Chiral monoterpenes reveal forest emission mechanisms and drought responses

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Abstract

Monoterpenes exist in mirror image forms called enantiomers, but their individual formation pathways in plants and ecological functions are poorly understood, as enantiomers are usually measured and modelled together. Here we present enantiomerically separated atmospheric monoterpene and isoprene data from an enclosed tropical rainforest ecosystem without photo-chemistry during a four-month controlled drought and rewetting experiment. Surprisingly, the enantiomers showed distinct diel emission peaks, which responded differently to progressive drying. Isotopic labelling established that vegetation emitted (-)- α -pinene mainly *de novo* while (+)- α -pinene was emitted from storage pools. As drought stress increased, (-)- α -pinene emissions shifted to storage pools, which are released later in the day, favouring cloud formation. The α -pinene enantiomers each correlated better with other monoterpenes than with each other, indicating different enzymatic controls. These results show that enantiomeric distribution is key to understanding the processes driving monoterpene emission from ecosystems and to predicting atmospheric feedbacks in response to climate change.

Introduction

Monoterpenes ($C_{10}H_{16}$) are emitted in large quantities by vegetation to the atmosphere ($>100 \text{ Tg C yr}^{-1}$), where they react with hydroxyl radicals and ozone to form new particles and hence clouds, impacting the Earth's radiative budget and climate change¹⁻³. Little attention has been given to their different chiral forms ((+) and (-)), as both monoterpene enantiomers have identical physical properties and rates of reaction with OH and O_3 ⁴; therefore, most atmospheric field and modelling studies do not differentiate them. However, this implicitly assumes that the sources and sinks of both enantiomers are identical, even though the individual enantiomer production pathways and drivers are uncertain. Recent forest measurements showed unequal (non-racemic) concentrations of enantiomers that sometimes do not even correlate with each other^{5,6}, indicating distinct source mechanisms. Although some reports suggest that the biosynthesis of enantiomers is homogenous throughout an individual plant⁶, leaves, bark and soil litter within a homogenous forest have distinct chiral signatures⁷, strongly suggesting that the emission and removal processes of these chiral species (and hence monoterpenes generally) are not adequately understood.

Isoprene emission is better understood than monoterpene emission, with generally precise model-prediction and measurement agreement^{8,9}. Isoprene synthesis occurs by the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, where photosynthetically assimilated CO_2 is converted to the isoprene precursor, isopentenyl diphosphate, and directly emitted from the leaf (*de novo* emission)⁸. Monoterpene synthesis also occurs by the MEP pathway, but some monoterpenes are synthesized by the mevalonate pathway. Both pathways result in the production of isopentenyl diphosphate, which combines with its isomer, dimethylallyl diphosphate, to form the common monoterpene precursor geranyl diphosphate (GPP)^{10,11}. Enzymes known as terpene synthases transform GPP into an array of monoterpenes such that chiral monoterpenes produced by a particular enzyme are typically in one chiral form, (-) or (+)^{12,13}.

Monoterpenes can be emitted by *de novo* emission, or released from storage pools, thus, decoupled from time of biosynthesis. Broad leaf plant species typical of the tropics usually store monoterpenes non-specifically throughout the leaves, mainly in the lipid phase but also a small amount in the aqueous phase within the leaf^{14,15}. The processes regulating monoterpene production and potential storage likely determine the plant's overall chiral emission signature, and it is unclear how these will change in response to extreme climate events such as drought. Droughts are expected to become more frequent throughout the 21st century¹⁶, causing disruptions to the functioning of ecosystems¹⁷ and emissions of volatile organic compound from forests¹⁸. Reported monoterpene emission responses to drought are highly variable and dependent on the individual plant, making empirically based achiral emission inventories intractable¹⁹⁻²³. However, since chiral compounds link directly to the underlying enzymatically driven processes they could form the basis of an improved emission scheme.

We separated and measured the enantiomers for α -pinene, camphene and limonene as well as measuring (-)- β -pinene, γ -terpinene, and isoprene at hourly intervals over almost four months within the enclosed Biosphere 2 Tropical Rain Forest (B2-TRF) by on-line gas chromatography-mass spectrometry (GC-MS) (**Fig. 1**). Trans- β -ocimene and β -myrcene were also detected but could not be resolved using the method for the online GC-MS. The B2-TRF was continually flushed with outside air and all incident light wavelengths below 385 nm were filtered by the internal mylar sheet within the surrounding glass panels²⁴. After three weeks of ambient measurements to establish the normal pre-drought condition (day of year (doy) 252–280), a 9.5-week drought was imposed to cause physiological change and drive differential responses in the biochemical processes (doy 281-337). The subsequent drought stage developed two phases (mild and severe drought), with respect to two relative humidity minima, soil moisture decline, and vegetation response. At the end of the drought, water was added to the deep soil for 12 days (doy 337-346), followed by “rain” delivered from overhead sprinklers (doy 347-356). Isotopically labelled $^{13}\text{CO}_2$ was twice added to the enclosed atmosphere during pre-drought and severe drought, enabling differentiation between *de novo* and storage pool emissions using an off-line GC-isotope ratio MS (GC-IRMS). ^{13}C labelling combined with long-term atmospheric flux monitoring allowed us to precisely determine how drought impacts fluxes and sources of distinct monoterpene enantiomers.

Results

Distinct trends of enantiomers

Total monoterpenes (consisting of (-)- α -pinene, (+)- α -pinene, (-)- β -pinene, (-)-limonene, (+)-limonene, (-)-camphene, (+)-camphene, and γ -terpinene) stayed relatively constant during pre-drought but peaked after 33 days (in Early Drought) and again, more strongly, after 66 days (in Severe Drought) (**Fig. 2a**). During deep-water rewet, when water was reintroduced to the lowest soil levels, the total monoterpene concentrations started to decrease. This decrease continued after rain (rain-rewet), but did not quite recover to pre-drought levels by the end of the measurement period. The same pattern of two concentration peaks (corresponding to mild and severe drought periods) was also observed when the

enantiomeric monoterpenes were separated; however, the enantiomer peak sizes showed strongly contrasting behaviour. Daytime concentrations of (-)- α -pinene were higher in the mild drought, whereas for (-)- β -pinene the severe drought concentrations were 10 times that of the mild (**Fig. 2b**). The (+)- α -pinene peak concentrations were approximately equal in both mild and severe stress conditions. For nighttime concentrations (-)- α -pinene, (+)- α -pinene, and (-)- β -pinene all showed higher concentrations during severe drought (**Fig. 2c**). After rewetting by rain, nighttime values of (-)- β -pinene, (-) and (+)- α -pinene all returned to pre-drought levels (**Fig. 2c**). Although daytime concentrations of the enantiomers also all decreased from their severe drought maxima they did not reach pre-drought levels. Another clear contrast in behaviour was that the enantiomers correlated well during nighttime, while during daytime they exhibited independent patterns (when *de novo* emissions were important) (**Ext. Data Fig. 1**). Strikingly, the current monoterpene emission model-based expectation that drought would elicit equivalent responses in (-) and (+)- α -pinene was not true.

Although (-)- α -pinene consistently dominated the total monoterpene emissions, (-)- β -pinene overtook (+)- α -pinene to become the second most abundant monoterpene during severe drought (**Fig. 2e**). Thus, the ratio of (+)- α -pinene to (-)- β -pinene could be used as a proxy of drought severity in this experiment. It should be noted that fluxes of monoterpenes from the soil did not affect these enantiomeric ratios as samples taken periodically throughout the experiment showed that the soil maintained a modest steady uptake of enantiomeric monoterpenes throughout (**Ext. Data Fig. 2**).

These responses in monoterpenes were in contrast to isoprene. During pre-drought, isoprene in the tree canopies increased by a factor of 3 over 26 days, reaching average concentrations of ~ 300 ppb (**Fig. 2a**), likely because the top soil moisture (5 cm SM) decreased from 35% to 26% and the strong soil uptake of isoprene weakened before the drought started^{25,26}. By the severe drought period the top soil moisture had decreased from $\sim 28\%$ to 15%, whilst average isoprene concentrations decreased and plateaued at ~ 100 ppb, equivalent to initial pre-drought values. This means that in the severe drought the monoterpene to isoprene ratio was significantly higher than under mild drought.

Distinct enantiomer emission sources

On two days during the experiment (pre-drought and severe drought), CO_2 labelled with the heavy ^{13}C isotope ($^{13}\text{CO}_2$) was introduced into the B2-TRF atmosphere to distinguish between *de novo* and storage type monoterpene emissions (**Fig. 3**). For (-)- α -pinene, the emissions became more enriched in ^{13}C during both pulses. Atmospheric samples taken post-pulse show that, on average, the baseline $\epsilon^{13}\text{C}$ values of (-)- α -pinene did not decline rapidly to pre-pulse values. This shows that (-)- α -pinene emissions are predominately *de novo* but that a small fraction also enters the storage pools from which it is emitted after the labelled CO_2 is flushed from the TRF. In contrast, no significant incorporation of ^{13}C was observed for (+)- α -pinene and (+)-limonene, indicating that these enantiomers were generated separately and emitted primarily from storage pools²⁷. Therefore, the daytime increases of these monoterpenes during drought (**Fig. 2b**) comes from an increase in storage pool emissions.

The observed increases in $\epsilon^{13}\text{C}$ values clearly indicate that fresh photosynthetic carbon was directly used to produce (-)- α -pinene, trans- β -ocimene and β -myrcene. The incorporation of ^{13}C by (-)- α -pinene, trans- β -ocimene and β -myrcene increased during the second pulse, likely because more freshly assimilated carbon is used for the production of these specific compounds. Trans- β -ocimene and β -myrcene are important emissions since they react quicker with harmful reactive oxygen species than other monoterpenes. This agrees with measurements from the Amazon rainforest, where emissions of ocimene and β -myrcene increased from heat stressed leaves²⁸.

Distinct diel cycles of enantiomers

Two distinct types of diel cycle were observed during pre-drought. One, followed by (-)- α -pinene and (-)- β -pinene, was aligned to photosynthetically active radiation (PAR) and assimilation rate (A); while the second, followed by (+)- α -pinene and (+)-limonene, was aligned to atmospheric water potential (AWP) and temperature. Current atmospheric models predict all monoterpene emissions as a function of temperature and light and therefore would erroneously place peak monoterpene emission midway between the real peaks, and be unable to reproduce the drought induced changes revealed by resolving enantiomers²⁹.

During pre- and mild drought, (-)- α -pinene and (-)- β -pinene peaked with maximum assimilation rate (A) between 11:00 and 12:00. In contrast, (+)- α -pinene and (+)-limonene peaked between 14:00 and 15:00, coincident with maximum temperature and atmospheric water potential (**Fig. 4c**). With the transition into severe drought, the (-)- α -pinene and (-)- β -pinene diel cycles shifted progressively later in the day, merging with the diel cycles of (+)- α -pinene in the afternoon, while the assimilation rate (A) declined (less carbon uptake by the vegetation) and atmospheric water potential (AWP) and PAR decreased. With rewetting the assimilation rate began to recover (increased carbon uptake), concurrent with a shift in peak (-)- α -pinene and (-)- β -pinene from 14:00 back to 12:00. Hence, the shift of the daily maximum of (-)- α -pinene to the afternoon with progressive drying potentially suggests that the emissions are less *de novo* and more storage pool in character (**Fig. 4a**). Since the diel cycles of (-)- β -pinene followed the same temporal pattern as (-)- α -pinene, it is likely that (-)- β -pinene also transitioned from *de novo* emission to storage pool emission.

Monoterpenes are generally stored in the lipid phase rather than the aqueous phase within the leaf^{14,15}. Monoterpenes are relatively water insoluble and partition rapidly between the aqueous and gas phase, according to their Henry's constant³⁰. The aqueous phase storage is small and empties quickly in the morning when the stomata open and water is lost from the leaf to the atmosphere therefore any monoterpene emissions from the aqueous phase are likely to be negligible¹⁴. Monoterpenes possess large octanol/water partition coefficients (K_{ow}) (≈ 20000 – 30000), meaning they can be stored in relatively large quantities in the lipid phase from where they are more slowly emitted to the atmosphere¹⁴. A plausible explanation of the emission behaviour is that throughout the measurement period, (+)- α -pinene and (+)-limonene were stored in the lipid phase, leaking slowly into the atmosphere peaking later

in the day than the *de novo* emissions. As photosynthesis decreased and biomass degradation increased, the emission of (-)- α -pinene and (-)- β -pinene transitioned from mainly *de novo* synthesis to emission from storage pools.

By grouping enzymes, which share similar attributes (i.e. light and temperature dependency, major monoterpene product), a three enzyme group – two reservoir model can explain the observed isoprene and monoterpene emissions (**Ext. Data Fig. 4**). Isoprene is generated by light activated isoprene synthase (enzyme group 1) and (-)- α -pinene and (-)- β -pinene are generated by light activated enzyme group 2 and emitted directly (*de novo*). However, both (-)- α -pinene and (-)- β -pinene are also partitioned to the lipid phase where they are released upon drought stress. Enzyme group 3 is responsible for synthesizing (+)- α -pinene and (+)-limonene continuously without light activation, which also partition to the lipid phase.

The changes in ecosystem emissions in relation to potential particle formation and growth are important since increased condensation nuclei (CCN) production efficiency, cloud formation and subsequent rain would represent a possible negative biosphere-atmosphere feedback to the drought. In early drought *de novo* monoterpene emissions increase. High fluxes of reactive isoprene will act to suppress OH radical levels above the canopy allowing emissions to reach higher, cooler altitudes, where the newly formed aerosols may invigorate convection³¹. In severe drought, isoprene emissions, which do not produce particles or extremely low volatile organic compounds (ELVOC) efficiently, decrease, whereas monoterpene emissions increase further. Among the monoterpenes, the most prolific increase is of α -pinene, which oxidizes to ELVOC and particles with high yield. The results presented here are therefore consistent with the negative feedback mechanism hypothesized above. Furthermore, the shift in monoterpene emissions during drought to later in the afternoon means they are released in periods when higher sensible heat flux and turbulence which would facilitate the transport of monoterpene rich air parcels to the levels of cloud formation.

The emission characteristics of enantiomers were deciphered because the B2-TRF provides a unique venue to conduct a drought under controlled conditions, with comprehensive biological measurements, in addition to atmospheric isotopic labelling in an environment of greatly reduced atmospheric chemistry. These results show that the degree of drought stress in a tropical rainforest can be gauged by either the afternoon-to-morning ratios of (-)- α -pinene or by the fractional contribution of (-)- β -pinene to the sum of monoterpenes, which almost tripled from pre- to severe drought. Unexpectedly, the (-) enantiomers from different monoterpenes exhibited the same diel behaviour whereas opposite enantiomers from the same monoterpenes exhibited different diel cycles. It is remarkable that the *de novo* and storage pool sources of monoterpene emissions from an ecosystem, and the carbon cycling changes in response to drought, can be assessed externally from air measurements provided that individual enantiomers are considered. This underlines that a new enantiomerically specific approach to emission modelling, based on the enzyme group model proposed here, would be more accurate and biologically founded than current approaches³², in particular with regards to a possible feedback between emission composition and particle production efficiency. We conclude that enantiomerically resolved monoterpenes are required to

accurately assess how key tropical regions, such as the Amazon rainforest, emit monoterpenes and respond to the predicted increase in future extreme drought events.

Declarations

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Author contributions

JB had the idea, performed the on-line GC-MS sampling, analysed the data and wrote the paper. JK performed the off-line GC-IRMS laboratory analysis and calculated the $\epsilon^{13}\text{C}$ values. GP planned and performed the atmospheric cartridge sampling. JvH calculated the assimilation rate and atmospheric water potential data, provided the meteorological data and helped conduct the drought experiment. NL, LM and CW supervised and planned the project, performed the drought experiment and $^{13}\text{CO}_2$ labelling experiment and commented the paper. JW had the idea, supervised the project and wrote the paper. All authors contributed to writing and editing the manuscript.

Additional Information

Supplementary Information is available for this paper.

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Methods

Tropical rainforest mesocosm (TRF)

The tropical rainforest mesocosm at the Biosphere 2 covers ca. 1950 m² and is representative of a managed tropical rainforest ecosystem similar to those found in South America. It is contained under a 26700 m³ glass ziggurat enclosure²⁵ (**Fig. 1**). Within the enclosure, there are 95 species of tropical plants, including 23 species of trees and 67 species of understory plants. The most dominant species are *Clitoria fairchildiana*, *Phytolacca dioica*, *Arenga pinnata*, *Ficus benjamina*, *Syngonium podophyllum*, *Piper sp.*, *Musa sp.* and *Pachira aquatica*. To recreate real rainforest conditions, such as low light intensity beneath the canopy, larger trees and understory plants (*Musa sp.*, *Piper sp.*, *Hibiscus rosa-sinensis*) were planted around the edges of the glass enclosure to block out direct sunlight. The soil profile consists of an up to 4 m deep subsoil layer and a topsoil layer of variable thickness (70-100 cm)²⁶. Located at the centre of the rainforest is a small artificial “mountain” within which a laboratory was constructed to house the on-line GC-MS and where the ambient temperature and humidity inside could be controlled.

Drought experiment

This experiment was conducted during the Water, Atmosphere, and Life Dynamics (WALD) campaign. Prior to the commencement of the drought period, the tropical rainforest was wetted from above with a sprinkler system to simulate rainfall, using ~20,000 liters of water 3 times a week. After watering on 7th October 2019 the rainforest biome was left to dry. During some stages air handler units were employed for the removal of humidity by condensation, otherwise the rainforest biome was left to dry naturally, until 3rd December 2019. The first water to the rainforest was introduced at the bottom through a network of drainage pipes under the soil on top of the concrete and steel structure underlying Biosphere 2. The

rainforest was again watered from above using the sprinkler system, on 12th December 2019, 19th December 2019, and every 2 days afterwards. The rainforest temperature was controlled throughout the experiment and the temperature at 13 m was on average between 28 and 32 °C during daylight hours and between 21 and 24 °C during nighttime hours.

The photosynthetically active radiation (PAR), temperature and relative humidity (RH) were recorded every 15 minutes using sensors connected to a datalogger (PAR sensors (Apogee SQ110, Campbell Scientific, Logan, UT, USA), temperature and RH with Vaisala HMP 45c sensors (Vaisala Oyi., Vantaa, Finland; purchased through Campbell Scientific). Sensors reported every 15 minutes to a centralized CR1000 datalogger (Campbell Scientific, Logan, UT, USA) with an AM16/32B multiplexer (Campbell Scientific, Logan, UT, USA). The dataloggers were connected to a centralized database with NL100 communications modules (Campbell Scientific, Logan, UT, USA) and the data is available through the Biosphere 2 website (www.biosphere2.org/data-models/rainforest-data). The PAR sensor was located at 13 m on the central measurement tower and the humidity and temperature sensors were located at a height of 13 m on the north-eastern measurement tower together with the sampling inlet. The soil moisture data presented in **Fig. 2d** is an average of measurements that were recorded every 15 minutes from four different soil pits (soil moisture and temperature sensors (Truebner SMT100, Truebner GmbH, Neustadt, Germany) and water potential sensors (TEROS 21, Meter Group, Pullman, WA, USA) in all four pits at 5 cm depth and at the soil-concrete interface (subsoil bottom)). Since the sensors are 30 mm wide and inserted vertically into the soil with the soil depth indicated at the midpoint, each depth is ± 1.5 cm.

The rainforest enclosure acted as a semi-enclosed system where there was constant air exchange with the outside environment. The air-exchange rate from the tropical rainforest enclosure was measured using sulfur hexafluoride (SF₆) at low ppb levels as a tracer gas, as it is completely anthropogenic and its concentration is < 10 ppt in background air and therefore it is only be affected by leakage and flushing. More information on using SF₆ as a tracer gas in the tropical rainforest mesocosm at the Biosphere 2 can be found elsewhere²⁵. Once the percentage exchange rate of SF₆ was obtained and interpolated, the measured data was corrected by using the equation

$$VMR_c = VMR_u + (VMR_u * ER)$$

Where the VMR_u was the uncorrected data, ER was the exchange rate percentage and VMR_c was the corrected data. The incoming VMR of all VOC's were assumed negligible.

Determination of isoprene and monoterpene ambient mixing ratios

From 9th September to 23rd December 2019, the ambient air from 13 m high within Biosphere 2 rainforest was continuously drawn at a flow of approximately 800 ml min⁻¹ through a main Teflon inlet line which comprised of 37 m of 0.625 cm (¼ ") Teflon tubing. 13 m was chosen as the sampling height as this was the height within the enclosure that had the greatest leaf area index. The main inlet line was fitted with a

(Cole Palmer, EW-02915-31) filter. After approximately 26 m, a t-piece was connected to the main inlet line which was connected to a thermal desorption unit (TD) (TT247-xr, MARKES International Ltd., U.K.) using 7 m of $\phi = 3.175$ mm (1/8 ") Teflon tubing. All sampling lines were insulated and heated to 50 °C to avoid water condensation within the lines. The line to the TD was continuously purged to avoid the sampling of a dead volume with a pump situated behind the TD in the flow path. During sampling, the pump behind the TD drew air from the main inlet line at flows ranging between 70 and 200 ml min⁻¹ for 10 minutes. The collected air was sampled first through a water condenser (kori-xr, MARKES International Ltd., U.K.). This allowed for the removal of water whilst leaving the target VOCs unchanged. The dehumidified sample was then pre-concentrated onto a cold injection trap at 30 °C (Material emissions, MARKES International Ltd., U.K.). After sampling, the injection trap was purged for 1 minute with helium with a flow of 50 ml min⁻¹ before being rapidly heated to 300 °C and desorbed for 3 minutes. The sample was removed from the cold trap with a Helium flow of 3 ml min⁻¹ including a split flow of 2 ml min⁻¹ and injected into the separating column.

The rainforest ambient air was analysed using a Gas Chromatograph (GC) (6890A, Agilent Technologies, U.K.). The carrier gas used was research 6.0 grade helium (Airgas®, USA.) Separation of the sampled compounds was achieved using a 30 m β -DEX™ 120 column (Sigma-Aldrich GmbH, Germany) with 0.25 mm internal diameter and a 0.25 μ m film thickness. The temperature program used was as follows, 40 °C for 5 minutes then 40 °C to 150 °C at 4 °C min⁻¹ and 150 °C to 200 °C at 30 °C min⁻¹. The column flow was set to 1 ml min⁻¹.

The GC was coupled with a quadrupole mass spectrometer (MS) (5973N, Agilent Technologies, U.K.), operated in selected ion mode for the identification of mass ions 68, 69, 93, 94, 119, 120, 136, 137 each with a dwell time of 60 ms.

The identification of the target compounds was achieved by first operating the MS in scan mode to obtain full mass spectra to be able to compare with the NIST 70 eV electron ionization library. For further confirmation, a gas standard mixture (Apel-Riemer Environmental Inc., 2019) containing the target compounds was injected into the GC-MS system. The same gas standard mixture was also injected onto sorbent cartridges and subsequently desorbed into a GC - time of flight - mass spectrometer (GC-TOF-MS) operated with identical conditions to the online GC-MS. Using liquid standards, the headspace of the individual compounds was taken onto sorbent cartridges and also desorbed into the GC-TOF-MS. The retention times from the chromatograms of the individual compounds was then crosschecked with the chromatogram of the gas standard mixture.

The MS was tuned on a weekly basis and the linearity was checked throughout the campaign. The gas standard mixture was injected into the system after each tuning and after 10 samples were analysed. Routine calibrations were performed by initially flushing the TD system with the gas standard mixture at a flow of 20 ml min⁻¹ for 2 minutes in order to remove the dead volume. The calibration gas was then injected with a flow of 20 ml min⁻¹ for 5 minutes directly onto the cold injection trap within the TD. The calibration gas sample was then treated with the same TD-GC-MS parameters as the routine sampling.

This step was repeated three times before sampling continued. The MS responses to the injected calibration gas samples were then plotted against the time since the last MS tune to track the MS sensitivity drop, which allowed for the correction and calibration of the raw data. To check the linearity, the same procedure was used with the calibration gas injection time being increased in stepwise intervals of 2.5 minutes from 0.5 to 12.5 minutes.

Data management

The highest individual VMRs measured were in excess of 3 ppb for (-)-alpha-pinene and 400 ppt for (+)-alpha-pinene were measured during ED (**Ext. Data Fig. 5 and 6**). However, to evaluate the general trends of all measured compounds through each stage, the hourly total monoterpene and isoprene data was smoothed by applying a Savitzky-Golay filter to keep long-term trends whilst removing short-term fluctuations. The dataset was further split into daytime and nighttime, using photosynthetically active radiation (PAR) data collected at the point of measurement. The smooth function (MATLAB) was then used to suppress noise in the trend line for each compound and the uncertainties were propagated using the same functions. To obtain average diel cycles for each compound, and the average composition all data in each of the five stages of the campaign were taken. A moving median calculation with a window length of 5 data points was applied to each group. The diel cycles were averaged over 435, 526, 349, 136 and 193 data points for PD, ED, SD, DRW and RRW respectively. β -Myrcene, (+)- β -pinene, α -terpinene and terpinolene, were also observed but not included as they amounted to an average of less than 5 % of the average total monoterpene for the entire measurement period. Ocimene was also observed but not calibrated with the on-line GC-MS system.

The atmospheric water potential was calculated from the Vaisala temperature and relative humidity measurements according to:

$$\psi \text{ (Pa)} = -\frac{RT}{V_w^0} \ln\left(\frac{\%RH}{100}\right)$$

where Ψ is the water potential (in Pa), R the gas constant ($8.3144 \text{ J mol}^{-1} \text{ K}^{-1}$), T the temperature ($^{\circ}\text{C}$), V_w^0 the molar volume of water at 293 K, and %RH is the relative humidity (%)³³. Zone 3 and 4 are the two height zones that contain the majority of the canopy in the Biosphere 2 rainforest (**Ext. Data Fig. 3**). The environmental conditions were averaged over all the sensors that were located within these zones. NEE was calculated every 15 minutes based on the change in moles of CO_2 within the rainforest ecosystem and the amount of CO_2 lost or gained with the air-exchange with the outside:

$$NEE = \frac{((CO_2^t - CO_2^{t-1}) + (CO_2^{27m} - CO_2^{Outside}) * ER)}{Area}$$

where CO_2^t , CO_2^{t-1} , CO_2^{27m} , and $CO_2^{outside}$ are the moles of CO_2 at the time calculated, previous time-step, 27m or top of the rainforest where the air flows out, and the outside air coming into the rainforest. Area stands for the soil surface area of the rainforest. The moles of CO_2 were calculated based on the ideal gas law and the CO_2 concentration:

$$\text{moles of } CO_2 = \frac{V * P}{(273.15 + T_{Ave}) * R} * \frac{[CO_2]}{10^6}$$

where V denotes the representative volume for the CO_2 measurement (either the rainforest volume fraction or the volume of air exchanged), P denotes the pressure (measured inside and outside the Biosphere 2 rainforest using WeatherHawk WXT 530, Vaisala Oyi., Vantaa, Finland), T_{Ave} denotes the average air temperature for the measurement zone or the outside air temperature (measured using HMP45c temperature and humidity sensors (Vaisala Oyi., Vantuu, Finland), R denotes the gas constant, and $[CO_2]$ denotes the CO_2 concentration measured inside Biosphere 2 with GMP343 CO_2 sensors (Vaisala Oyi., Vantuu, Finland) and outside Biosphere 2 with GMP220 sensors (Vaisala Oyi., Vantuu, Finland) and inside the air inflow with an Aerodyne Dual QCL (Aerodyne Research Inc., Billerica, MA, USA). The ecosystem assimilation (A , $mmoles\ m^{-2}\ s^{-1}$) was calculated from the Net Ecosystem Exchange (NEE) and assuming that the nighttime respiration (R) was representative for the daytime respiration (a reasonable assumption in tropical forest ecosystems^{34,35}):

$$A = NEE - R$$

$^{13}CO_2$ pulse labelling experiment

The $^{13}CO_2$ pulses were carried out on 5th October at 8:00 am (MST) and 23rd November 2019 at 9:00 am (MST) to coincide with peak photosynthetic activity³⁶. A deliberate effort was made to proceed with the pulse experiments on days with high amounts of direct sunlight, when there would be a high rate of photosynthesis, to maximize CO_2 uptake. During the first pulse, the rainforest was fumigated with 10 lpm of 99% $^{13}CO_2$ (Millipore Sigma, Burlington, MA, USA) for 15 minutes. In order to balance reduced carbon assimilation rates during the drought, 20 lpm of 99% $^{13}CO_2$ was released over 15 minutes during the second pulse. The $\delta^{13}C$ value of atmospheric CO_2 within the rainforest was monitored throughout each pulse using a Tunable Infrared Laser Direct Absorption Spectrometer (TILDAS, Aerodyne Research, Billerica, MA, U.S.A). After 4 hours during the first pulse, and 5.2 hours during the second pulse, the flow of air through the rainforest was increased and, at midday, windows were temporarily removed from the enclosure of the B2-TRF and excess $^{13}CO_2$ was ventilated to the outside air, so that the entry of ^{13}C into the mesocosm could be more accurately traced back to a fixed point in time.

Determining enantiomer ^{13}C isotope ratios

Pairs of monoterpene enantiomers abundant in the air were analysed in the TRF ecosystem in a height of 13m at the atmosphere tower. 5, 16, 36, 11 and 14 glass cartridges were sampled during pre-pulse, first pulse, first post-pulse, second pulse and second post pulse respectively. For terpene accumulation, ambient air was drawn through glass cartridges filled with about 100 mg Tenax (Sigma, Germany) as an adsorbent at a controlled flow rate of 200 ml min⁻¹ for 90 min using a handheld pump (SKC Ltd., Dorset, U.K). Glass cartridges were kept at 4 °C until analysis. The samples were analyzed at the university of Freiburg on a system consisting of a gas chromatograph (GC 7980, Agilent Technologies, Germany) coupled to a mass selective detector (MSD 5975C, Agilent Technologies, Germany) and equipped with a thermodesorption unit (TDU, Gerstel, Germany) and a cold injection system (CIS, Gerstel, Germany). For analysis of ¹³C isotope ratios, this system was coupled to an isotope ratio mass spectrometer (IRMS, Isoprime precisiON, Elementar Analysensysteme GmbH, Langenselbold, Germany) via a combustion furnace (GC 5 interface, Elementar Analysensysteme GmbH, Langenselbold, Germany). For analysis, air sampling glass cartridges were heated to 220 °C for 5 min to thermodesorb terpenes and channel them into the CIS which was kept at -70 °C. By heating the CIS to 240 °C for 3 min, terpenes were directed onto the GC separation column (BetaDex 120 Chirality, 60 m x 250 µm x 0.25 µm, Supelco, USA) with a He stream of 1 ml min⁻¹. The oven program started at 45 °C which was kept for 1 min, temperature was then stepwise increased to 60 °C, 150 °C and 210 °C at rates of 2 °C min⁻¹, 1 °C min⁻¹ and 3.5 °C min⁻¹, respectively. The eluate was split and ca. 10 % was directed into the MSD for terpene identification and quantification. For this purpose, the MSD was run in SIM mode detecting m/z 68, 93, 119 and 136. The remainder eluate passed the combustion furnace where at a temperature of 850 °C the terpenes were oxidized to form CO₂ and H₂O. After elimination the H₂O by a Nafion water trap, the ¹³C/¹²C ratios of the CO₂ were measured by the IRMS.

Statistical information

The t-tests used on the data presented in **Fig. 3**, were one-tailed, two sample unequal variance t-tests that were performed using the MATLAB R2017B software (**Ext. Data Table 1**).

GC-IRMS data processing

¹³C isotopologues elute slightly faster from the GC than their ¹²C counterparts, meaning that δ¹³C values are not homogenous across the peak³⁷. For chromatographically unresolved compounds such as (-)-α-pinene (**extended data figure 6**), integrating from the beginning of the peak to the trough between it and the subsequent unidentified co-eluting peak results in δ¹³C values that appear artificially enriched in ¹³C. Absolute δ¹³C values for such compounds cannot be reported. We therefore report relative offsets between measured δ¹³C values during the ¹³CO₂ pulses and ambient conditions

$$\varepsilon^{13}\text{C} = \frac{\left(\frac{^{12}\text{C}}{^{13}\text{C}}\right)_{\text{pulse}}}{\left(\frac{^{12}\text{C}}{^{13}\text{C}}\right)_{\text{ambient}}} - 1$$

While the relative abundance of (-)- α -pinene to its coeluter changes its apparent $\delta^{13}\text{C}$ value, we are confident that these chromatographic effects cannot account for the relative ^{13}C -enrichment of this compound during the $^{13}\text{CO}_2$ pulses for two reasons. First, the ^{13}C -enrichment of the samples from the $^{13}\text{CO}_2$ pulses are significantly enriched relative to the variability in ambient $\delta^{13}\text{C}$ values, which are subject to a greater range of relative peak heights. Second, ^{13}C enrichment is only apparent during the $^{13}\text{CO}_2$ pulses when (-)- α -pinene is integrated in various combinations with the other three peaks in the co-eluting hump, but not when any of these other peaks are individually integrated trough-to-trough (**Ext. Data Fig. 6**).

There is no indication that (+)- α -pinene becomes enriched in ^{13}C during either $^{13}\text{CO}_2$ pulse. However, given its small size and poor resolution from the preceding unlabeled peak, we cannot definitively rule out slight ^{13}C -enrichment for (+)- α -pinene. Myrcene, trans- β -ocimene, and (+)-limonene all have well resolved fronts and poorly resolved tails. These compounds display either high ^{13}C -enrichment during the $^{13}\text{CO}_2$ pulses (trans- β -ocimene during both pulses, myrcene during the second) or no enrichment at all (myrcene during the first pulse, (+)-limonene during both). Since these chromatographically similar peaks only display large ^{13}C -enrichment in some compounds and not others, we consider apparent presence or absence of label uptake to be robust results for these compounds (**Ext. Data Fig. 8 & 9**).

Soil uptake and emission of monoterpenes experiment

For the investigation of how monoterpenes are taken up and emitted by the soil, 3 soil chambers made from polyvinyl chloride were placed on pre-installed soil collars around the B2-TRF (**Ext. Data Fig. 2**). Using sorbent cartridges, samples were taken from the atmosphere, located at the inlet (MR_{atm}) of the soil chamber, and at the same time from the outlet (MR_{soil}). Samples were collected at 200 ml min^{-1} for ~ 10 minutes using a handheld pump (SKC Ltd., Dorset, U.K). The sorbent cartridges were made from inert coated stainless steel (SilcoNert 2000 (SilcoTek™, Germany)). The sorbent consisted of 150 mg of Tenax® TA followed by 150 mg of Carbograph™ 5 TD ($560 \text{ m}^2/\text{g}$). The size of the Carbograph™ particles was in the range of 20-40 mesh. The Carbograph™ 5 was supplied by L.A.R.A s.r.l. (Rome, Italy) and Buchem BV (Apeldoorn, The Netherlands) supplied the Tenax®.

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Figures

Figure 1

a. Schematic of the Biosphere 2 Tropical Rainforest biome. b. A photograph taken from within the biome (Photo J. Byron).

Figure 2

Long-term total monoterpenes (MTs) and isoprene trends throughout the experiment show strongly differing trends for monoterpene enantiomers, especially during daylight hours. Monoterpene and isoprene data is divided into 5 stages, indicated by the grey dashed lines: pre-drought (PD), early drought (ED), severe drought (SD), deep-water rewet (DRW) and rain rewet (RRW). The timing of the $^{13}\text{CO}_2$ pulses is indicated by the black (••) lines. a. Daytime isoprene and total monoterpene volume mixing ratios (VMR). The shaded region around the line represents the absolute measurement uncertainty. b. Daytime (-) and (+) alpha-pinene and other monoterpenes. c. Night-time (-) and (+) alpha-pinene and other monoterpenes. d. soil moisture (SM) and relative humidity (RH). Note different scales for enantiomers. e. Pie charts showing the daytime composition of the enantiomeric monoterpenes during each stage. Note different scales for enantiomers. The shaded region around the line represents the absolute measurement

uncertainty. Other monoterpenes includes (-)-camphene, (+)-camphene, (-)-limonene, (+)-limonene and γ -terpinene.

Figure 3

Carbon sources (de novo or storage) for monoterpene emissions were clearly separated by $\epsilon^{13}\text{C}$ values of monoterpenes and their enantiomers after ^{13}C -enriched CO_2 was added during one morning in pre-drought (1st $^{13}\text{CO}_2$) and severe drought (2nd $^{13}\text{CO}_2$) phases. $^{13}\text{CO}_2$ gas was introduced into the atmosphere so that plants taking up CO_2 and directly producing immediate monoterpene emissions (de novo) would produce emissions enriched in ^{13}C . Therefore, emissions that did not become enriched in ^{13}C came from storage pools. a. Enrichment of chiral monoterpenes. b. Enrichment of non-chiral monoterpenes. Grey shading represents the standard deviation of the $\epsilon^{13}\text{C}$ values of the compounds in ambient air when there is no $^{13}\text{CO}_2$ pulse. The black line through the grey boxes represents the mean. The box plots present median, and 25th and 75th percentiles. The small squares represent the mean and the whiskers represent the maximum and minimum acquired data points that are not considered outliers. Significantly ^{13}C -enriched values are indicated by the asterisk (*) above the box (i.e. results are significant if $P \leq 0.05$).

Figure 4

Comparison of diel cycles of selected monoterpenes and their enantiomers with light, temperature and soil moisture across the experiment suggest emission driver changes in some monoterpenes. a. Average diel cycles for (-) and (+)- α -pinene, (-)- β -pinene and (+)-limonene. The monoterpene diel cycles were normalized by their respective maxima across all averaged diel bins, which was found to be during SD for both compounds. The shaded region around the lines is the average absolute uncertainty. b. Assimilation (A) and photosynthetically active radiation (PAR). The shaded region around the assimilation rate line represents 1σ . c. Atmospheric water potential (AWP) and temperature. Zones 3 and 4 were the uppermost sections of the rainforest enclosure (Ext. Data Fig. 3). The shaded region around the atmospheric water potential lines represents 1σ .

Supplementary Files

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- [ExtendedDatafinal.docx](#)