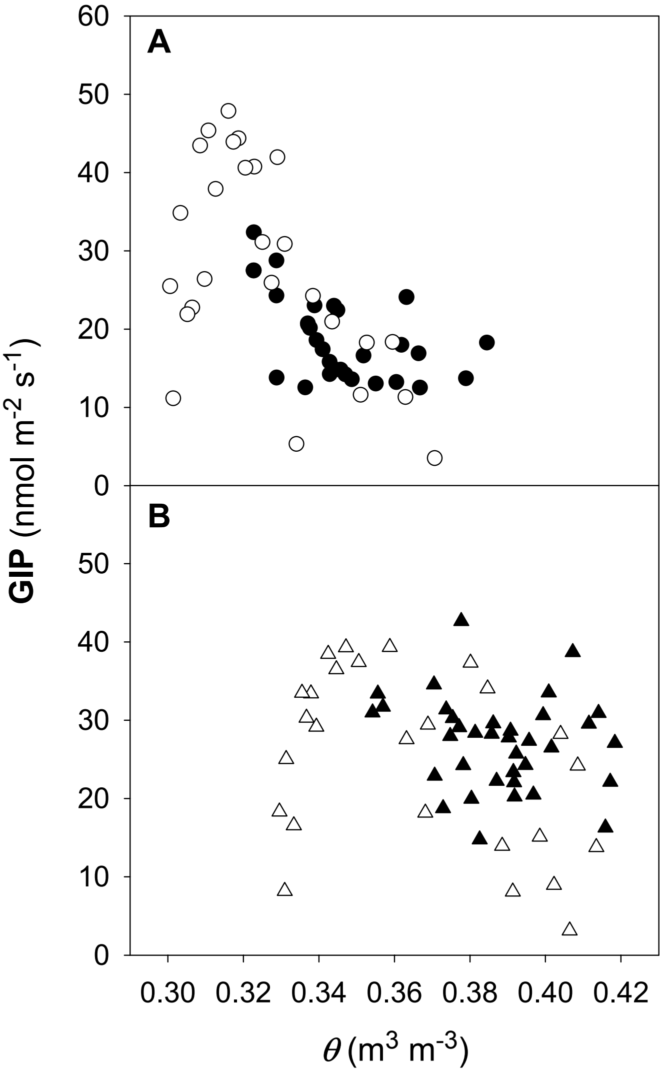


Figure 4.5. Relationship between central daytime (10:45 to 15:45) average of GIP and θ for the elevated (A) and ambient (B) CO_2 treatments. Data are shown for the low (*ca.* 1 kPa) (black dots) and the high (*ca.* 3 kPa) (white dots) VPD phases.

However, from November 20 ($\theta = 0.31\ m^3\ m^{-3}$) and November 22 ($\theta = 0.34\ m^3\ m^{-3}$) for the ambient and the elevated CO_2 treatment, respectively, GIP decreased dramatically until the end of the drought. Because of the contrasting effects that water stress and VPD had on GIP and NEE (Figure 4.1A and B), GIP increased with decreasing NEE (Figure 4.6), until the magnitude of the soil water stress was severe, and as a result the cost in carbon emitted as isoprene with respect to the assimilated carbon also increased from *ca.* 2.5% and *ca.* 0.6% in well-watered conditions to a maximum of *ca.* 60% and *ca.* 40% for the ambient and the elevated CO_2 treatments, respectively.

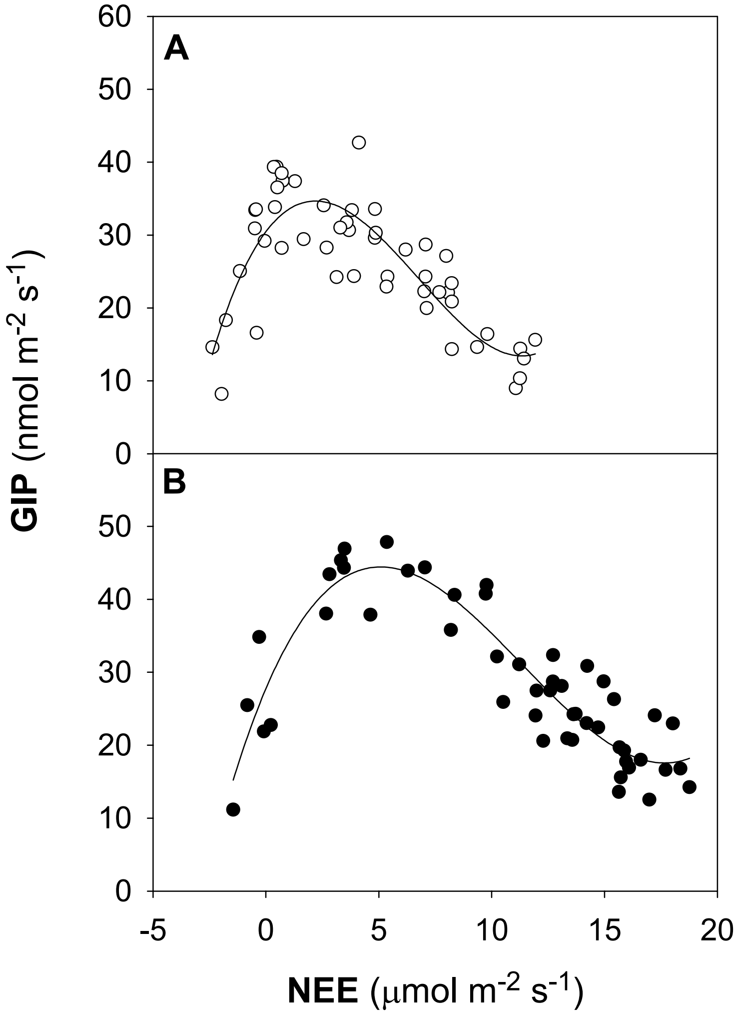


Figure 4.6. Relationship between central daytime (10:45 to 15:45) average of GIP and NEE for the ambient (A) and elevated (B) CO_2 treatments.

Although the overall effect of the drought and high VPD was of increasing GIP in both treatments (Figure 4.1A), as the drought progressed isoprene fluxes increased more in the elevated CO_2 treatment than in the ambient CO_2 treatment. As a result, the difference between the two treatments decreased gradually with the decrease in soil water availability and when the stress was severe, in the last seven days of the drought, the difference in isoprene fluxes between the two CO_2 treatments was only 3%.

4.3.1. Linear multiple regression model

Using multiple regression analysis, logarithmically transformed GIP data were regressed on the linear combination of PAR, T_{air}, θ and VPD. Parameter estimates, standard errors, *P* values and standardised parameters are presented in Table 4.1 and Table 4.2 for the ambient and the elevated CO_2 treatments, respectively.

Table 4.1. Parameter estimates, standard errors, *t* values, *P* values and standardised parameters estimates (β values), for the linear multiple regression model used on logarithm transformed GIP values in the 430 μmol mol^{-1} CO_2 treatment.

430 [CO_2]	Estimate	SE	*t* value	*P* (>|t|)	β
Intercept	0.375	$2.334*10^{-1}$	1.61	0.1132	0
PAR	$4.932*10^{-4}$	$8.875*10^{-5}$	5.56	<.0001	0.362
T_{air}	$6.583*10^{-2}$	$5.730*10^{-3}$	11.49	<.0001	0.699
θ	-3.985	$4.926*10^{-1}$	-8.09	<.0001	-0.392
VPD (dummy)	$-4.373*10^{-2}$	$3.140*10^{-2}$	-1.39	0.1686	-0.088

Table 4.2. Parameter estimates, standard errors, *t* values, *P* values and standardised parameters estimates (β values), for the linear multiple regression model used on logarithm transformed GIP in the 1200 μmol mol^{-1} CO_2 treatment.

1200 [CO_2]	Estimate	SE	*t* value	*P* (>|t|)	β
Intercept	-0.159	$3.331*10^{-1}$	-0.48	0.6321	0
PAR	$1.705*10^{-4}$	$9.982*10^{-5}$	1.71	0.0926	0.098
T_{air}	$8.370*10^{-2}$	$6.120*10^{-3}$	13.67	<.0001	0.757
θ	-4.494	$7.446*10^{-1}$	-6.03	<.0001	-0.303
VPD (dummy)	$1.871*10^{-1}$	$3.431*10^{-2}$	1.41	0.1633	0.082

The equation containing the four variables accounted for 89% ($P < 0.0001$, adjusted $R^2 = 0.88$) and 91% ($P <.0001$, adjusted $R^2 = 0.90$) of the variance in the GIP for the ambient and elevated CO_2 treatments, respectively. When comparing the observed values against the predicted values obtained by running the linear model using PAR, T_{air}, θ and VPD as descriptive variables, they showed a very good linear relationship with an R^2 of 0.79 and 0.86 for the ambient and the elevated CO_2 treatments, respectively (Figure 4.7).

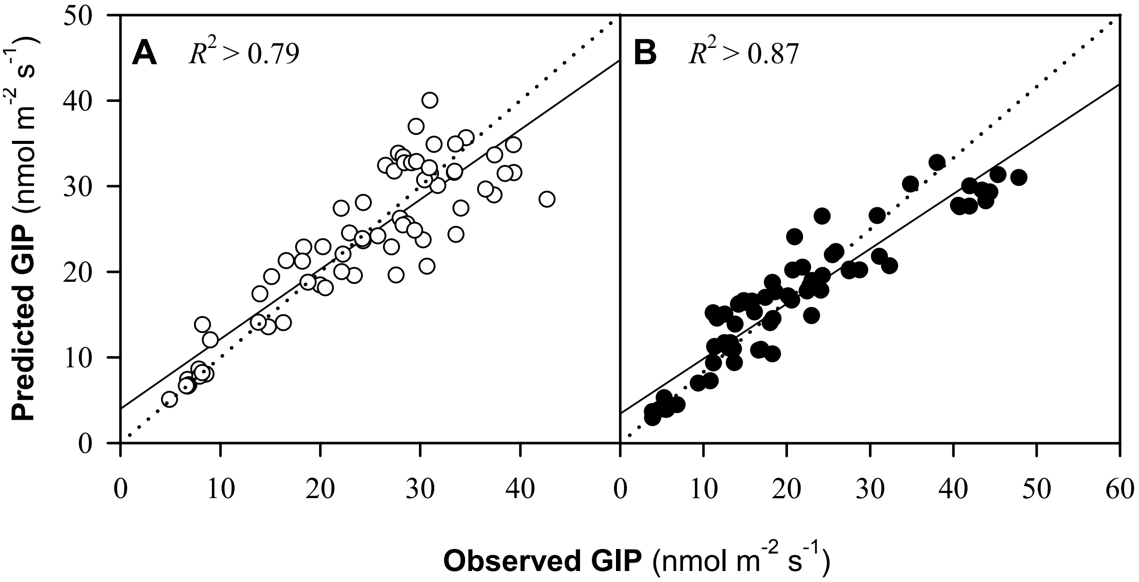


Figure 4.7. Linear multiple regression between observed and predicted values of GIP obtained from the linear regression model of LOG(GIP) as a function of PAR, T_{air}, θ and VPD, for the ambient (A) and the elevated (B) CO_2 treatments (n = 68). The dotted line represents the 1:1 line.

4.4. Discussion

The unique facility of the Biosphere 2 IFM gave us the unprecedented opportunity to observe the sensitivity of ecosystem level isoprene fluxes to changes in selected and controlled environmental variables such as: [CO_2], VPD and θ, and the effect of their interaction. As found in previous studies at both leaf (e.g. Monson and Fall 1989; Loreto and Sharkey 1990; Sharkey *et al.* 1991; Loreto *et al.* 2001; Scholefield *et al.* 2004) and canopy scale (Rosenstiel *et al.* 2003), exposure to elevated atmospheric [CO_2] reduced ecosystem isoprene production presumably by reducing substrate availability as a result of metabolic competition for phosphoenolpyruvate (Rosenstiel *et al.* 2003). In this experiment, elevated [CO_2] also inhibited isoprene emission. Furthermore, isoprene fluxes measured in the elevated CO_2 treatment were always higher than those measured in the ambient CO_2 treatment when compared at the same [CO_2], indicating that the inhibition caused by growth in elevated CO_2 conditions is a long-term adaptation of the plant metabolism. Our results indicate that in the short term, both water stress and high VPD counteracted the [CO_2] effect by stimulating isoprene emission. Furthermore the stimulating effect of water limitation was strongest in the elevated CO_2 treatment. As observed from leaf level data (Pegoraro *et al.* 2004b), the stimulation of isoprene emission was probably mainly the result of the decrease in intercellular [CO_2] (C_i) caused by stomatal closure, which led to a stronger decrease of the inhibitory effect in the elevated CO_2 treatment. Although the decrease in transpiration led to an increase in leaf temperature (data not shown), this was not large enough (*ca.* 4 ^oC in both CO_2 treatments) to explain the large increase in emission rates. The difference in the increase in isoprene emission during the drought from the two CO_2 treatments was also replicated during the VPD cycles, supporting the hypothesis that the stimulation of isoprene emission is linked mainly to a decrease in C_i. Furthermore, toward the middle of the experiment, the stimulation effect was larger with the combination of low θ and high VPD, which is consistent with the higher sensitivity of stomata to VPD in situations of water limitation.

Although GIP and NEE showed a similar response to light and temperature, their response to CO_2 and VPD was opposite in sign, with NEE being stimulated by elevated CO_2 and depressed by high VPD, while GIP was inhibited by elevated CO_2 and stimulated by high VPD. The difference in response between isoprene production and the photosynthetic process was also evident during the water stress experiment, with GIP being stimulated in the short term and declining only when the stress was severe, whereas NEE started to decrease from the beginning. This incomplete coupling between isoprene synthesis and photosynthesis derives from the existence of alternative slow turnover sources of carbon that the plant can use for isoprene production (Karl *et al.* 2002; Affek and Yakir 2003; Schnitzler *et al.* 2004). There is now ample evidence that part of the C contained in the isoprene molecule is derived from xylem transported C (Schnitzler *et al.* 2004) and leaf internal C pools such as starch (Karl *et al.* 2002; Affek and Yakir 2003). Previous studies have found that normally, under unstressed conditions, *ca.* 80% of the carbon in isoprene is derived from photosynthate and only *ca.* 20% is derived from alternative carbon sources (Sharkey *et al.* 1991; Delwiche and Sharkey 1993; Karl *et al.* 2002; Affek and Yakir 2003), whereas under stressed conditions the contribution of alternative carbon to isoprene production can increase to up to 30% and even more (Funk *et al.* 2004). Therefore, even though during water stress NEE was reduced, isoprene maintained high fluxes and continued to respond quickly to changes in [CO_2]. The opposite response of GIP and NEE to water limitation also led to a drastic increase in the fraction of fixed carbon lost as isoprene emission as water stress progressed. Although the relative contribution of the slow-turnover carbon sources may have increased as the drought progressed, providing the necessary carbon supply for the tree to maintain high isoprene emission rates, the prolonged depression of photosynthetic carbon flow may have ultimately drained the alternative carbon reservoirs. It is likely that the drop in GIP at the end of the drought period was a consequence of the ultimate depletion of the available carbon pool for isoprene production.

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

Drought effects on isoprene production and consumption in the Biosphere 2 tropical rainforest mesocosm

Pegoraro, E., Rey, A., Abrell, L., Vanharen, J., Guanghui, L.

(submitted to *Global Change Biology*)

CHAPTER 5

Drought effects on isoprene production and consumption in the Biosphere 2 tropical rainforest mesocosm

5.1. Introduction

Tropical rain forests are known to play a major role in the global carbon cycle (Malhi and Grace 2000; Monson 2002; Loescher *et al.* 2003). Much recent research has focussed on the influence of tropical forests on atmospheric [CO_2], but forests emit a number of other carbon compounds, particularly non-methane hydrocarbons (NMHCs), of which isoprene (C_5H_8) is the most important (estimated by Guenther *et al.* [1995] to comprise about 44% of global emissions). Recent estimates suggest that tropical regions account for the bulk (>80%) of global isoprene emissions (Jacob and Wofsy 1988; Zimmerman *et al.* 1988; Guenther *et al.* 1995). The total amount of carbon per unit area released through tropical isoprene emissions is believed to be in the range 0.1-0.3 t C ha^{-1} yr^{-1}(Harley *et al.* 2004). Physiological models (Lloyd 1999), global carbon budget considerations (Malhi and Grace 2000) and biomass studies (Phillips *et al.* 1998) all suggest that the tropical forest carbon sink is in the order of 1 t C ha^{-1} yr^{-1}. Hence, in order to obtain a more realistic relationship between measurements of net CO_2 uptake and the net carbon sink, it is important to understand and quantify the amount of carbon "lost" from the forest through isoprene and the role played by the climatic driving forces of this carbon loss.

Isoprene has a major role in regulating the oxidation potential of the troposphere because of its high reactivity with the hydroxyl radical (OH), the principal tropospheric oxidising agent (Wofsy 1976; Crutzen and Fishman 1977; Greenberg *et al.* 1985; Fehsenfeld *et al.* 1992). This has several consequences for the habitability of the biosphere, including production of atmospheric pollutants such as O_3 and PANs, and formation of organic acids. It also has a potential role in climate warming, reducing the effectiveness of methane removal from the troposphere (Zimmerman *et al.* 1988).

The exact physiological role of isoprene production in plants is still unknown, but several hypotheses have been proposed. The one that has received most attention is that isoprene serves as a thermal protectant, protecting the leaves against high temperature episodes (Sharkey and Singsaas 1995; Singsaas *et al.* 1997; Singsaas and Sharkey 1998; Singsaas 2000). This temperature-dependency may be linked to the effects of water stress on isoprene emission by plants. It has been observed that in the long-term, withholding water can reduce isoprene emission to 40% of the emission of normally watered plants (Lerdau *et al.* 1997). In the short-term, whereas photosynthesis is clearly suppressed, isoprene emission can be stimulated by water stress (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996). However, rather little work has been done on the effect of water stress on isoprene emission and the link between isoprene emission, water stress and leaf temperature is therefore unclear and requires further investigation.

Since isoprene emission is highly temperature sensitive, there is an obvious concern that future increases in global temperature and drought, as predicted in climate models (Cox *et al.* 2000), could result in enhanced isoprene fluxes. This, in turn, could result in a reduced potential for removing tropospheric methane, resulting in even further global warming. Ultimately these effects are further enhanced indirectly by other consequences of a global rise in temperature: a regional shift in precipitation, biomass/plant species redistribution and an increase in the length of the growing season (Turner *et al.* 1991; Lerdau *et al.* 1997; White *et al.* 1999).

Because tropical forest ecosystems are physiologically active year-round, and experience wet-dry season regimes, they are probably the largest single sources of isoprene. Nevertheless because of high species diversity and difficulty in access, there is still a substantial lack of information on the isoprene source strength from tropical systems and we still depend largely on model extrapolation based on leaf level measurements (Guenther *et al.* 1995). In Chapter 4 we have described ecosystem level measurements in the IFM of Biosphere 2 Laboratory (B2L). Here we report similar ecosystem level measurements from the tropical rainforest mesocosm. Taking advantage of a unique opportunity at the B2L controlled environment facilities, we established an experiment to quantify the effect of environmental variables on isoprene emission and its contribution to the carbon balance at the ecosystem level.

The specific objectives were: 1) to screen a range of tree species for their capacity to emit isoprene; 2) to understand the relationship between the isoprene flux and environmental variables such as light and temperature; 3) to investigate the effect of water stress on ecosystem scale isoprene flux; 4) to study the changes in the carbon balance of the model system during drought; 5) to explore the potential magnitude of isoprene uptake by soil and its sensitivity to water stress.

We will test the hypotheses that acclimation to water stress involves an increase in the potential to produce isoprene and that water stress strongly affect isoprene uptake by soil.

5.2. Material and Methods

5.2.1. Plant Material

The rainforest mesocosm of Biosphere 2 Centre was constructed in 1991 (Dempster 1999), and although it was never designed to represent any particular natural tropical forest ecosystem it is now structurally and functionally representative of disturbed humid tropical rainforests in South America, but with floristically diverse pan-tropical vegetation (Leigh *et al.* 1999; Lin *et al.* 1999; Prance, pers. comm.). The top canopy in this mesocosm has reached about 15 m high and covered major open space after about a decade of development, reaching in 2001 a leaf area index (LAI) of 4-5 (Leigh *et al.* 1999). Secondary canopy and understorey plants have also established under or between large trees.

The dominant canopy species include *Clitoria racemosa* Sesse & Moc., *Ceiba pentandra* (L.) Gaertn., *Cecropia schreberiana* Miq., *Arenga pinnata* (Wurmb) Merr., *Phytolacca dioica* L., *Pterocarpus indicus* Willd., *Hura crepitants* L., *Inga feuillei* DC. and *Hibiscus elatus* SW.. The most common understorey plants are *Costus spp.*, *Eppiperapium spp.*, *Dieffenbochia sp.*, *Ficus pumila* L., *Hedychium* spp., *Piper* spp., *Coffea spp.*. To reduce light penetration into the forest floor, many edge plants were grown along the four sides of the mesocosm, including *Alpinia spp*, *Musa spp.*, *Bambusa spp.*, *Zinger spectabile* Griff., etc.

The soil profile in the rainforest mesocosm of Biosphere 2 was assembled with a subsoil layer (up to 5 m deep) and a topsoil layer of variable depth (0.3 to 3.2 m in depth) (Leigh *et al.* 1999). Although soil bulk density, soil organic matter (SOM) content and major nutrient concentrations in the B2L's rainforest mesocosm were very similar to those of several Puerto Rico rainforests (Silver and Fall 1991), soils of Biosphere 2 rainforest were more alkaline (pH around 7.5 compared to pH of 5.1 found in Puerto Rico rainforests by Silver and Fall [1991]) and contain slightly higher P, K and other nutritional elements (Leigh *et al.* 1999; Lin *et al.* 1999). The soil fauna is, however, very limited (ants, cockroaches). Soil excavations revealed an abundance of earthworms in the agriforest mesocosm, but no census of soil macrofauna was undertaken (Karl Bil, pers. comm.).

5.2.2. Growth conditions

The rainforest mesocosm is encased in a glass and metal shell and was operated as a semi-closed system with control of temperature, atmospheric gas composition and precipitation. Arrays of sensors allowed the continuous monitoring of the atmospheric composition, climatic conditions (light, temperature, leaf temperature, soil moisture and humidity) and energy and trace gas fluxes throughout the canopy. The [CO_2] in the rainforest, the savannah and outside atmosphere were measured continuously using a LI-6262 gas analyser (Licor Inc., Lincoln, NE, USA), which was calibrated periodically with five standard CO_2 cylinders. The CO_2 injection rate was monitored using Sierra 840M-3-OV1-SV1-E-V4-S4-MP mass flow controllers (Sierra Instruments, Inc., Monterey, CA, USA) and the fan speeds were measured using Panametrics GM868 Ultrasonic gas flowmeters (GE Panametrics, Waltham, MA, USA) (for more details see: Lin *et al.* 1999).

The environmental conditions in the glasshouse were artificially controlled except for light. Photosynthetically active radiation (PAR) reaching the top of the canopy inside the mesocosm was *ca.* 75% of the outside PAR. The daily mean air temperature for this tropical mesocosm was set at 27°C with air temperature range of 35°C/20°C day/night, and relative humidity (RH) of *ca.* 85%. Temperature stratification in the upper canopy, identified by Arain *et al.* (2000) as the principal artefact of enclosure, was minimised by high speed fans mounted in the structure to ensure adequate mixing of the mesocosm atmosphere. The mesocosm was regularly watered with an average daily precipitation of 3.6 mm.

5.2.3. Experimental setup

Before the experiment started, the rainforest mesocosm was heavily wetted for 2 weeks (7.7 mm d^{-1}), to reach field capacity. From September 23 (day 1) to October 28 2002 (day 36) no water was added and the soil was left to dry naturally. At the beginning of the recovery, we rewatered for two weeks using the pre-drought regime. These drought treatments were calibrated to result in mild stress, still permitting rapid and reversible recovery the rainforest mesocosm. On day 32 an isolated soil compartment (approx. 20% of the whole soil surface) was watered with 30000 l (36 mm) to test the effect of a singular, isolated watering event.

In order to monitor water stress, soil volumetric water content (θ) was continuously monitored over the duration of the entire experiment by Time Domain Reflectometry (TDR) probes (CS165, Campbell Scientific Instruments, Logan, UT, USA) inserted at five different locations (N, NW, S, SE and center) in the soil at two depths: 0-30cm and 30-60 cm. As a parameter for plant sensitivity to water stress, pre-dawn and midday leaf water potential (Ψ_{leaf}) measurements were performed on four of the large tree species: *Ceiba pentandra*, *Clitoria racemosa*, *Hibiscus elatus* and *Hura crepitants*, using a PMS 1003 digital pressure-bomb (PMS Instruments, Corvallis, OR, USA). For each tree species, four leaves of the outer canopy were sampled at the time of each Ψ_{leaf} measurement. Leaf temperature of sun leaves was also monitored with infrared sensors (Apogee Instruments, Logan, UT, USA) pointed at sun leaves at the top of the canopy of the three main isoprene emitter species. The instrument has a field of view of 6.45 cm^2 m^{-1} and was mounted at *ca.* 2 m from the canopy thus monitoring a leaf area of *ca.* 12 cm^2. All data were collected as 15 minutes averages with a CR10 datalogger (Campbell Scientific, USA).

Because the glass-enclosed mesocosm is free of UV light, inside the mesocosm atmosphere O_3 production and OH radical generation is minimised (Cockell *et al.* 2000). Therefore, the lack of atmospheric oxidative destruction of isoprene (more details are given in Chapter 6) permitted the measurement of isoprene consumption at night by the soil.

5.2.4. Leaf gas exchange measurements

Photosynthetic rate, stomatal conductance and intercellular [CO_2] were measured using a LI 6400 open path gas exchange measurement system (Li-Cor, Lincoln, NE, USA). All measurements were made under the same standard conditions: leaf temperature of 32°C, PAR of 1200 µmol m^{-2} s^{-1} and air flow of 400 µmol s^{-1}. After a leaf was placed in the cuvette, a minimum of 10 min was allowed for equilibration, and all measurements were made after steady-state conditions had been realised, as indicated by continuous monitoring of CO_2 and H_2O fluxes. To avoid interference of 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.

In 2002, changes in isoprene concentration inside the LI 6400 cuvette were monitored by collecting an air sample by attaching a Teflon® bag (of 2.5 dm^3 volume) to the cuvette exhaust. The bag was then brought to the laboratory and isoprene concentration determined by gas chromatography (for more details see Chapters 2 and 3). In 2003, changes in isoprene concentrations were measured on-line via a 5 m length of Teflon® PFA tubing (1.6 mm inside diameter), inserted through a "T" connection to the leaf cuvette exhaust and connected to a proton-transfer-reaction mass spectrometer (PTR-MS, Ionicon GmbH, Innsbruck, Austria). Operational details of the PTR-MS are described elsewhere (Hansel *et al.* 1995; Lindinger *et al.* 1998; Hayward *et al.* 2002). The air sample was pulled by the PTR-MS at a constant flow rate of *ca.* 20 ml min^{-1} and isoprene concentration was determined using a dwell time of 2 s, with a high temporal resolution between successive measurements of the same mass (about 7 s). The instrument was calibrated before and after the experiment (for more details see Chapter 6).

5.2.5. Ecosystem level gas exchange measurements

5.2.5.1. *Isoprene fluxes*

One-minute averages of ecosystem isoprene atmospheric concentration from the mesocosm were measured and recorded with a Fast Isoprene Sensor (FIS-02-AUTO, Hills Scientific, Boulder, CO, USA) mounted in an adjacent laboratory. The mesocosm was sampled continuously for 15 min each hour. The FIS was calibrated before, during and after the experiments. A more detailed description of the measurement system and flux calculation is given in Chapter 4. All flux calculations were performed exclusively for a closed system, excluding the periods when the pull/push fans where exchanging air with the outside. The *net isoprene exchange* (NIE) (isoprene emission minus isoprene consumption), was calculated every 15 min as:

$$NIE = \frac{\Delta C}{\Delta t} = \frac{(C_{t+1} + (C_{t+1} * L) - (C_{t-1} + (C_{t-1} * L))}{2 \times \Delta t}$$

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, *L* is the leak rate and Δt is the time period (15 min in this case). Soil isoprene uptake was measured both at the whole mesocosm level and in the small soil chambers (Pegoraro *et al.*, in review) and was used to calculate the *soil isoprene uptake flux* (IF_{soil}) (nmol m^{-2} s^{-1}) ($IF_{\mathrm{soil}} = -k*C$), where *k* is the isoprene deposition velocity. *Gross isoprene production* (GIP) was then calculated as:

$$\mathrm{GIP} = \mathrm{NIE} - IF_{\mathrm{soil}}$$

5.2.5.2. *Ecosystem CO_2 uptake*

Net ecosystem exchange (NEE) was calculated as moles of CO_2 exchange per m^2 of ground area at 15 min intervals continuously for the entire experimental periods according to,

$$\mathrm{NEE} = F_{in} + F_{tank} - F_{out} - F_{leak} - F_{atm}$$

where F_{atm} is the flux due to change in CO_2 storage in the mesocosm, F_{leak} is the flux of CO_2 leakage across the isolation curtain, F_{tank}, is CO_2 injected from the CO_2 tank, F_{in} and F_{out} are the rates of CO_2 exchange by the pull/push fans (for more details see: Lin *et al.* 1999). Following the tradition of ecophysiological research, we denote positive NEE values for the net CO_2 uptake by the whole ecosystem. Leakage through the curtain separating the rainforest from the adjacent mesocosm (the savannah) was estimated to be about 1.6% of total air volume per hour based on sulphur hexafluoride (SF_6) tracer experiments (see Chapter 6).

Day time respiration was estimated using night time NEE values and gross primary productivity (GPP) was then calculated by subtracting daytime respiration to NEE (Lin *et al.* 1999).

5.2.6. Soil gas exchange

The absence of atmospheric isoprene oxidation and the minimisation of isoprene leak to the outside by regulation of the mesocosm exhaust system resulted in elevated isoprene concentrations inside the rainforest mesocosms (between 200 and 1200 nmol mol^{-1} (ppb)). These high ambient isoprene concentrations in turn resulted in high isoprene consumption fluxes by the soil, which allowed a detailed exploration of this process. Isoprene consumption in the dark at whole ecosystem level and in small soil chambers always followed an exponential decay function. It was possible to calculate the deposition velocity as: $k = \ln (C_2/C_1)/(t_2-t_1)$. k has been called "soil activity factor" to include all physical and biological capabilities of soil to take up isoprene (see Chapter 6).

For *in situ* soil isoprene uptake measurements, five soil collars were set up in five different locations (mainly along a North to South transect), two weeks before the start of the experiment to give the soil the time to recover from disturbance. Soil isoprene exchange measurements were made with small (30 x 30 x 40 cm) aluminium static soil chambers (a more detailed description of the system is given in Chapter 6). Isoprene concentration inside the chamber was determined in real time by a PTR-MS connected to the outlet of the chamber via 10 m long Teflon® PFA tubing (1.6 mm inside diameter). All measurements were made at isoprene concentrations attained in the mesocosm as a whole at the time of measurements.

5.3. Results

The relatively easy canopy access inside the tropical rainforest mesocosm of B2L allowed screening for the production and emission of isoprene under standard conditions (i.e. leaf temperature of 32°C and PAR of 1200 µmol m^{-2} s^{-1}) by the large canopy trees and the most common understorey and edge plants. None of the screened understorey and edge plants were found to be potential isoprene emitters. In Table 5.1 are reported the potential emitters of the canopy layer together with their average isoprene emission rate in non-stressed conditions.

Table 5.1. Average leaf isoprene emission rate ± SE (measured at leaf temperature of 32°C and PAR of 1200 µmol m^{-2} s^{-1}) in non-stressed conditions for five canopy dominant species in the tropical rainforest mesocosm (*n* = number of leaves tested). All measurements were performed between 12:00 and 14:00.

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)

5.3.1. Growth conditions

Before and after the drought experiment of 2002, in non-stressed conditions, soil water content (θ) in the top 30 cm of soil was maintained at *ca.* 0.27 m^3 m^{-3} (Figure 5.1A).

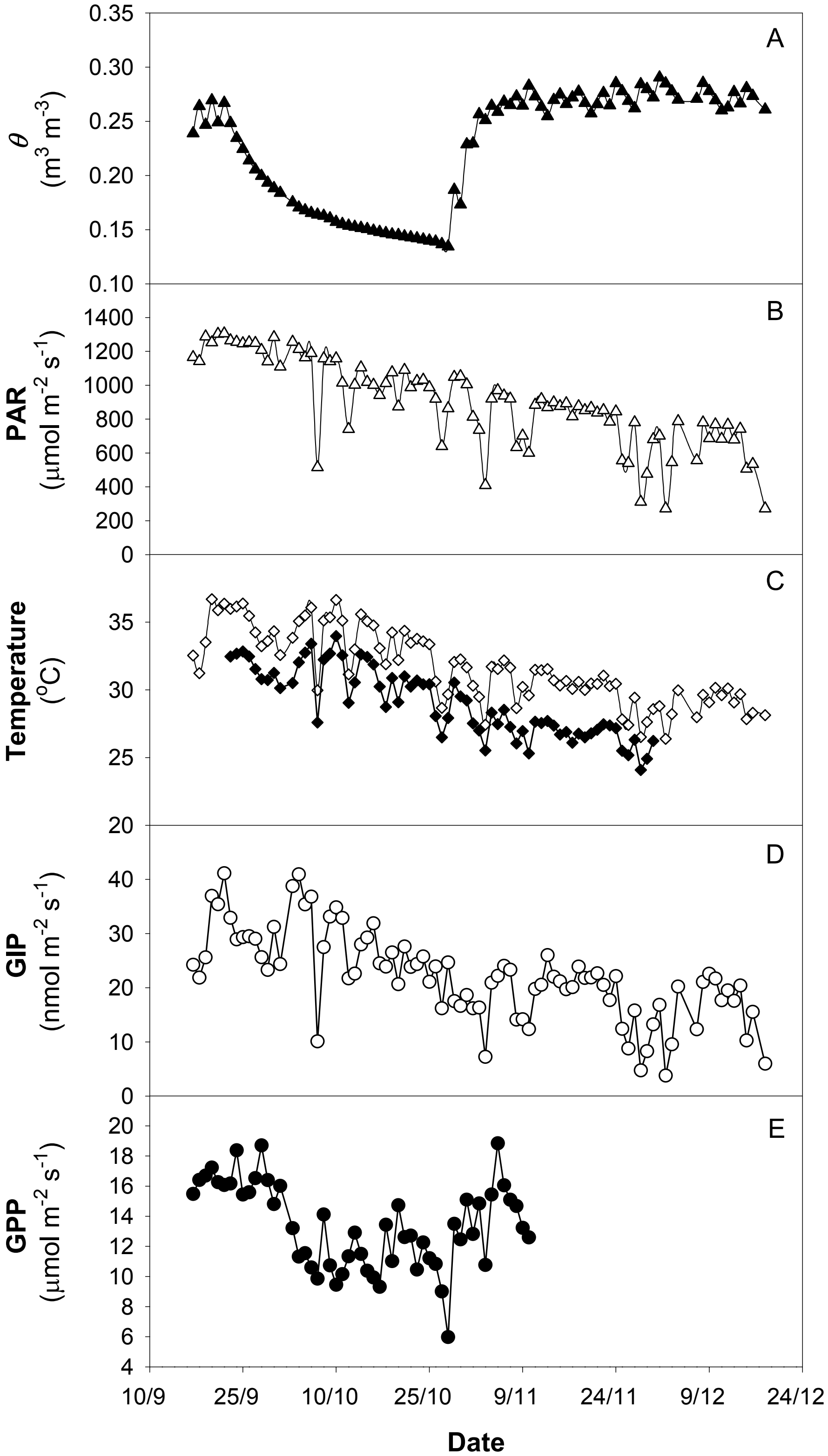


Figure 5.1. Time course of outside PAR (A), θ (B), air temperature (C, white diamonds), leaf temperature (C, black diamonds), GIP (D) and GPP (E), for the synthetic model tropical rainforest mesocosm. Data are daytime (from 8:00 to 17:00) averages.

After the last rain, θ decreased, rapidly reaching 0.13 m^3 m^{-3} on the last day of the drought. However, the decrease in θ was particularly strong in the top 30 cm of soil; below 60 cm soil depth the decrease in θ was much less pronounced (for more details see Rascher *et al.* 2004).

Over the three months period comprising the drought experiment and the recovery period, average daytime outside PAR decreased steadily from 1300 µmol m^{-2} s^{-1} at the end of September to *ca.* 750 µmol m^{-2} s^{-1} (40% less) in mid-December (Figure 5.1B). Both air temperature inside the mesocosm and leaf temperature of one of the major emitting species closely followed the variations in the outside light, which also showed a decreasing trend (Figure 5.1C). Leaf temperature was always a few degrees Celsius lower than air temperature, although this difference was reduced to a minimum in the last days of the drought when it was *ca.* 1^oC.

GIP also decreased steadily over the experimental period (Figure 5.1D) tracking changes in light and temperature. However, it did not show any response to water stress. As expected, GPP also decreased following the decrease in light and temperature (Figure 5.1E). Furthermore, it decreased more rapidly during the water stress period and showed a rapid recovery at the end of the drought period on the day after the first rain. A detailed analysis of drought effects on CO_2 exchange can be found in Rascher *et al.* (2004).

5.3.2. Drought effect on gas exchange

The strong decrease in θ observed in the top 30 cm of soil did not affect isoprene emission from the large tree canopy species (Figure 5.2A). However, the decrease in soil moisture had a strong effect on GPP, which started to decline noticeably when θ was lower than *ca.* 0.22 m^3 m^{-3} (Figure 5.2B).

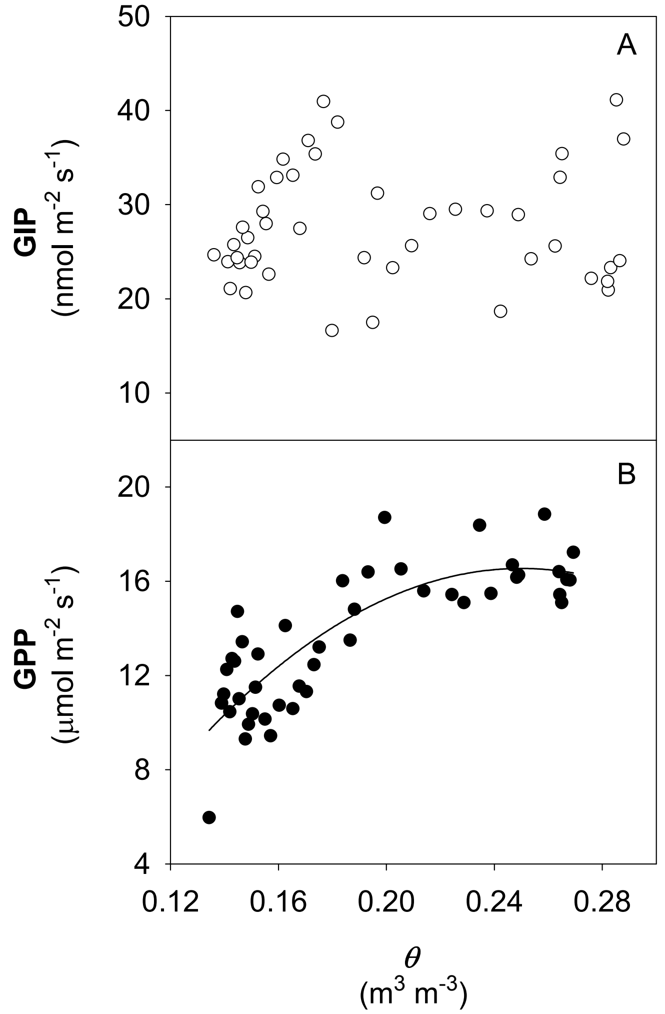


Figure 5.2. Relationship between daytime average GIP (A) and GPP (B), and θ measured in the top 30 cm of soil. Days with low light levels (cloudy days) were not considered. The regression line in (B) is only shown to indicate the trend.

GIP variations were essentially explained by variations in light and temperature (Figure 5.3A and B). However, most of the GIP variation was mainly a result of changes in light. Although temperature inside the biome is largely controlled, the two variables showed a strong correlation with each other as strong variations in the outside solar radiation always resulted in a variation in temperature. GPP also showed a strong relationship with light but a less strong one with temperature (Figure 5.4A and B). GPP increased very quickly with increasing light until light levels of *ca.* 600 µmol m^{-2} s^{-1}, when it continued to increase but at a much lower rate, then remaining practically constant between light levels of 1400 and 1800 µmol m^{-2} s^{-1}.

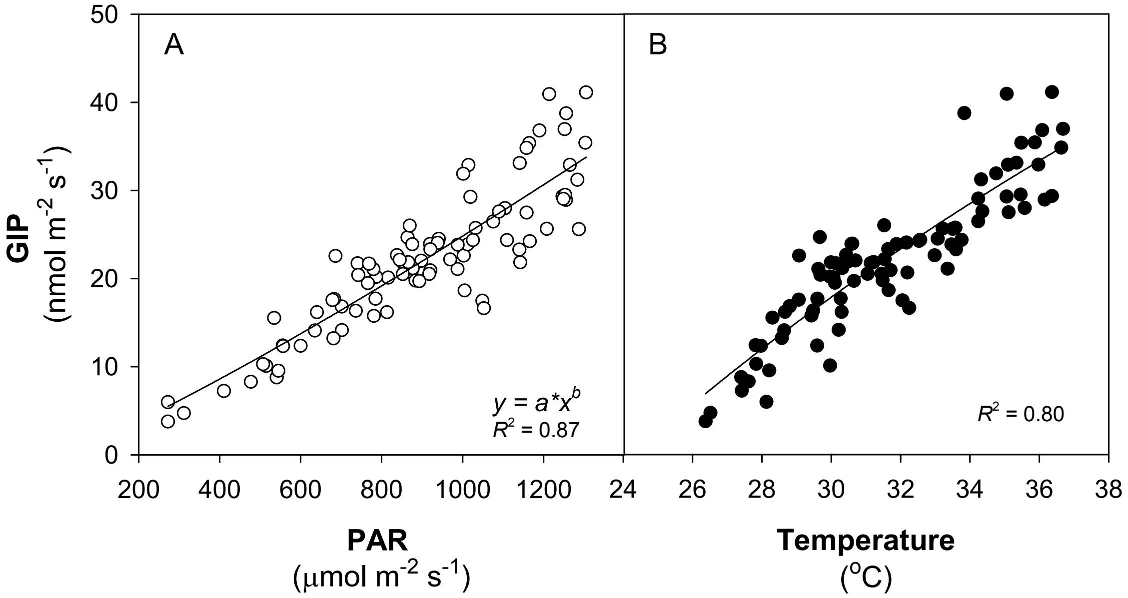


Figure 5.3. Relationship between day average outside PAR (A) and air temperature (B), and day average GIP. Data are daytime (from 8:00 to 17:00) averages.

Although GPP clearly showed a saturation level also in the relationship with temperature, reaching its optimum at *ca.* 35^{o}C, it then started to decrease quickly with increasing temperature.

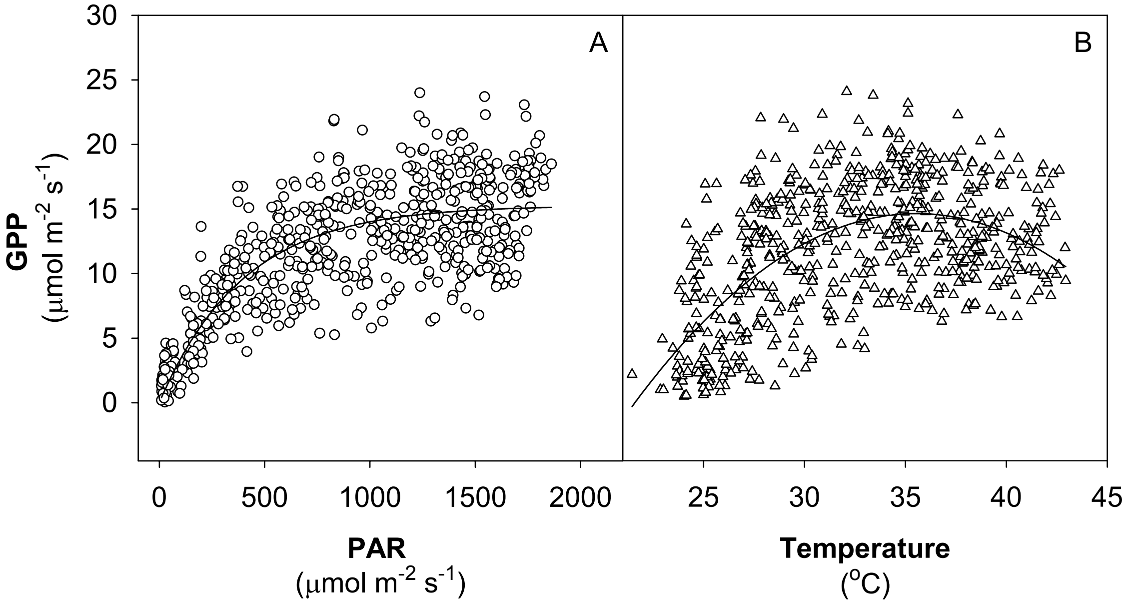


Figure 5.4. Relationship between PAR (A) and temperature (B), and GPP inside the tropical rainforest mesocosm during the drought experiment in 2002. Data points are calculated 15 minute fluxes.

The isoprene carbon loss as a fraction of total photosynthesis varied remarkably over the drought period. Before the drought started GIP represented the 0.94% of the carbon assimilation. The carbon loss increased rapidly at the beginning of the drought and on day 12 it was 100% higher. It then tended to decrease slowly until the last day of the drought, when GPP dropped to its minimum and consequently the carbon loss reached its peak (2%). Because of the lower light intensity and as a consequence of the difference in the response to light between GPP and GIP (the first clearly showing saturation at medium-high light intensities and the second showing no saturation even at high light intensities), over the recovery period the carbon loss stabilised at a lower level (0.66%) than before the pre-drought period.

When compared to the results obtained in the cottonwood plantation grown at ambient CO_2, GIP from the rainforest was noticeably higher. However, the relationship with both light and air temperature was very similar although the GIP measured in the tropical rainforest mesocosm seemed to have higher light and temperature saturation levels (Figure 5.5).

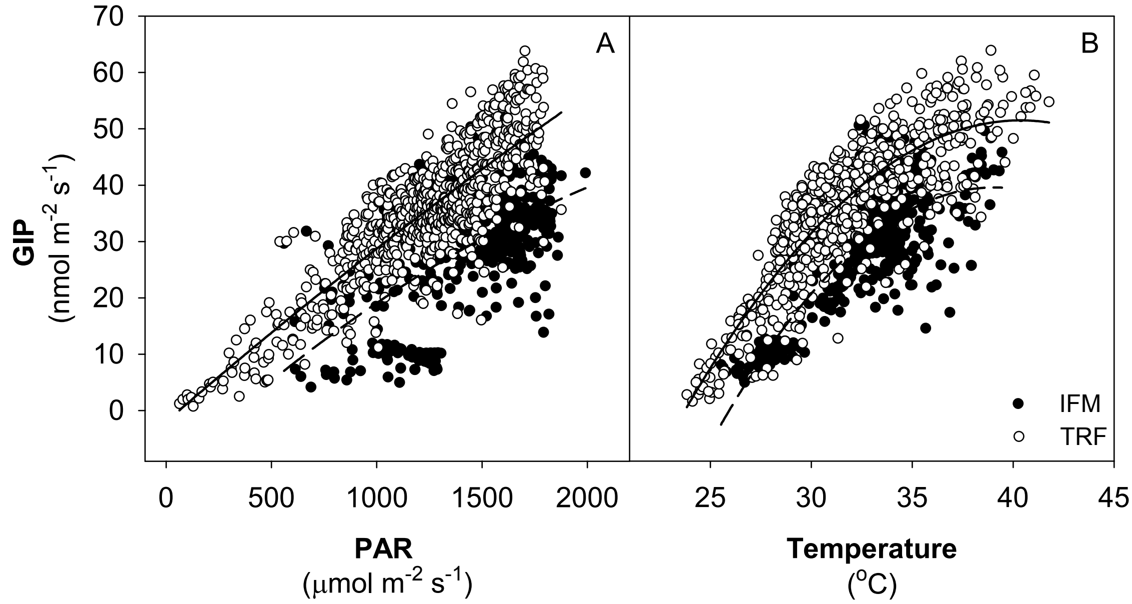


Figure 5.5. Relationship between PAR (A) and temperature (B), and GIP for the intensive forestry mesocosm (IFM) (black circles) and for the tropical rainforest mesocosm (TRF) (white circles). Data points are calculated 15 minute fluxes.

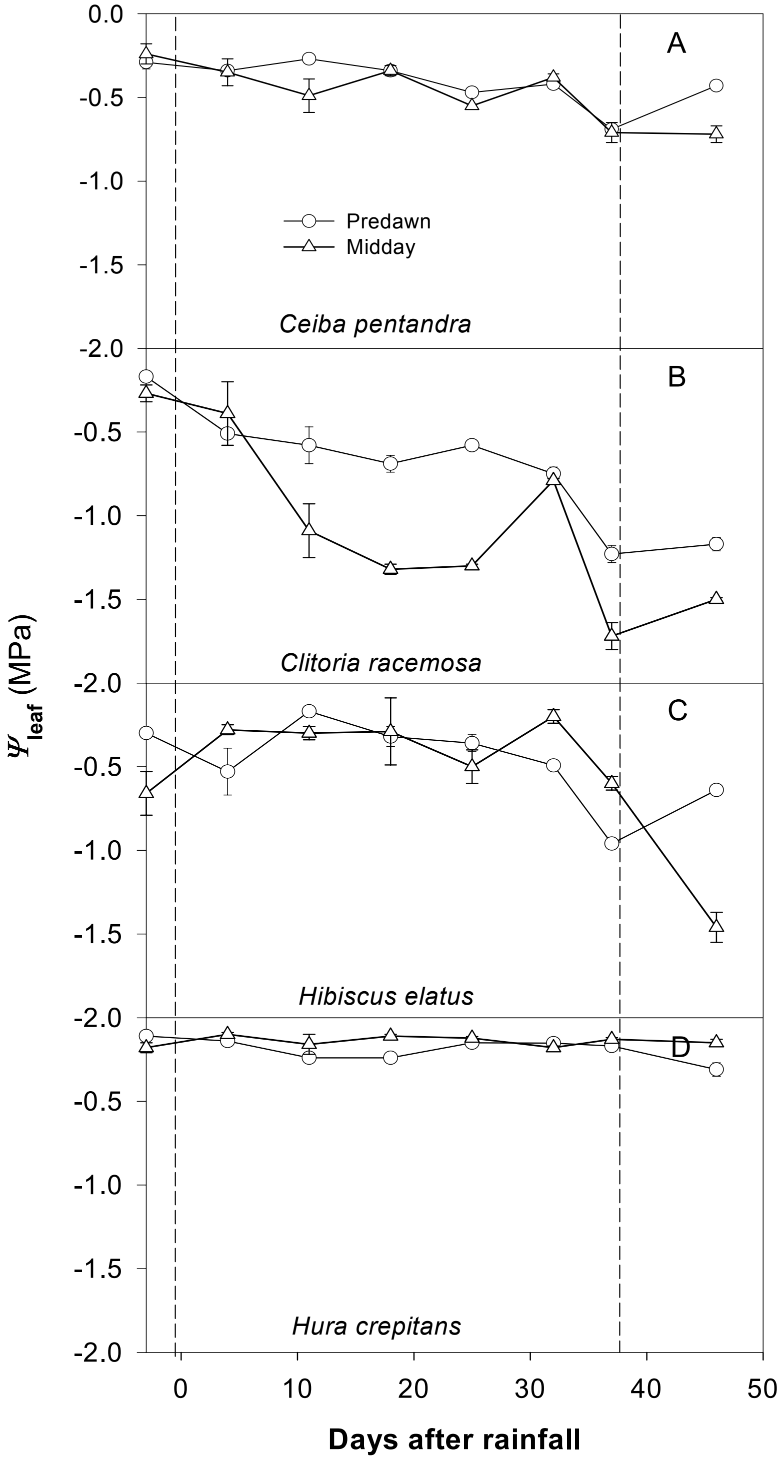


Figure 5.6. Pre-dawn (circle) and midday (triangle) Ψleaf for the four main tree species of the synthetic model tropical rainforest mesocosm: *Ceiba pentandra* (A), *Clitoria racemosa* (B), *Hibiscus elatus* (C) and *Hura crepitants* (C), during the drought experiment. Dashed lines indicate the day of the last “rainfall” before the drought and the first “rainfall” after the drought. Data are averages ± SE (n=4).

Responses of pre-dawn and midday leaf water potential (Ψ_{leaf}) to drought and recovery after drought were variable among the four investigated tree species, although in general they tended not to decrease significantly until the very end of the drought (Figure 5.6).

Ψ_{leaf} did not vary between pre-dawn and midday for either *Ceiba pentandra* or *Hura crepitans* and neither species exhibited a noticeable response to drought. For *Ceiba pentandra*, during drought Ψ_{leaf} decreased of *ca.* -0.44 MPa, but while pre-dawn Ψ_{leaf} recovered from -0.69 MPa to -0.43 MPa after drought, midday Ψ_{leaf} did not recover (Figure 5.6A). Of the four species, *Clitoria racemosa* displayed the largest decrease in Ψ_{leaf} during the drought with pre-dawn Ψ_{leaf} decreasing below -1.2 MPa and midday Ψ_{leaf} dropping below -1.5 MPa, with no appreciable recovery in either pre-dawn or midday Ψ_{leaf} after rainfall commenced (Figure 5.6B). Leaves of *Clitoria racemosa* strongly responded to the single, isolated watering experiment at day 32, with midday Ψ_{leaf} equalling pre-dawn values. Pre-dawn and midday Ψ_{leaf} of *Hibiscus elatus* were very variable and did not show any significant decreasing trend throughout the drought (Figure 5.6C). Both dropped drastically only in the last day of the drought, and whereas pre-dawn showed signs of recovery midday Ψ_{leaf} continued to decrease after rainfall commenced. Both, pre-dawn and midday Ψ_{leaf} of *Hura crepitans* were constant at -0.2 MPa throughout, showing no effect to the drought (Figure 5.6D).

Although drought did not affect isoprene production, it had a large effect on the soil activity of the top soil layer. When soil was wet ($\theta > 0.24$ m^3 m^{-3}) isoprene soil uptake fluxes rapidly increased with increasing atmospheric isoprene concentration, whereas when measured in dryer conditions ($\theta < 0.21$ m^3 m^{-3}) soil fluxes showed a slower response to increases in concentration (Figure 5.7).

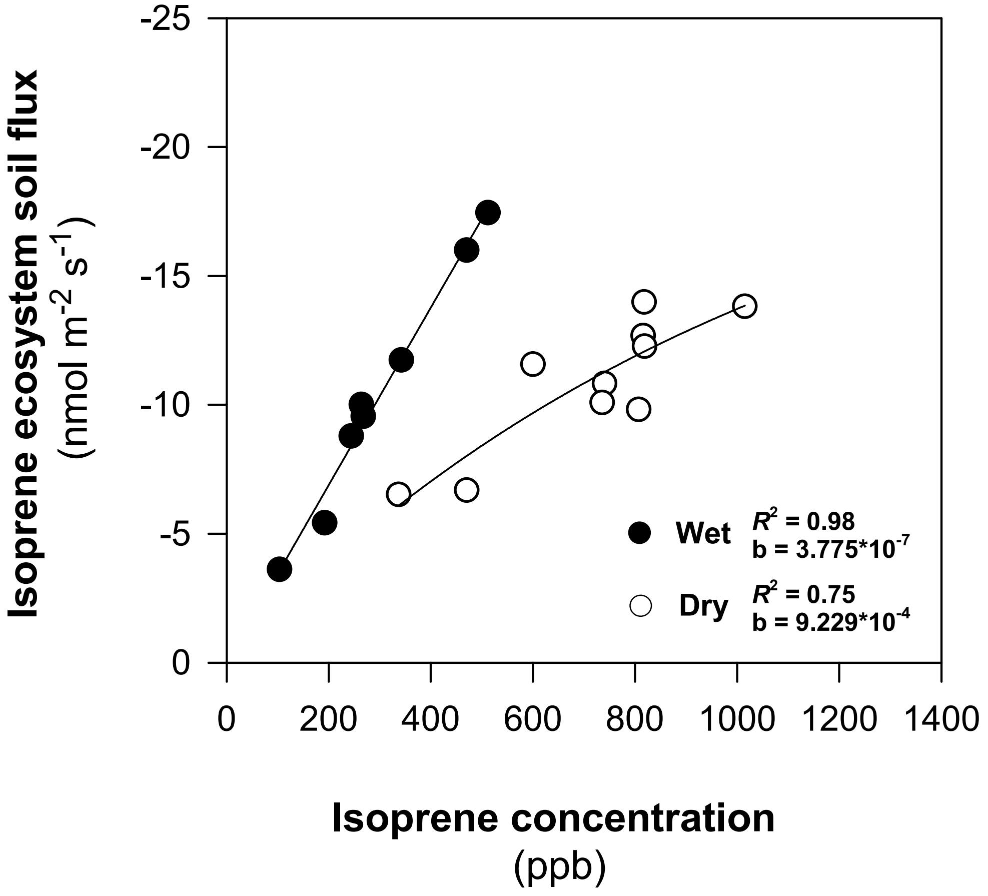


Figure 5.7. Relationship between average night-time mesocosm isoprene uptake flux and maximum initial atmospheric isoprene concentration in a synthetic model tropical rainforest mesocosm. The figure shows the relationship for wet (black circles) and dry (white circles) conditions during the 2002 drought experiment, for selected days when the mesocosm was left closed at night. All data were fitted to an exponential regression model ($F = a*(1\text{-exp}(-b*C)))$). Values for coefficient b and R^2 are also given.

The relationship between soil activity factor[1] k (m min^{-1}) in the tropical rainforest mesocosm and soil moisture (Figure 5.8A and B) also shows that this soil system was evidently very sensitive to soil water content. Soil activity factor k was estimated for each day of the period 17 September, 2002 – 20 June, 2003 by using an exponential function of the regression that fitted the relationship between k_{observed} and

---

[1] Although the proportionality constant k of the exponential decay is commonly called *deposition velocity* (Cleveland and Yavitt 1997), using the term *soil activity factor* we intended to draw attention to the fact that the uptake process is likely to be of biological origin and thus k would represent a measure of the microbial population activity.

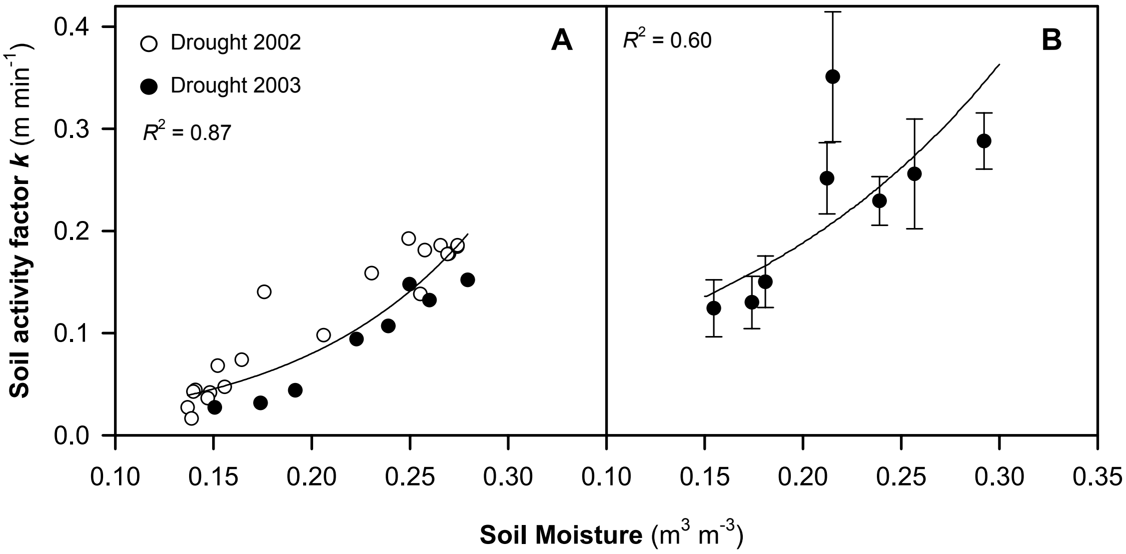


Figure 5.8. Relationships between ecosystem soil activity factor *k* (m min^{-1}) and soil moisture (m^3 m^{-3}) during two drought experiments in the years 2002 (white circles) and 2003 (black circles) (A), and between the static chamber measurements of soil activity factor *k* (m min^{-1}) and soil moisture (m^3 m^{-3}) during the drought experiments of 2003 (B), for the synthetic model tropical rainforest.

soil moisture. This relationship was found by using *k* measured in the whole system (k_{observed}) during days when the system was closed. Observed and modelled *k* for the 2002 period are presented in Figure 5.9 showing that the model predicts well the soil activity factor during the drought period although it overestimates it when soil moisture reached its maximum level after the drought.

The assumption that most of isoprene consumption occurs in the top 5 cm of soil was also confirmed by soil profile measurements showing that only *ca.* 2% of the isoprene atmospheric concentration reached 5 cm depth during the wet period. During the dry period, the decrease in *k* slowed down isoprene uptake and *ca.* 23% of the atmospheric isoprene concentration reached 5 cm depth.

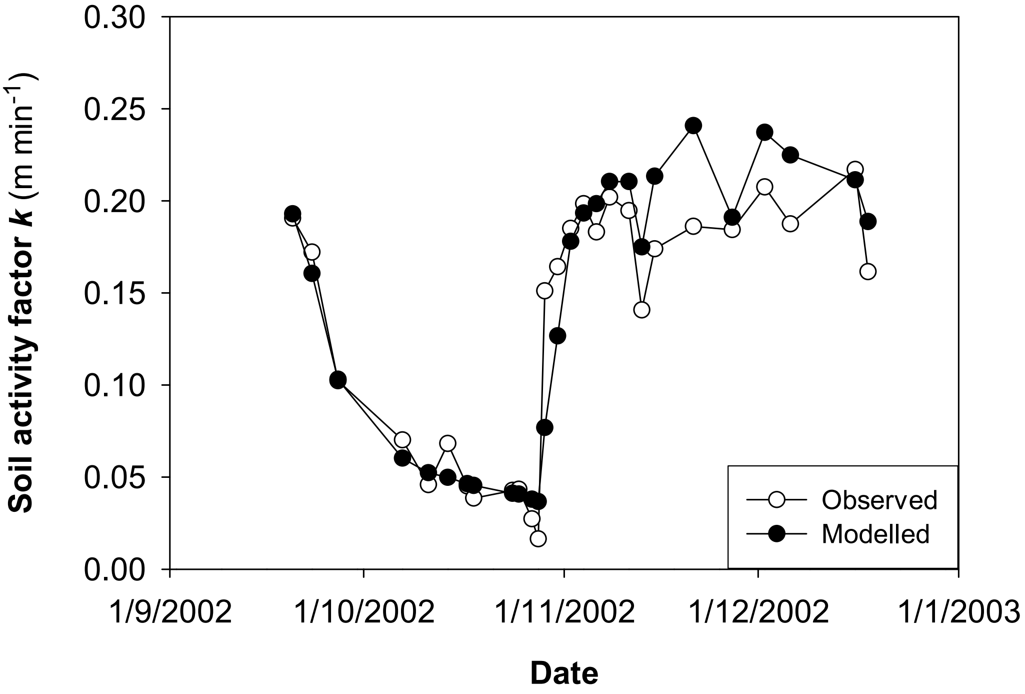


Figure 5.9. Time course of observed (white circles) and modelled (black circles) ecosystem soil activity factor *k* (m min^{-1}) for the drought experiment and the post-stress recovery period.

5.4. Discussion

Because of difficulties in access and the high species diversity of tropical ecosystems, studies on isoprene emission both at the leaf and whole canopy level have mainly focused on temperate ecosystems. Current models estimating isoprene emissions for tropical regions were often developed before any leaf-level data on tropical emissions had been collected (Guenther *et al.* 1995) and therefore rely mostly on data from temperate plants for their leaf-level controls and constrains. This may lead to important bias: for example, previous work (Lerdau and Keller 1997; Keller and Lerdau 1999; Lerdau and Throop 2000) indicated that unlike temperate plants, isoprene emission from tropical species does not saturate with increasing light intensity. Therefore there is urgent need for more information from tropical ecosystems to verify current estimates of tropical isoprene emissions. Although leaf-level measurements can provide detailed information on the physiological mechanism of the response of isoprene production from leaves to changes in the external environment, the final result at the whole ecosystem level may be very different because of the number of different variables and their interactions that intervene in the processes of both production and consumption. Recently, new technology (such as: eddy covariance both by proton-transfer reaction mass spectrometry [Karl *et al.* 2003] or by fast isoprene sensor [Guenther and Hills 1998], and disjunct eddy accumulation [Rinne *et al.* 2000], etc.) has become available, facilitating above-canopy measurements of trace gases such as isoprene, and the number of studies on isoprene emission from tropical ecosystems has been rapidly increasing in the last few years. However, it is often difficult in the field to control all the variables and therefore data often represent a simple observation more than a targeted experiment. The large-scale facility of Biosphere 2 Laboratory gave us the unique opportunity to explore the response of ecosystem isoprene emissions to selected environmental variables in a tropical rainforest mesocosm, a model system of some 110 species developed over 12 years under controlled environmental conditions. Although the response of isoprene production and emission to light and temperature has been extensively studied (Monson and Fall 1989; Harley *et al.* 1999; Fuentes *et al.* 2000; Singsaas and Sharkey 2000), we were able to measure the instantaneous response of isoprene fluxes from tropical species, something that is difficult in natural situations because of practical constraints. The variations in GIP were mostly driven by variation in light. The intensity of the water stress used for this experiment was not sufficiently severe to significantly affect isoprene production, probably because the larger change in θ affected mainly the top 30 cm of soil. As indicated by our screening of a large number of species within both the top canopy and the understorey vegetation layers, the isoprene emitters were mainly distributed among the large trees of the canopy layer which are likely to have developed a root system extending well below 60 cm depth, as is observed in natural Amazonian forests, where big trees utilize deep water resources during drought (Nepstad *et al.* 1994; Meinzer *et al.* 1999). Although isoprene production was not affected by the water stress, the mild water limitation had several other important consequences on the system. As found in previous leaf level and whole canopy studies (Tingey *et al.* 1981; Sharkey and Loreto 1993; Fang *et al.* 1996; Guenther *et al.* 1999; Pegoraro *et al.* 2004a; Pegoraro *et al.* 2004b), opposite to isoprene production, CO_2 uptake is very sensitive to water stress. As reported in detail by Rascher *et al.* (2004), even the mild water stress imposed in this study had a significant effect on the deep-rooted canopy trees. Moreover, it strongly affected the shallow-rooted understorey. As a consequence, over the drought period GPP was strongly reduced and the isoprene carbon loss as a fraction of total photosynthesis increased remarkably. Furthermore, during the drought soil isoprene uptake decreased dramatically, leading to a large increase in daytime net isoprene fluxes and to accumulation of isoprene in the mesocosm atmosphere.

While the drought intensity used in this experiment was not severe enough to significantly affect isoprene production, it may well represent an extended dry season in the Amazon. Similar dry periods are observed in Amazonia in El Niño years (Trenberth and Hoar 1997) and may be more likely if deforestation continues (Nepstad *et al.* 2004). Tropical rainforests play an important role in global carbon and water cycles, representing *ca.* 35% of the global net primary production (Loescher *et al.* 2003). In order to reliably predict the long term responses of tropical rainforests to a changing environment it is important to better understand the processes that underlie whole ecosystem response to local climate and feedbacks expected on the regional climate (Field *et al.* 1995; Cox *et al.* 2000). The effects of drought on tropical rainforests are potentially large and complex, but only a few studies have addressed so far the mechanistic effects of drought in a rainforest ecosystem (Nepstad *et al.* 2004). Furthermore, the implications deriving from effects on trace gas emissions have not been considered. Experimentally the tropical rainforest within the Biosphere 2 Laboratory provided an ideal model system, as the easy canopy access enabled leaf-level measurements in tight correlation with whole ecosystem carbon budgeting (Lin *et al.* 1999; Lin *et al.* 2002), and NEE values measured in the tropical rainforest mesocosm were comparable to those reported for field sites in the wet tropics (Andreae *et al.* 2002; Osmond *et al.* 2004).

Effects of elevated atmospheric [CO_2] and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm

Pegoraro, E., Abrell, L., Vanharen, J., Malhi, Y., Guanghui, L. and Murthy, R.

(*Global Change Biology*, in press)

CHAPTER 6

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

6.1. 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 and Holland 2001). Since Went (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 recognised 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 and Fall 1989; Singsaas and 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 and Wofsy 1988; Zimmerman *et al.* 1988; Guenther *et al.* 1995). Future increases in atmospheric [CO_2] 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 (Rapparini *et al.* 2004; Pegoraro *et al.* 2004b) 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] 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 (Sharkey and Yeh 2001; Monson and Holland 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 and Loreto 1993; Fang *et al.* 1996; Guenther *et al.* 1999; Bruggemann and Schnitzler 2002) have been investigated at the leaf level. Effects of elevated CO_2 have involved both leaf and stand level studies (Monson and Fall 1989; Sharkey *et al.* 1991; Guenther *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 and Yavitt 1997; Cleveland and Yavitt 1998), the significance of soil uptake in the overall isoprene budget of forest systems is still conjectural (Fall and 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 and Odum 1999; Walter and Lambrecht 2004; Osmond *et al.* 2004). The tightly sealed glass and steel enclosure excluded ultraviolet (UV) light (Cockell *et al.* 2000) thereby minimising isoprene depletion by atmospheric oxidative reactions such as those involving OH. Attainment of high concentrations of isoprene from natural vegetation, and observation of large fluxes into defined, temperature regulated soil systems in response to controlled [CO_2] and drought was 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 (three-years old) of a strong isoprene emitter, *Populus deltoides* Bartr., grown under three atmospheric [CO_2]: 430, 800 and 1200 μmol mol^{-1} (ppm); the second was a twelve year-old synthetic model tropical rainforest with several strong isoprene emitting species. Specifically, we explored the relationship between isoprene uptake and atmospheric [CO_2] and drought.

6.2. Material and Methods

6.2.1. 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 Biosphere 2 Laboratory (B2L), Oracle, Arizona, USA. The design and operation of B2L are described in details elsewhere (Lin *et al.* 1999; Zabel *et al.* 1999; Griffin *et al.* 2002).

Intensive forestry management mesocosm (IFM): The intensive forestry management mesocosm comprises three agriforest cottonwood plantations (*Populus. deltoides* Bartr.) grown in three separated experimental bays (*ca.* 550 m^2, 12000 m^3 each) operated as semi-closed 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] (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 and Johnson 2001), comparable to those used for agriforestry in the SE United States. It now shows metabolic (Murthy *et al.* 2004) and microbiological properties (Lipson *et al.*, 2004; in review) “within a reasonable range for natural soils” (Kudeyarov *et al.* 2002), with a soil organic carbon content of *ca.* 2-3% and a carbon:nitrogen ratio of 8.3.

Tropical rainforest mesocosm (TRF): the synthetic model tropical rainforest of the TRF (*ca.* 1950 m^2, 27000 m^3) comprises *ca.* 130 plant species (Leigh *et al.* 1999) and was also operated as a semi-closed 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; Prance, pers. comm.). 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 tropical rainforest mesocosm 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 tropical rainforest mesocosm has a subsoil layer (up to 5 m deep) and a topsoil layer (0.3-3.2 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 concentrations of P, K and other nutrient elements (Scott 1999).

6.2.2. 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 in 2002 (agriforest drought experiment 1) and from May 13 until rewatering on June 4 in 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 tropical rainforest mesocosm water was withheld from September 23 to October 28 in 2002 (TRF drought experiment 1) and from April 21 to May 6 in 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, Logan, UT, USA) 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.

6.2.3. 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 on-line 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] 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 μmol s^{-1}. All measurements were made under the same standard conditions: leaf temperature of 32^{o}C and photosynthetic active radiation (PAR) of 1200 μmol 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 μmol s^{-1}. Inside the PTR-MS reaction cell H_3O^+ ions produced from pure water vapour 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, San Ramon, CA, USA), a dilution of the standard (25 ppb) and zero air from a compressed air cylinder. Environment data collected inside the TRF by the 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, Figure 6.1). FIS is highly selective for isoprene (Guenther and Hills 1998), therefore good agreement between signals detected by the two instruments indicates that any interference at m/e 69 by other compounds in the PTR-MS is minimal, if any.

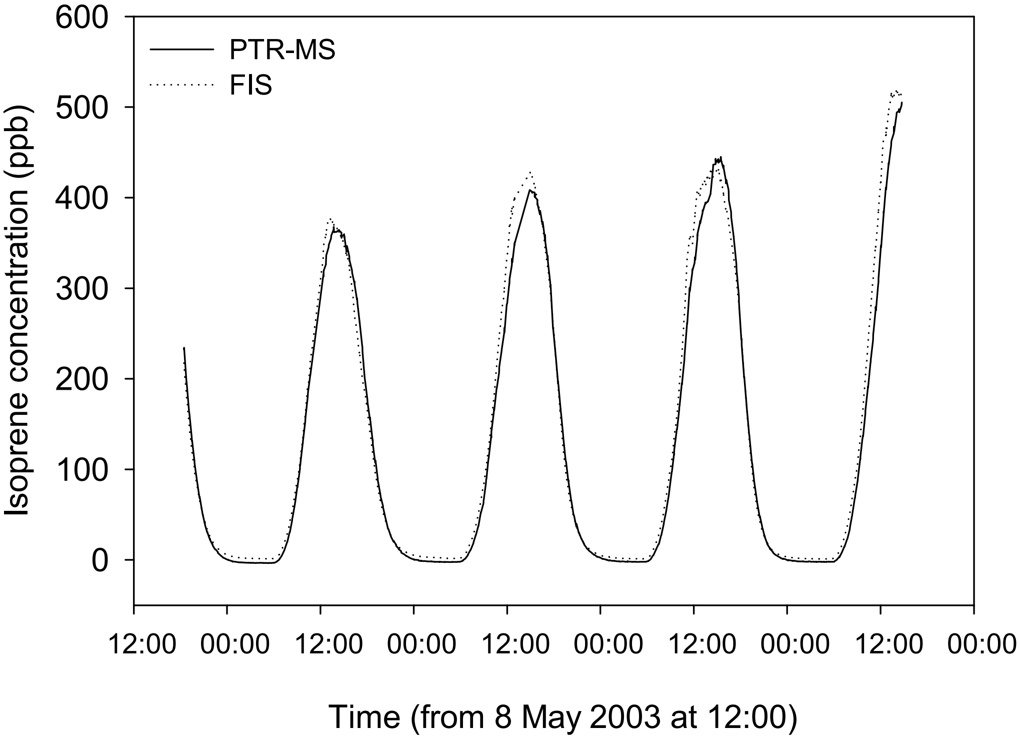


Figure 6.1. Trend of isoprene concentration measured with FIS and measured as m/e 69 with PTR-MS inside the tropical rainforest mesocosm over four days (May 8-12, 2003).

6.2.4. 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 two weeks before the start of the experiment to allow the soil recovery time from disturbance. The PTR-MS was connected by a 9 m long Teflon line (1.6 mm inside diameter) to aluminium 30 x 30 x 40 cm 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 μmol 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 minimised (*ca.* 2 min) to flush the tubing and collect a significant sample of air.

6.2.5. 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 hours. 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 meant that the lowest [CO_2] 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 a FIS based on chemiluminescence detection. Operational details of the instrument have been described in details elsewhere (Hills and Zimmerman 1990; Guenther and 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, 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 μmol mol^{-1}, Scott-Marrin, Riverside, CA, USA) over the range of 50 nmol mol^{-1}–1 μmol mol^{-1} isoprene. FIS instrument stability throughout the experiment was monitored by running an automated calibration cycle each midnight using a standard (100 nmol mol^{-1}) 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 point of each sampling period was automatically discarded to allow complete flushing of the short inlet line from the manifold of valves entering the FIS. In order to have similar datasets from the different mesocosms, the one minute raw isoprene concentration data were averaged by sampling period. A spline model was then used to fill gaps smaller than two hours 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: $F_{\mathrm{P+S}}$), 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:

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

where C_{t+1} is the concentration in the mesocosm for the following 15 min period with respect to time “t“, $C_{t\text{-}1}$ 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 centring 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 (sulphur 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% air-tight, calculated leak rates were taken into account in the isoprene flux calculations by adding the leak flux to the calculated isoprene flux. Diffusion into the soil was also determined by tracer gas injections. During soil profile measurements, after SF_6 addition to the mesocosms, substantial increases in 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 to the total volume of the bay only *ca.* 0.2% of the total leak rate could be the result of diffusion into the soil.

6.2.6. 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 type:

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

The constant k of the equation was calculated as: $k = \mathrm{Ln}\ (C_2/C_1)/(t_2\text{-}t_1)$. 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.

6.2.7. 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]. 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 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.

6.3. 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]. 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 and 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; Greenberg and Zimmerman, unpublished data; Pegoraro, Guenther and 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 and Wang 1999; Fuentes *et al.* 1999) with peak values of as much as 140 ppb (B. Hopkins, Washington State University, Pullman, pers. comm.). 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.

6.3.1. 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 Figure 6.2.

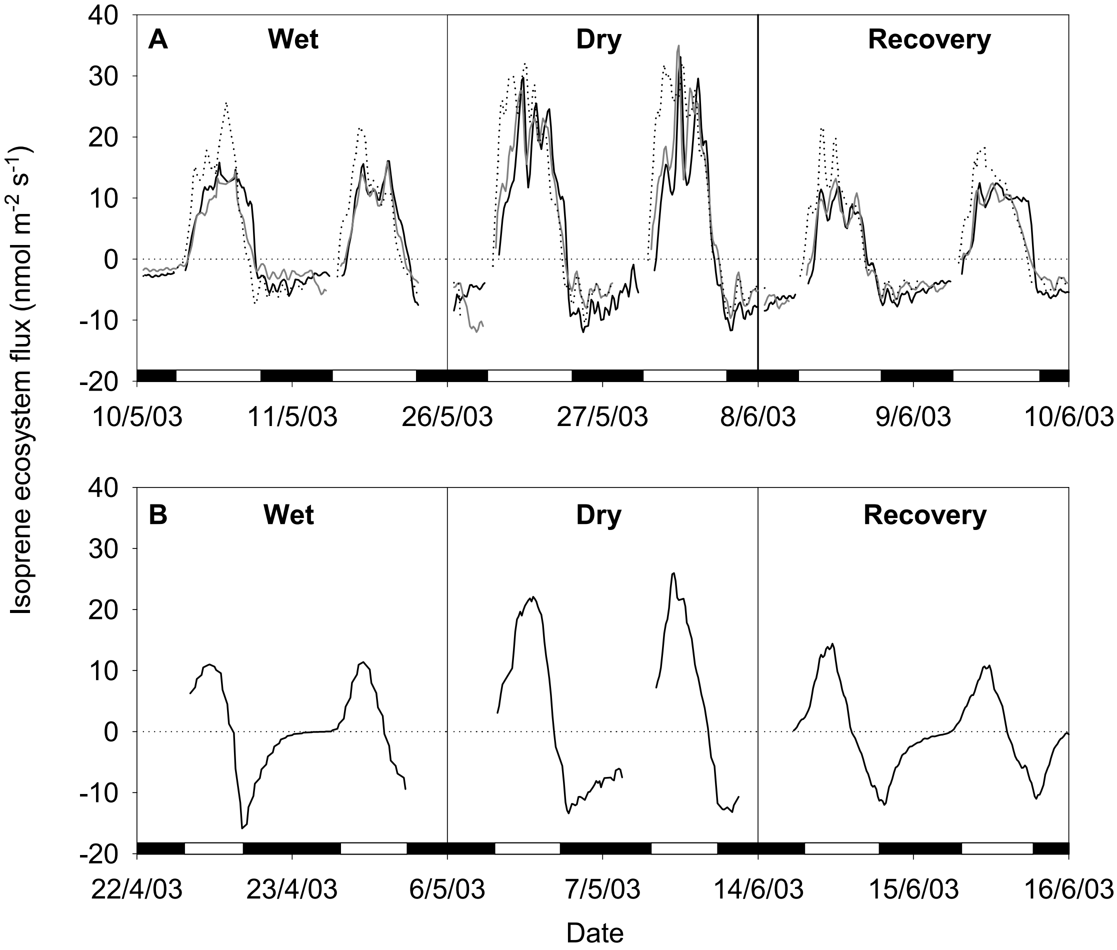


Figure 6.2. Net isoprene fluxes over two wet, dry and recovery days during a drought experiment in agriforest cottonwood plantations grown in three different atmospheric CO_2 conditions: 430 (dotted line), 800 (grey line) and 1200 (solid line) ppm (A), and in a synthetic model tropical rainforest mesocosm (B). Fluxes are given per unit area of soil surface. Day-time (white bar) and night-time (black bar) periods are indicated at the bottom of the chart.

The figure shows selected days in May 2003 with almost identical external incident photon fluxes. As expected from the well-characterised light-dependent 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 photosynthetically active radiation (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 (Figure 6.2A) and the highest emission fluxes of 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 to 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], reducing the inhibitory effect of atmospheric [CO_2] (Pegoraro *et al.* 2004b). 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 those of pre-drought controls within three days. A detailed evaluation of the effects of drought on leaf level isoprene emission from cottonwoods is given elsewhere (Pegoraro *et al.* 2004b).

Experiments in the tropical rainforest mesocosm maintained at 400 ppm CO_2 also revealed rapid emission and uptake of isoprene measured using the FIS method (Figure 6.2B). The tropical rainforest had many isoprene-emitting species that achieved rates, based on leaf area, approaching those of the cottonwoods (Table 6.1). 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 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 to *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.

Table 6.1. Average leaf isoprene emission rate (measured at leaf temperature of 32°C and PAR of 1200 μmol m^{-2} s^{-1}) in non-stressed 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)

6.3.2. 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 (Figure 6.3A, B and C). Experiments in the tropical rainforest also showed a positive relationship between uptake rate and atmospheric isoprene concentration (Figure 6.3D).

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] has a large effect on isoprene emission, it does not alter the isoprene sink capacity of the soil in the litter-free agriforest stands.

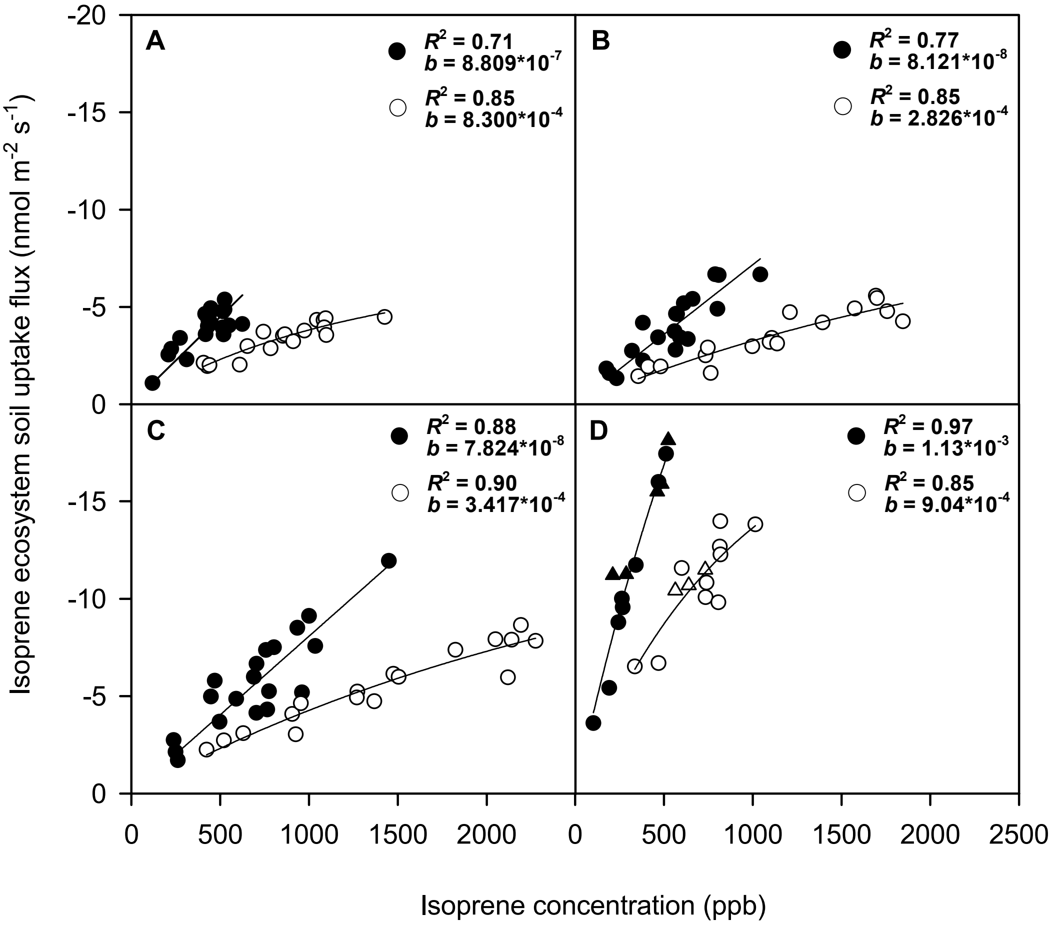


Figure 6.3. Relationship between night-time mesocosm isoprene uptake flux (nmol m^{-2} s^{-1}) and maximum initial atmospheric isoprene concentration (ppb) in agriforest cottonwood plantations grown in three different atmospheric CO_2 treatments (430 (A), 800 (B) and 1200 ppm (C)), and in a synthetic 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 ($F = a*(1-\exp(-b*C))$). Values for coefficient b and R^2 are also given.

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 Figure 6.4 (A 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 of soil may be responsible for most of the isoprene uptake.

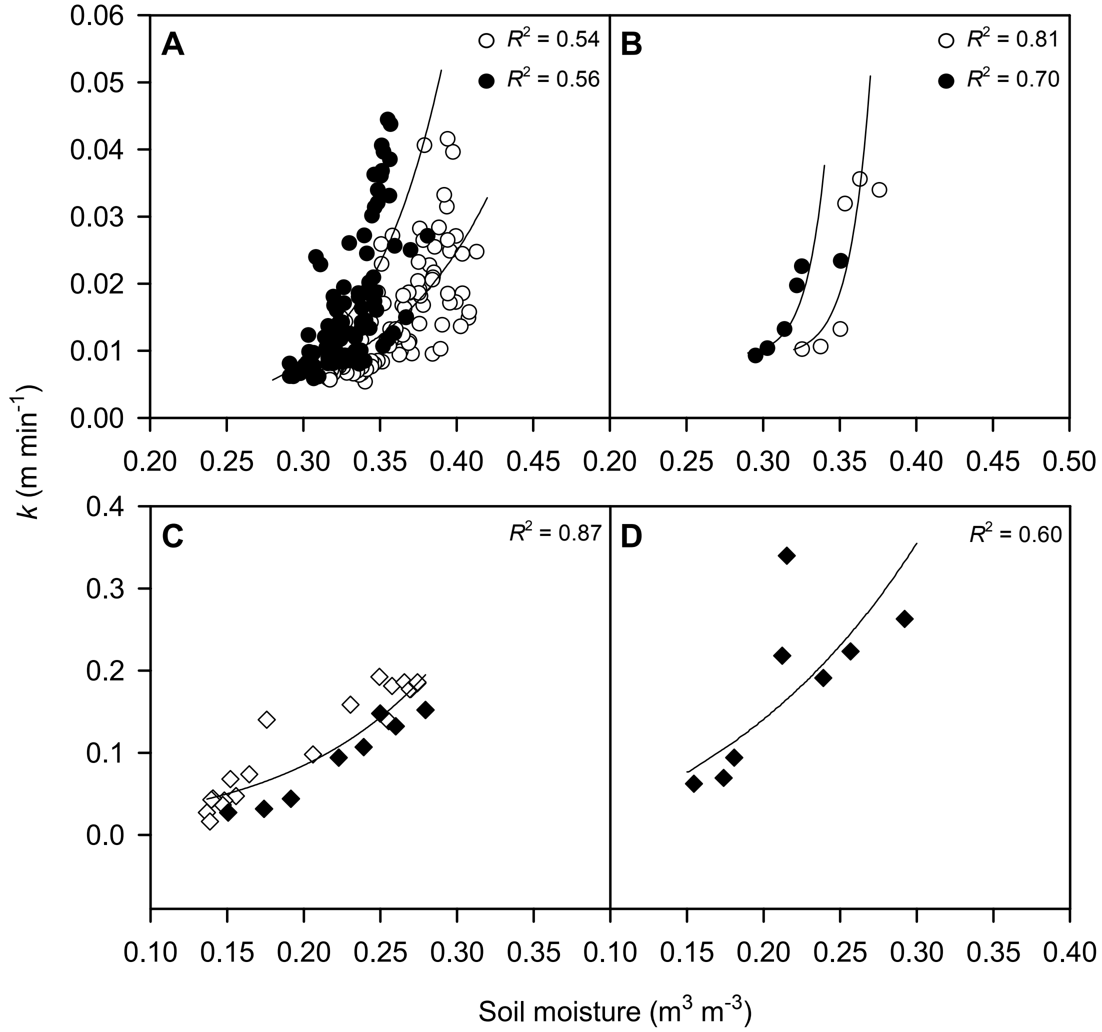


Figure 6.4. Relationship between ecosystem soil activity factor *k* (m min^{-1}) and soil moisture (m^3 m^{-3}) in agriforest cottonwood plantations grown under two atmospheric CO_2 treatments: 430 (white circles) and 1200 (black circles) ppm (A), and in a synthetic model tropical rainforest mesocosm 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.

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 (Figure 6.5).

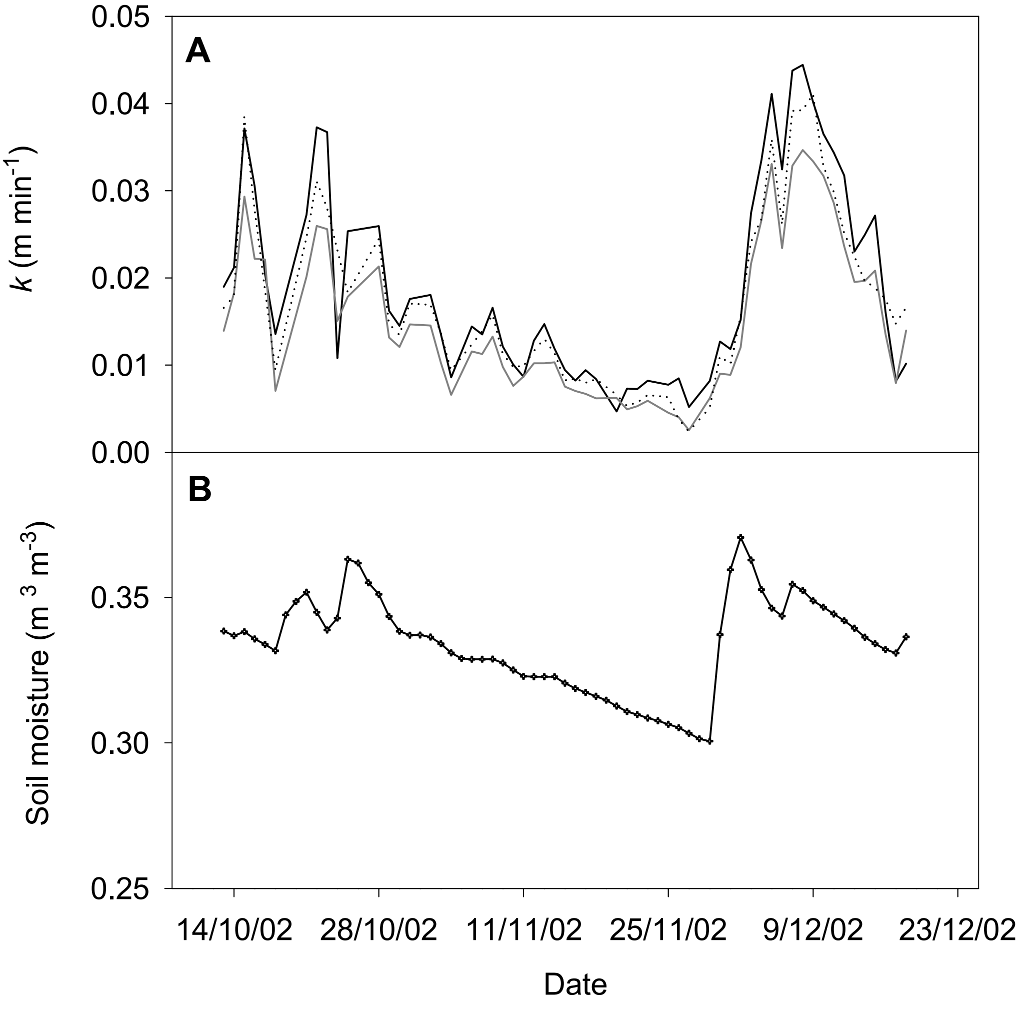


Figure 6.5. Hourly average mesocosm soil activity factor *k* (m min^{-1}) in agriforest cottonwood plantations grown under three different atmospheric [CO_2]: 430 (dotted line), 800 (grey line) and 1200 ppm (dotted line), during the 2002 drought experiment (A). Soil moisture (m^3 m^{-3}) (solid line plus cross) is also shown (B).

These were caused by unavoidable rewetting of the top centimetres of soil caused by condensation from mist used for controlling vapour pressure deficit (VPD) during a series of three-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 (Figure 6.5) 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 (Lipson *et al.*, 2004; in review) and isoprene became available again after leaf expansion (data not shown).

The relationship between soil activity factor *k* in the tropical rainforest mesocosm and soil moisture (Figure 6.4C 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 centimetres 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.

6.3.3. 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] (Figure 6.6). At the beginning of the 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).

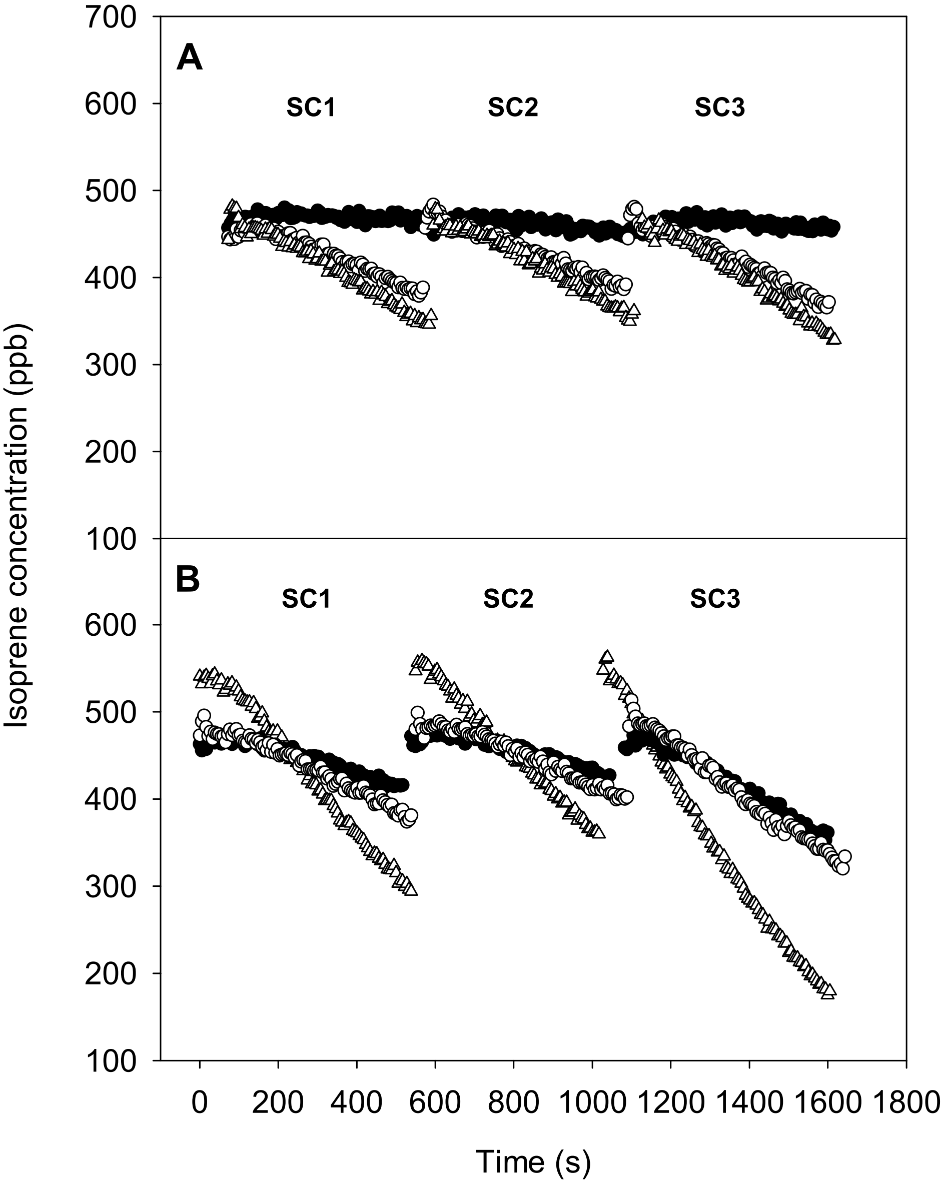


Figure 6.6. Kinetics of isoprene uptake after rewetting dry soil in the agriforest cottonwood mesocosm (430 ppm CO_2). The data shown are real time measurement by PTR-MS of isoprene concentration changes inside the three replicate soil chambers (SC1, SC2 and SC3) for the different phases of the local 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).

In all of 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 centimetres 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 and Birch (1961) showed that microbial populations are able to respond very quickly (within a few hours) to the re-wetting of very dry soils. Isoprene utilisation 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 well represented in clone libraries from B2L soil environmental DNA (Lipson *et al.*, in review). However, given the diverse physiological nature of bacteria, it is impossible to infer isoprene utilising phenotypes based on similarity to known organisms from these data with any certainty (D. Lipson, San Diego State University, San Diego, pers. comm.) and clearly much further research is needed.

6.4. 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] 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 and 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 and Yavitt 1997; Cleveland and Yavitt 1998) and suggests that the soil bacterial metabolism of this hydrocarbon is not limited to recycling of soil-derived substrate (Fall and Copley 2000). 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 and Khalil 1988; Zimmerman *et al.* 1988; Rinne *et al.* 2002) and 16 hours 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 and Wang 1999; Fuentes *et al.* 1999) as an average ambient concentration and 16 hours 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 which 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 (Figure 6.3) 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, Washington State University, Pullman, pers. comm.). 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.* 2004b). The large-scale controlled environment experiments described here will help parameterise 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.

CHAPTER 7

Conclusions

In this thesis I have explored the effect of environmental variables such as: light, temperature, atmospheric [CO_2], soil water content and leaf-to-air vapour pressure deficit on isoprene emission, both at the leaf and whole ecosystem level. Furthermore I explored the possible role of a soil sink in temperate and tropical ecosystems and the controls that elevated CO_2 and soil water content exert on soil isoprene uptake.

The major findings are as follows:

1- Light and Temperature controls on isoprene emission

Isoprene production and emission showed a similar strong positive response to both light and temperature at the whole ecosystem level in both the temperate and tropical mesocosms (Chapter 4 and 5). However, gross isoprene production from the tropical ecosystem seemed to saturate at higher light and temperature levels. The results also show that high leaf-to-air vapour pressure deficits (VPD) significantly stimulate isoprene emission response to light and temperature (Chapter 4). The response to light showed higher isoprene emission rates but a slightly lower saturation light level, and in the response to temperature isoprene emission showed a stronger exponential increase with increase of temperature.

2- Drought controls on isoprene emission

In general isoprene emission was much less sensitive to water stress than photosynthesis (Chapter 2 and 5). However, when water stress was severe (θ measured at 30 cm soil depth smaller than 0.38 m^3 m^{-3} in ambient atmospheric

[CO_2]) it strongly stimulated isoprene emission (Chapter 3 and 4) while photosynthesis was strongly depressed.

3- Leaf-to-air vapour pressure deficit (VPD) controls on isoprene emission

High VPD had a strong stimulating effect on both leaf and whole ecosystem level isoprene emission (Chapter 3 and 4). This stimulation effect by both water stress and high VPD was the result of a decrease in intercellular [CO_2] (C_i) caused by the stomatal closure, which led to a decrease the inhibitory effect of elevated CO_2 on isoprene emission (Chapter 3).

4- CO_2 controls over isoprene emission

Instantaneous increases in [CO_2] always resulted in a depression of isoprene emission, and long-term growth under atmospheric elevated [CO_2] led to a permanent inhibition of isoprene production (Chapter 3 and 4).

5- A parameter linking isoprene emission to water stress

We found that leaf water potential (Ψ_{leaf}) showed a strong correlation with isoprene emission during drought (Chapter 2). This relationship may prove very useful for improving existing isoprene emission models that so far do not take into account the water stress effect on isoprene emission.

6- Isoprene consumption by soils

The experiments presented here confirm the existence of a soil sink in an agriforest plantation and a tropical rainforest synthetic ecosystem (Chapter 6). It was shown that this soil sink is insensitive to atmospheric [CO_2] but responds very quickly to soil moisture changes: while drought suppressed the sink capacity, the full sink capacity of dry soil was recovered within a few hours upon rewetting. Although the soil sink may be modest in a natural environment, in the presence of very low isoprene concentration, nevertheless it is significant when taken into account in global isoprene emission estimates.

The results presented in this work indicate that changes in the global climate may have important repercussions in the response of isoprene emissions from terrestrial ecosystems and offer some novel information to help improve existing models trying to predict the response of global isoprene emission to climate change. Although the metabolic pathway for isoprene production has been largely elucidated (Wildermuth and Fall 1996; Schnitzler *et al.* 1997; Lichtenthaler 1998; Karl *et al.* 2002; Affek and Yakir 2003; Schnitzler *et al.* 2004), there are still unknowns (Logan *et al.* 2000). Existing models rely on the well-known response of isoprene emission to temperature and light, but so far models do not include any parameter describing the effect of elevated CO_2, water stress and elevated VPD, and they do not allow for isoprene deposition to soil. In a world dominated by a human civilization characterized by increasing energy requirements and still relying mainly on fossil fuel burning, CO_2 atmospheric concentration is likely to continue to rise well above the current levels (Keeling *et al.* 1995; Watson R.T. *et al.* 1996). In a possible climate change scenario, with significant increases in temperature and extended drought periods, the repercussions on global isoprene emissions are complex: the inhibition effect by elevated CO_2 may well be off-set by the increase in emitting biomass as a result of increased leaf area index, and by reduced C_i as a result of reduced stomatal conductance due to high VPD and water stress. This situation may be further complicated by a shift in species composition in favour of isoprene emitters and by increased growing season length (Turner *et al.* 1991; Lerdau *et al.* 1997; White *et al.* 1999). Therefore, we can expect that the overall 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 balance (Monson *et al.* 1991; Guenther 2002).

Isoprene emission estimates for tropical rainforests are highly uncertain due to enormous species diversity, difficulty in the accessibility, uncharacterised landscapes and the limited amount of emission data from tropical vegetation. However, tropical regions are believed to represent the major source of isoprene emission to the atmosphere (Guenther 1997; Lerdau *et al.* 1997). Therefore, understanding the response of tropical rainforest to possible global climate change scenarios is crucial in order to be able to predict the consequences on global isoprene emission. As a synthesis of the results of this work, the average gross isoprene production (GIP) and soil isoprene sink in a tropical rainforest were calculated for different climate scenarios, focusing on two environmental variables: soil water availability and atmospheric [CO_2].

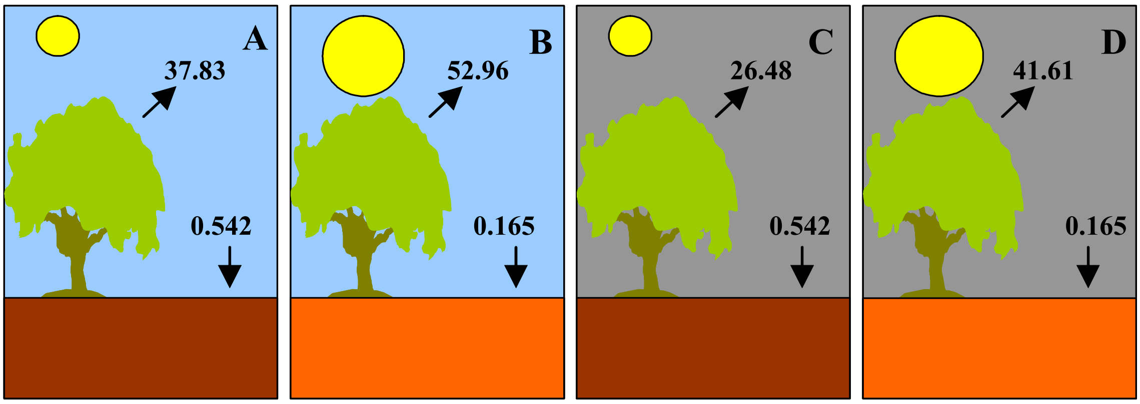


Figure 7.1. Predicted central daytime (10:00 to 15:00) average GIP (nmol m^{-2} s^{-1}) and soil isoprene uptake (nmol m^{-2} s^{-1}) flux for different climatic scenarios: no drought and ambient (400 ppm) atmospheric [CO_2] (A), severe drought and ambient atmospheric [CO_2] (B), no drought and elevated (700 ppm) atmospheric [CO_2] (C), and severe drought and elevated atmospheric [CO_2] (D).

The experiments carried out in this thesis present several limitations. Although studies on leaf level responses using potted plants may provide useful mechanistic information about small scale processes, they may lead to biased responses compared to field grown plants (Medlyn *et al.* 1999). Both the limitations to a normal root system development and the different developmental stage of the plant may have strong effects on its metabolism. Scaling-up through modelling is the way to combine our knowledge of separate small-scale processes in order to understand the effects of interacting environmental variables and their feedbacks on a changing climate on a global scale. However, this methodology relies on precise parameterization and it is often difficult to take into account the infinite complexity of natural ecosystems. Long-term experiments in large-scale facilities capable of precise manipulation of selected environmental variables such as Biosphere 2

Laboratory (B2L) represent a step further in investigating the complexity of ecological interactions (Osmond *et al.* 2004). However, associated with such large scale enclosures there are a number of problems such as the lack of UV light and lack of naturally random events such as wind and rain. Moreover, as we have already seen (Chapter 1), there is a limitation on the ability to replicate experiments, and some difficulty in maintaining all the ecosystem components at the desired setting, etc. These limitations may be turned to advantage in some cases (e.g. allowing the study of the dynamics of UV-photolabile trace gases, something that is often difficult in natural conditions) or can be partly compensated by the technology (e.g. using large-capacity fans, special sprinkler systems, etc.) or overcome by judicious experimental design (e.g. using replicates in time).

In this study I decided to tackle the problem of understanding the response of isoprene emission to environmental variables both at the small (leaf level measurements) and at the large scale (whole ecosystem measurements). The main reason was that while leaf level measurements are very helpful in providing information on the details of the process (such as the relationship with leaf water potential), collecting the amount of information allowing reliable estimates of whole ecosystem responses would be a life-time’s work because of the number of variables and interactions among them that come together to give the final ecosystem result (canopy structure, leaf position, metabolism of sun- and shade-leaves, changes in leaf biomass, etc.). The study conducted in B2L gave me the opportunity to verify that leaf level responses still hold, notwithstanding the bigger complexity of the system.

It is clear that more work is needed in order to improve our knowledge of the mechanisms underlying the process of production and emission of isoprene in response to changes of environmental variables such as CO_2, soil water content and VPD. I believe that the results reported in this study contain valuable indications, but the picture is still incomplete. The next step would be to use the results obtained in this work to improve some of the existing models, linking ecosystem models to models of global atmospheric chemistry. I also believe that more field work is necessary to confirm some of the findings of this thesis in natural ecosystems, using state-of-the-art instrumentation that was only just emerging when this work was in progress.

REFERENCE LIST

Affek, H.P. and Yakir, D. (2003). Natural abundance carbon isotope composition of isoprene reflects incomplete coupling between isoprene synthesis and photosynthetic carbon flow. *Plant Physiology* **131,** 1727-1736.

Andreae, M.O., Artaxo, P., Brandao, C. et al. (2002). Biogeochemical cycling of carbon, water, energy, trace gases, and aerosols in Amazonia: the LBA-EUSTACH experiments. *Journal of Geophysical Research-Atmospheres* **107,** 8054-8066.

Andreae, M.O. and Crutzen, P.J. (1997). Atmospheric aerosols: biogeochemical sources and role in atmospheric chemistry. *Science* **276,** 1052-1058.

Arain, M.A., Shuttleworth, W.J., Farnsworth, B. et al. (2000). Comparing micrometeorology of rain forests in Biosphere-2 and Amazon basin. *Agricultural And Forest Meteorology* **100,** 273-289.

Atkinson, R. (1997). Gas-phase tropospheric chemistry of volatile organic compounds .1. Alkanes and alkenes. *Journal of Physical and Chemical Reference Data* **26,** 215-290.

Atkinson, R. (2000). Atmospheric chemistry of VOCs and NO_x. *Atmospheric Environment* **34,** 2063-2101.

Atkinson, R. and Arey, J. (1998). Atmospheric chemistry of biogenic organic compounds. *Accounts of Chemical Research* **31,** 574-583.

Bach, T.J. and Lichtenthaler, H.K. (1983). Inhibition by mevinolin of plant-growth, sterol formation and pigment accumulation. *Physiologia Plantarum* **59,** 50-60.

Baldocchi, D., Guenther, A.B., Harley, P.C. et al. (1995). The fluxes and air chemistry of isoprene above a deciduous hardwood forest. *Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences* **351,** 279-296.

Baldocchi, D.D., Fuentes, J.D., Bowling, D.R. et al. (1999). Scaling isoprene fluxes from leaves to canopies: test cases over a boreal aspen and a mixed species temperate forest. *Journal of Applied Meteorology* **38,** 885-898.

Bombelli, A. and Gratani, L. (2003). Interspecific differences of leaf gas exchange and water relations of three evergreen Mediterranean shrub species. *Photosynthetica* **41,** 619-625.

Bowes, G. (1991). Growth at elevated CO_2 - photosynthetic responses mediated through rubisco. *Plant, Cell and Environment* **14,** 795-806.

Brown S, Sathaye J, Cannell M, Kauppi P (1996) 'Climate Change in 1995 - Impacts, Adaptations and Mitigation of Climate Change: Scientific-Technical Analyses. Contribution of Working Group II to the Second Assessment Report of the Intergovernmental Panel on Climate Change.' (Cambridge Univ. Press: Cambridge)

Bruggemann, N. and Schnitzler, J.P. (2002). Comparison of isoprene emission, intercellular isoprene concentration and photosynthetic performance in water-limited oak (*Quercus pubescens* Willd. and *Quercus robur* L.) saplings. *Plant Biology* **4,** 456-463.

Centritto, M., Nascetti, P., Petrilli, L. et al. (2004). Profiles of isoprene emission and photosynthetic parameters in hybrid poplars exposed to free-air CO_2 enrichment. *Plant, Cell and Environment* **27,** 403-412.

Chameides, W.L., Lindsay, R.W., Richardson, J. et al. (1988). The role of biogenic hydrocarbons in urban photochemical smog: Atlanta as a case-study. *Science* **241,** 1473-1475.

Chen, X., Hulbert, D. and Shepson, P.B. (1998). Measurement of the organic nitrate yield from OH reaction with isoprene. *Journal of Geophysical Research* 25,563-25,568.

Claeys, M., Graham, B., Vas, G. et al. (2004). Formation of secondary organic aerosols through photooxidation of isoprene. *Science* **303,** 1173-1176.

Cleveland, C.C. and Yavitt, J.B. (1997). Consumption of atmospheric isoprene in soil. *Geophysical Research Letters* **24,** 2379-2382.

Cleveland, C.C. and Yavitt, J.B. (1998). Microbial consumption of atmospheric isoprene in a temperate forest soil. *Applied and Environmental Microbiology* **64,** 172-177.

Cockell, C.S., Southern, A. and Herrera, A. (2000). Lack of UV radiation in Biosphere 2 - practical and theoretical effects on plants. *Ecological Engineering* **16,** 293-299.

Cox, P.M., Betts, R.A., Jones, C.D. et al. (2000). Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* **408,** 184-187.

Crutzen, P.J. and Fishman, J. (1977). Average concentrations of OH in the troposphere and the budgets of CH_4, CO, H_2 and CH_3CCl_3. *Geophysical Research Letters* **4,** 321-324.

Curtis, P.S. (1996). A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment* **19,** 127-137.

Delwiche, C.F. and Sharkey, T.D. (1993). Rapid appearence of C-13 in biogenic isoprene when (CO_2)-C-13 is fed to intact leaves. *Plant, Cell and Environment* **16,** 587-591.

Dempster, W.F. (1999). Biosphere 2 engineering design. *Ecological Engineering* **13,** 31-42.

Di Carlo, P., Brune, W.H., Martinez, M. et al. (2004). Missing OH reactivity in a forest: Evidence for unknown reactive biogenic VOCs. *Science* **304,** 722-725.

Evans, R.C., Tingey, D.T., Gumpertz, M.L. et al. (1982). Estimates of isoprene and monoterpene emission rates in plants. *Botanical Gazette* **143,** 304-310.

Fall, R. and Copley, S.D. (2000). Bacterial sources and sinks of isoprene, a reactive atmospheric hydrocarbon. *Environmental Microbiology* **2,** 123-130.

Fall, R.R. and Monson, R.K. (1992). Isoprene emission rate and intercellular isoprene concentration as influenced by stomatal distribution and conductance. *Plant Physiology* **100,** 987-992.

Fang, C.W., Monson, R.K. and Cowling, E.B. (1996). Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long- term drying cycles. *Tree Physiology* **16,** 441-446.

FAO. Forest Products 1991-1995. [30]. 1995. Rome, Food and Agricolture Organization of the United Nations. FAO Forestry Series.

Fehsenfeld, F., Calvert, J., Fall, R.R. et al. (1992). Emission of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochemical Cycles* **6,** 389-430.

Fenning, M. and Gershenzon, J. (2002). Where will the wood come from? Plantation forests and the role of biotechnology. *Trends in Biotechnology* **20,** 291-296.

Field, C.B., Jackson, R.B. and Mooney, H.A. (1995). Stomatal responses to increased CO_2 - implications from the plant to the global-scale. *Plant, Cell and Environment* **18,** 1214-1225.

Fuentes, J.D., Lerdau, M., Atkinson, R. et al. (2000). Biogenic hydrocarbons in the atmospheric boundary layer: A review. *Bulletin of the American Meteorological Society* **81,** 1537-1575.

Fuentes, J.D. and Wang, D. (1999). On the seasonality of isoprene emissions from a mixed temperate forest. *Ecological Applications* **9,** 1118-1131.

Fuentes, J.D., Wang, D., DenHartog, G. et al. (1995). Modeled and field measurements of biogenic hydrocarbon emission from a canadian deciduous forest. *Atmospheric Environment* **29,** 3003-3017.

Fuentes, J.D., Wang, D. and Gu, L. (1999). Seasonal variations in isoprene emissions from a boreal aspen forest. *Journal of Applied Meteorology* **38,** 855-869.

Funk, J.L., Mak, J.E. and Lerdau, M.T. (2004). Stress-induced changes in carbon sources for isoprene production in *Populus deltoides*. *Plant, Cell and Environment* **27,** 747-755.

Gao, W., Wesely, M.L. and Doskey, P.V. (1993). Numerical modeling of the turbulent-diffusion and chemistry of NO_x, O_3, isoprene and other reactive gases in and above a forest canopy. *Journal of Geophysical Research-Atmospheres* **98,** 18339-18353.

Geron, C.D., Nie, D., Arnts, R.R. et al. (1997). Biogenic isoprene emission: Model evaluation in a southeastern United States bottomland deciduous forest. *Journal of Geophysical Research-Atmospheres* **102,** 18889-18901.

Goldstein, A.H., Goulden, M.L., Munger, J.W. et al. (1998). Seasonal course of isoprene emissions from a midlatitude deciduous forest. *Journal of Geophysical Research-Atmospheres* **103,** 31045-31056.

Greenberg, J.P., Guenther, A., Harley, P. et al. (2003). Eddy flux and leaf-level measurements of biogenic VOC emissions from mopane woodland of Botswana. *Journal of Geophysical Research-Atmospheres* **108,** art-8466.

Greenberg, J.P., Guenther, A.B., Petron, G. et al. (2004). Biogenic VOC emissions from forested Amazonian landscapes. *Global Change Biology* **10,** 651-662.

Greenberg, J.P., Zimmerman, P.R. and Chatfield, R.B. (1985). Hydrocarbons and carbon-monoxide in African savannah air. *Geophysical Research Letters* **12,** 113-116.

Griffin, K.L., Turnbull, M., Murthy, R. et al. (2002). Leaf respiration is differentially affected by leaf vs. stand- level night-time warming. *Global Change Biology* **8,** 479-485.

Griffin, R.J., Cocker, D.R., Flagan, R.C. et al. (1999). Organic aerosol formation from the oxidation of biogenic hydrocarbons. *Journal of Geophysical Research-Atmospheres* **104,** 3555-3567.

Griffiths, E. and Birch, H.F. (1961). Microbiological changes in freshly moistened soil. *Nature* **189,** 424-427.

Guenther, A.B. (1997). Seasonal and spatial variations in natural volatile organic compound emissions. *Ecological Applications* **7,** 34-45.

Guenther, A.B. (2002). The contribution of reactive carbon emissions from vegetation to the carbon balance of terrestrial ecosystems. *Chemosphere* **49,** 837-844.

Guenther, A.B., Archer, S., Greenberg, J.P. et al. (1999). Biogenic hydrocarbon emissions and landcover/climate change in a subtropical savanna. *Physics and Chemistry of the Earth Part B-Hydrology Oceans and Atmosphere* **24,** 659-667.

Guenther, A.B., Geron, C.D., Pierce, T. et al. (2000). Natural emissions of non-methane volatile organic compounds; carbon monoxide, and oxides of nitrogen from North America. *Atmospheric Environment* **34,** 2205-2230.

Guenther, A.B., Hewitt, C.N., Erickson, D. et al. (1995). A global model of natural volatile organic compound emissions. *Journal of Geophysical Research-Atmospheres* **100,** 8873-8892.

Guenther, A.B. and Hills, A.J. (1998). Eddy covariance measurement of isoprene fluxes. *Journal of Geophysical Research-Atmospheres* **103,** 13145-13152.

Guenther, A.B., Monson, R.K. and Fall, R.R. (1991). Isoprene and monoterpene emission rate variability - observations with *Eucalyptus* and emission rate algorithm development. *Journal of Geophysical Research-Atmospheres* **96,** 10799-10808.

Guenther, A.B., Zimmerman, P., Klinger, L. et al. (1996). Estimates of regional natural volatile organic compound fluxes from enclosure and ambient measurements. *Journal of Geophysical Research-Atmospheres* **101,** 1345-1359.

Guenther, A.B., Zimmerman, P. and Wildermuth, M. (1994). Natural volatile organic-compound emission rate estimates for United States woodland landscapes. *Atmospheric Environment* **28,** 1197-1210.

Guenther, A.B., Zimmerman, P.R., Harley, P.C. et al. (1993). Isoprene and monoterpene emission rate variability - model evaluations and sensitivity analyses. *Journal of Geophysical Research-Atmospheres* **98,** 12609-12617.

Hansel, A., Jordan, A., Holzinger, R. et al. (1995). Proton-transfer reaction mass-spectrometry - online trace gas- analysis at the ppb level. *International Journal of Mass Spectrometry and Ion Processes* **150,** 609-619.

Hansen, J., Fung, I., Lacis, A. et al. (1988). Global climate changes as forecast by goddard institute for space studies 3-dimensional model. *Journal of Geophysical Research-Atmosphere* **93,** 9341-9364.

Hanson, D.T., Swanson, S., Graham, L.E. et al. (1999). Evolutionary significance of isoprene emission from mosses. *American Journal of Botany* **86,** 634-639.

Harley, P., Vasconcellos, P., Vierling, L. et al. (2004). Variation in potential for isoprene emissions among Neotropical forest sites. *Global Change Biology* **10,** 630-650.

Harley, P.C., Guenther, A.B. and Zimmerman, P. (1996). Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiology* **16,** 25-32.

Harley, P.C., Guenther, A.B. and Zimmerman, P. (1997). Environmental controls over isoprene emission in deciduous oak canopies. *Tree Physiology* **17,** 705-714.

Harley, P.C., Litvak, M.E., Sharkey, T.D. et al. (1994). Isoprene emission from velvet bean leaves - Interactions among nitrogen availability, growth photon flux density, and leaf developement. *Plant Physiology* **105,** 279-285.

Harley, P.C., Monson, R.K. and Lerdau, M.T. (1999). Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia* **118,** 109-123.

Hayward, S., Hewitt, C.N., Sartin, J.H. et al. (2002). Performance characteristics and applications of a proton transfer reaction-mass spectrometer for measuring volatile organic compounds in ambient air. *Environmental Science & Technology* **36,** 1554-1560.

Hills, A.J. and Zimmerman, P.R. (1990). Isoprene measurement by ozone-induced chemiluminescence. *Analytical Chemistry* **62,** 1055-1060.

Hoffmann, T., Odum, J.R., Bowman, F. et al. (1997). Formation of organic aerosols from the oxidation of biogenic hydrocarbons. *Journal of Atmospheric Chemistry* **26,** 189-222.

Houghton JT, Ding Y, Griggs DJ, van der Linden PJ, Xiaosu D (2001) 'Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC).' (Cambridge University Press: Cambridge)

Isebrands, J.G., McDonald, E.P., Kruger, E. et al. (2001). Growth responses of Populus tremuloides clones to interacting elevated carbon dioxide and tropospheric ozone. *Environmental Pollution* **115,** 359-371.

Jacob, D.J. and Wofsy, S.C. (1988). Photochemistry of biogenic emissions over the Amazon forest. *Journal of Geophysical Research-Atmospheres* **93,** 1477-1486.

Karl, T., Fall, R., Rosenstiel, T.N. et al. (2002). On-line analysis of the (CO_2)-C-13 labeling of leaf isoprene suggests multiple subcellular origins of isoprene precursors. *Planta* **215,** 894-905.

Karl, T., Hansel, A., Mark, T. et al. (2003). Trace gas monitoring at the Mauna Loa Baseline observatory using proton-transfer reaction mass spectrometry. *International Journal of Mass Spectrometry* **223,** 527-538.

Keeling, C.D., Whorf, T.P., Wahlen, M. et al. (1995). Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* **375,** 666-670.

Keller, M. and Lerdau, M. (1999). Isoprene emission from tropical forest canopy leaves. *Global Biogeochemical Cycles* **13,** 19-29.

Kesselmeier, J., Kuhn, U., Rottenberger, S. et al. (2002). Concentrations and species composition of atmospheric volatile organic compounds (VOCs) as observed during the wet and dry season in Rondonia (Amazonia). *Journal of Geophysical Research-Atmospheres* **107,** art-8053.

Kudeyarov, V.N., Ponizovskii, A.A., Bil, K.Y. et al. (2002). Soil in the intensive forestry biome at the Biosphere 2 station, Columbia University (Arizona, United States). *Eurasian Soil Science* **35,** S34-S45.

Kulmala, M., Suni, T., Lehtinen, K.E.J. et al. (2004). A new feedback mechanism linking forests, aerosols, and climate. *Atmospheric Chemistry and Physics* **4,** 557-562.

Kuzma, J. and Fall, R.R. (1993). Leaf isoprene emission rate is dependent on leaf development and the level of isoprene synthase. *Plant Physiology* **101,** 435-440.

Lamb, B., Gay, D., Westberg, H. et al. (1993). A biogenic hydrocarbon emission inventory for the USA using a simple forest canopy model. *Atmospheric Environment Part A-General Topics* **27,** 1673-1690.

Lamb, B., Pierce, T., Baldocchi, D. et al. (1996). Evaluation of forest canopy models for estimating isoprene emissions. *Journal of Geophysical Research-Atmospheres* **101,** 22787-22797.

Lawlor, D.W. and Mitchell, R.A.C. (1991). The effects of increasing CO_2 on crop photosynthesis and productivity - a review of field studies. *Plant, Cell and Environment* **14,** 807-818.

Leigh, L.S., Burgess, T., Marino, B.D.V. et al. (1999). Tropical rainforest biome of Biosphere 2: Structure, composition and results of the first 2 years of operation. *Ecological Engineering* **13,** 65-93.

Lerdau, M., Guenther, A.B. and Monson, R. (1997). Plant production and emission of volatile organic compounds. *Bioscience* **47,** 373-383.

Lerdau, M. and Keller, M. (1997). Controls on isoprene emission from trees in a subtropical dry forest. *Plant, Cell and Environment* **20,** 569-578.

Lerdau, M. and Throop, H.L. (2000). Sources of variability in isoprene emission and photosynthesis in two species of tropical wet forest trees. *Biotropica* **32,** 670-676.

Lichtenthaler, H.K. (1998). The plants' 1-deoxy-D-xylulose-5-phosphate pathway for biosynthesis of isoprenoids. *Fett-Lipid* **100,** 128-138.

Lin, G.H., Adams, J., Farnsworth, B. et al. (1999). Ecosystem carbon exchange in two terrestrial ecosystem mesocosms under changing atmospheric CO_2 concentrations. *Oecologia* **119,** 97-108.

Lin G.H., Berry J.A., Kaduk J. et al. (2002) Sensitivity of photosynthesis and carbon sinks in world tropical rainforests to projected atmospheric CO_2 and associated climate changes. In 'Proceedings of 12th International Congress on Photosynthesis'. CSIRO Publishing: Melbourne, Australia.

Lin, G.H., Marino, B.D.V., Wei, Y.D. et al. (1998). An experimental and modeling study of responses in ecosystems carbon exchanges to increasing CO_2 concentrations using a tropical rainforest mesocosm. *Australian Journal of Plant Physiology* **25,** 547-556.

Lindinger, W., Hansel, A. and Jordan, A. (1998). Proton-transfer-reaction mass spectrometry (PTR-MS): on-line monitoring of volatile organic compounds at pptv levels. *Chemical Society Reviews* **27,** 347-354.

Litvak, M.E., Loreto, F., Harley, P.C. et al. (1996). The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. *Plant, Cell and Environment* **19,** 549-559.

Lloyd, J. (1999). The CO_2 dependence of photosynthesis, plant growth responses to elevated CO_2 concentrations and their interaction with soil nutrient status, II. Temperate and boreal forest productivity and the combined effects of increasing CO_2 concentrations and increased nitrogen deposition at a global scale. *Functional Ecology* **13,** 439-459.

Loescher, H.W., Oberbauer, S.F., Gholz, H.L. et al. (2003). Environmental controls on net ecosystem-level carbon exchange and productivity in a Central American tropical wet forest. *Global Change Biology* **9,** 396-412.

Logan, B.A., Anchordoquy, T.J., Monson, R.K. et al. (1999). The effect of isoprene on the properties of spinach thylakoids and phosphatidylcholine liposomes. *Plant Biology* **1,** 602-606.

Logan, B.A. and Monson, R.K. (1999). Thermotolerance of leaf discs from four isoprene-emitting species is not enhanced by exposure to exogenous isoprene. *Plant Physiology* **120,** 821-825.

Logan, B.A., Monson, R.K. and Potosnak, M.J. (2000). Biochemistry and physiology of foliar isoprene production. *Trends in Plant Science* **5,** 477-481.

Loreto, F., Mannozzi, M., Maris, C. et al. (2001). Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiology* **126,** 993-1000.

Loreto, F., Pinelli, P., Brancaleoni, E. et al. (2004). C-13 labeling reveals chloroplastic and extrachloroplastic pools of dimethylallyl pyrophosphate and their contribution to isoprene formation. *Plant Physiology* **135,** 1903-1907.

Loreto, F. and Sharkey, T.D. (1990). A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. *Planta* **182,** 523-531.

Malhi, Y. and Grace, J. (2000). Tropical forests and atmospheric carbon dioxide. *Trends in Ecology & Evolution* **15,** 332-337.

Manabe, S. and Stouffer, R.J. (1994). Multiple-century response of a coupled ocean-atmosphere model to an increase of atmospheric carbon dioxide. *Journal of Climate* **7,** 5-23.

Marino, B.D.V. and Odum, H.T. (1999). Biosphere 2. Introduction and research progress. *Ecological Engineering* **13,** 3-14.

Martinez-Vilalta, J., Mangiron, M., Ogaya, R. et al. (2003). Sap flow of three co-occurring Mediterranean woody species under varying atmospheric and soil water conditions. *Tree Physiology* **23,** 747-758.

McGarvey, D.J. and Croteau, R. (1995). Terpenoid metabolism. *Plant Cell* **7,** 1015-1026.

Mead R, Curnow RN (1983) 'Statistical Methods in Agriculture and Experimental Biology.' (Chapman & Hall: London)

Medlyn, B.E., Badeck, F.W., De Pury, D.G.G. et al. (1999). Effects of elevated [CO_2] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant, Cell and Environment* **22,** 1475-1495.

Meinzer, F.C., Andrade, J.L., Goldstein, G. et al. (1999). Partitioning of soil water among canopy trees in a seasonally dry tropical forest. *Oecologia* **121,** 293-301.

Mgaloblishvili, M.P., Khetsuriani, N.D., Kalandadze, A.N. et al. (1979). Localization of isoprene biosynthesis in poplar chloroplasts. *Plant Physiology* **25,** 837-842.

Monson, R.K. (2002). Volatile organic compound emissions from terrestrial ecosystems: A primary biological control over atmospheric chemistry. *Israel Journal of Chemistry* **42,** 29-42.

Monson, R.K. and Fall, R.R. (1989). Isoprene emission from aspen leaves - influence of environment and relation to photosynthesis and photorespiration. *Plant Physiology* **90,** 267-274.

Monson, R.K., Harley, P.C., Litvak, M.E. et al. (1994). Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. *Oecologia* **99,** 260-270.

Monson, R.K., Hills, A.J., Zimmerman, P.R. et al. (1991). Studies of the relationship between isoprene emission rate and CO_2 or photon flux density using a real time isoprene analyzer. *Plant, Cell and Environment* **14,** 517-523.

Monson, R.K. and Holland, E.A. (2001). Biospheric trace gas fluxes and their control over tropospheric chemistry. *Annual Review of Ecology and Systematics* **32,** 547-560.

Monson, R.K., Jaeger, C.H., Adams, W.W. et al. (1992). Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. *Plant Physiology* **98,** 1175-1180.

Murthy, R., Barron-Gafford, G., Dougherty, P.M. et al. (2004). Increased leaf area dominates carbon flux response to elevated CO_2 in stands of *Populus deltoides* (Bartr.) and underlies a switch from canopy light-limited CO_2 influx in well-watered treatments to individual leaf, stomatally-limited influx under water stress. *Global Change Biology*.

Murthy, R., Griffin, K.L., Zarnoch, S.J. et al. (2003). Carbon dioxide efflux from a 550 m^3 soil across a range of soil temperatures. *Forest Ecology and Management* **178,** 311-327.

Nepstad, D., Lefebvre, P., Da Silva, U.L. et al. (2004). Amazon drought and its implications for forest flammability and tree growth: a basin-wide analysis. *Global Change Biology* **10,** 704-717.

Nepstad, D.C., Decarvalho, C.R., Davidson, E.A. et al. (1994). the role of deep roots in the hydrological and carbon cycles of amazonian forests and pastures. *Nature* **372,** 666-669.

Niinemets, U., Loreto, F. and Reichstein, M. (2004). Physiological and physico-chemical controls on foliar volatile organic compound emissions. *Trends in Plant Science* **9,** 180-186.

Norby, R.J., Wullschleger, S.D., Gunderson, C.A. et al. (1999). Tree responses to rising CO_2 in field experiments: implications for the future forest. *Plant, Cell and Environment* **22,** 683-714.

Oren, R., Ellsworth, D.S., Johnsen, K.H. et al. (2001). Soil fertility limits carbon sequestration by forest ecosystems in a CO_2 enriched atmosphere. *Nature* **411,** 469-472.

Osmond, B., Ananyev, G., Berry, J.A. et al. (2004). Changing the way we think about Global Change research: Scaling up in experimental ecosystem science. *Global Change Biology* **10,** 1-16.

Owens, M.K. and Schreiber, M.C. (1992). Seasonal gas-exchange characteristics of 2 evergreen trees in a semiarid environment. *Photosynthetica* **26,** 389-398.

Pegoraro, E., Rey, A., Greenberg, J. et al. (2004a). Effect of drought on isoprene emission rates from leaves of *Quercus virginiana* Mill. *Atmospheric Environment* **38**, 6149-6156.

Pegoraro, E., Rey, A., Malhi, Y. et al. (2004b). Effect of CO_2 concentration and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought. *Functional Plant Biology* **31,** 1-11.

Phillips, O.L., Malhi, Y., Higuchi, N. et al. (1998). Changes in the carbon balance of tropical forests: Evidence from long-term plots. *Science* **282,** 439-442.

Poisson, N., Kanakidou, M. and Crutzen, P.J. (2000). Impact of non-methane hydrocarbons on tropospheric chemistry and the oxidizing power of the global troposphere: 3- dimensional modelling results. *Journal of Atmospheric Chemistry* **36,** 157-230.

Poorter, H. (1993). Interspecific variation in the growth-response of plants to an elevated ambient CO_2 concentration. *Vegetatio* **104,** 77-97.

Potvin, C., Lechowicz, M.J. and Tardif, S. (1990). The statistical-analysis of ecophysiological response curves obtained from experiments involving repeated measures. *Ecology* **71,** 1389-1400.

Ramanathan, V. (1988). The greenhouse theory of climate change - a test by an inadvertent global experiment. *Science* **240,** 293-299.

Rapparini, F., Baraldi, R., Miglietta, F. et al. (2004). Isoprenoid emission in trees of *Quercus pubescens* and *Quercus ilex* with lifetime exposure to naturally high CO_2 environment. *Plant, Cell and Environment* **27,** 381-391.

Rascher, U., Bobich, E.G., Lin, G. et al. (2004). Functional diversity of photosynthesis during drought in a model tropical rainforest - the contributions of leaf area, photosynthetic electron transport and stomatal conductance to reduction in net ecosystem carbon exchange. *Plant, Cell and Environment* **27,** 1239-1256.

Rasmussen, R. (1970). Isoprene: identified as a forest-type emission to the atmosphere. *Environmental Science & Technology* **4,** 667-671.

Rasmussen, R. and Khalil, M.A.K. (1988). Isoprene over the Amazon basin. *Journal of Geophysical Research-Atmospheres* **93,** 1417-1421.

Rey, A. and Jarvis, P.G. (1998). Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. *Tree Physiology* **18,** 441-450.

Rinne, H.J.I., Delany, A.C., Greenberg, J.P. et al. (2000). A true eddy accumulation system for trace gas fluxes using disjunct eddy sampling method. *Journal of Geophysical Research-Atmospheres* **105,** 24791-24798.

Rinne, H.J.I., Guenther, A.B., Greenberg, J.P. et al. (2002). Isoprene and monoterpene fluxes measured above Amazonian rainforest and their dependence on light and temperature. *Atmospheric Environment* **36,** 2421-2426.

Rosenstiel, T.N., Potosnak, M.J., Griffin, K.L. et al. (2003). Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. *Nature* **421,** 256-259.

Rosenthal, Y. (1998). Variations of ecosystem gas exchange in the rain forest mesocosm at Biosphere 2 in response to elevated CO_2. *Global Change Biology* **4,** 539-547.

Rosenthal, Y., Farnsworth, B., Romo, F.V.R. et al. (1999). High quality, continuous measurements of CO_2 in Biosphere 2 to assess whole mesocosm carbon cycling. *Ecological Engineering* **13,** 249-262.

Sanadze, G.A., Dzhaini, G.I. and Tevzadze, T.M. (1972). Incorporation into the isoprene molecule of carbon from $C^{13}O_2$ assimilated during photosynthesis. *Plant Physiology* **19,** 17-20.

Schnitzler, J.P., Graus, M., Kreuzwieser, J. et al. (2004). Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. *Plant Physiology* **135,** 152-160.

Schnitzler, J.P., Lehning, A. and Steinbrecher, R. (1997). Seasonal pattern of isoprene synthase activity in *Quercus robur* leaves and its significance for modeling isoprene emission rates. *Botanica Acta* **110,** 240-243.

Scholefield, P.A., Doick, K.J., Herbert, B.M.J. et al. (2004). Impact of rising CO_2 on emissions of volatile organic compounds: isoprene emission from *Phragmites australis* growing at elevated CO_2 in a natural carbon dioxide spring. *Plant, Cell and Environment* **27,** 393-401.

Scott, H.J. (1999). Characteristics of soils in the tropical rainforest biome of Biosphere 2 after 3 years. *Ecological Engineering* **13,** 95-106.

Sharkey, T.D. and Loreto, F. (1993). Water-stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia* **95,** 328-333.

Sharkey, T.D., Loreto, F. and Delwiche, C.F. (1991). High-carbon dioxide and sun shade effects on isoprene emission from oak and aspen tree leaves. *Plant, Cell and Environment* **14,** 333-338.

Sharkey, T.D. and Singsaas, E.L. (1995). Why plants emit isoprene. *Nature* **374,** 769.

Sharkey, T.D., Singsaas, E.L., Lerdau, M.T. et al. (1999). Weather effects on isoprene emission capacity and applications in emissions algorithms. *Ecological Applications* **9,** 1132-1137.

Sharkey, T.D. and Yeh, S.S. (2001). Isoprene emission from plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **52,** 407-436.

Silver, G.M. and Fall, R.R. (1991). Enzymatic-synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts. *Plant Physiology* **97,** 1588-1591.

Singsaas, E.L. (2000). Terpenes and the thermotolerance of photosynthesis. *New Phytologist* **146,** 1-2.

Singsaas, E.L., Lerdau, M., Winter, K. et al. (1997). Isoprene increases thermotolerance of isoprene-emitting species. *Plant Physiology* **115,** 1413-1420.

Singsaas, E. L. and Sharkey, T. (1998). The regulation of isoprene emission responses to rapid leaf temperature fluctuation. *Plant, Cell and Environment* **21**, 1181-1188.

Singsaas, E.L. and Sharkey, T.D. (2000). The effects of high temperature on isoprene synthesis in oak leaves. *Plant, Cell and Environment* **23,** 751-757.

Tans, P.P., Conway, T.J. and Nakazawa, T. (1989). Latitudinal distribution of the sources and sinks of atmospheric carbon dioxide derived from surface observations and an atmospheric transport model. *Journal of Geophysical Research-Atmosphere* **94,** 5151-5172.

Tingey, D.T., Evans, R.C., Bates, E.H. et al. (1987). Isoprene emissions and photosynthesis in 3 ferns - the influence of light and temperature. *Physiologia Plantarum* **69,** 609-616.

Tingey, D.T., Evans, R.C. and Gumpertz, M.L. (1981). Effects of environmental conditions on isoprene emission from live oak. *Planta* **152,** 565-570.

Tingey, D.T., McVeety, B.D., Waschmann, R. et al. (1996). A versatile sun-lit controlled-environment facility for studying plant and soil processes. *Journal of Environmental Quality* **25,** 614-625.

Torbert, H.A. and Johnson, H.B. (2001). Soil of the intensive agriculture biome of Biosphere 2. *Journal of Soil and Water Conservation* **56,** 4-11.

Trainer, M., Hsie, E.Y., McKeen, S.A. et al. (1987). Impact of natural hydrocarbons on hydroxyl and peroxy-radicals at a remote site. *Journal of Geophysical Research-Atmospheres* **92,** 11879-11894.

Trenberth, K.E. and Hoar, T.J. (1997). El Niño and climate change. *Geophysical Research Letters* **24,** 3057-3060.

Tubiello, F.N., Lin, G., Druitt, J.W. et al. (1999). Ecosystem-level evapotranspiration and water-use efficiency in the desert biome of Biosphere 2. *Ecological Engineering* **13,** 263-271.

Turner, D.P., Baglio, J.V., Wones, A.G. et al. (1991). Climate change and isoprene emissions from vegetation. *Chemosphere* **23,** 37-56.

van Ginkel, C.G., Dejong, E., Tilanus, J.W.R. et al. (1987). Microbial oxidation of isoprene, a biogenic foliage volatile and of 1,3-butadiene, an anthropogenic gas. *FEMS Microbiology Ecology* **45,** 275-279.

Vlieg, J.E.T.V., Kingma, J., Kruizinga, W. et al. (1999). Purification of a glutathione S-transferase and a glutathione conjugate-specific dehydrogenase involved in isoprene metabolism in *Rhodococcus* sp. strain AD45. *Journal of Bacteriology* **181,** 2094-2101.

Walter, A. and Lambrecht, S.C. (2004). Biosphere 2 Center as a unique tool for environmental studies. *Journal of Environmental Monitoring* **6,** 267-277.

Warneke, C., Holzinger, R., Hansel, A. et al. (2001). Isoprene and its oxidation products methyl vinyl ketone, methacrolein, and isoprene related peroxides measured online over the tropical rain forest of Surinam in March 1998. *Journal of Atmospheric Chemistry* **38,** 167-185.

Watson R.T., Zinyowerea M.C., Moss R.H. (1996) 'Climate Change 1995. Impacts, adaptations and mitigation of climate change: scientific-technical analyses. Contribution of working group II to the second assesment report of the Intergoveramental Panel on Climate Change.' (Cambridge University Press: Cambridge)

Went, F. (1960). Blue haze in the atmosphere. *Nature* **187,** 641-643.

White, M.A., Running, S.W. and Thornton, P.E. (1999). The impact of growing-season length variability on carbon assimilation and evapotranspiration over 88 years in the eastern US deciduous forest. *International Journal of Biometeorology* **42,** 139-145.

Wildermuth, M.C. and Fall, R.R. (1996). Light-dependent isoprene emission - Characterization of a thylakoid-bound isoprene synthase in *Salix discolor* chloroplasts. *Plant Physiology* **112,** 171-182.

Will, R.E. and Teskey, R.O. (1997). Effect of irradiance and vapour pressure deficit on stomatal response to CO_2 enrichment of four tree species. *Journal of Experimental Botany* **48,** 2095-2102.

Wofsy, S.C. (1976). Interactions of CH_4 and CO in the Earth's atmosphere. *Annual Review of Earth Planet Science* **4,** 441-469.

Zabel, B., Hawes, P., Stuart, H. et al. (1999). Construction and engineering of a created environment: Overview of the Biosphere 2 closed system. *Ecological Engineering* **13,** 43-63.

Zimmerman, P.R., Greenberg, J.P. and Westberg, C.E. (1988). Measurements of atmospheric hydrocarbons and biogenic emission fluxes in the Amazon boundary layer. *Journal of Geophysical Research-Atmospheres* **93,** 1407-1416.