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Measurement report: Age-dependent BVOC emissions in *Eucalyptus urophylla*: a comparison of leaf cuvette and branch chamber measurements

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S1. Leaf sampling

Leaf emissions were measured by a portable photosynthesis system (LI-6800, Li-Cor Inc., Lincoln, NE, USA) equipped with 6800-01A leaf chamber fluorometers and 6 cm^2 apertures. For each single measurement, a healthy, mature and sunlit leaf was clamped into the leaf chamber. Circulating air with flow rate of 500 μmol s^{-1} (ca. 0.75 L min^{-1}) was passed through an active charcoal VOCs-scrubber. We maintained environmental conditions within the leaf chamber at 30 °C the temperature, 1000 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR), 400 ppm carbon dioxide concentration, and 55% relative humidity, thus the emission rates represent E_s. Air exiting the leaf chamber was bifurcated: one part with flow rate of 200 μmol s^{-1} was analyzed by the built-in infrared gas analyzer, and the other was vented into the ambient at a flow rate of 300 μmol s^{-1} through the "SAM" port, from which BVOC samples were collected via a three-way valve by using adsorbent cartridges (Tenax TA/Carbograph 5TD, Markes International Ltd, Bridgend, UK) connected to a portable dual-channel sampler (ZC-QL, Zhejiang Hengda Instrumentation Ltd., Zhejiang, China) at a rate of 200 mL min^{-1} for 2 minutes. This setup allowed for the capture of BVOC samples five minutes post photosynthesis stabilization. Concurrently, the photosynthetic parameter like net photosynthetic rate (P_n) was recorded. After the measurement, the measured leaf was cut and taken to the laboratory where they were scanned, oven-dried at 60 °C for 48 hours to obtain the dry weight (g). The scanned images were analyzed by the ImageJ software (https://imagej.net/software/imagej/) to determine the leaf area (m^{-2}). Thus, leaf mass per area (LMA, g m^{-2}) was calculated as the ratio of dry weight to leaf area.

S2. Branch sampling

BVOC emissions were measured using a dynamic chamber constructed from polymethyl methacrylate, featuring an inner surface coated with fluorinated ethylene propylene (FEP) Teflon film (FEP 100, Type 200A; DuPont, CA, USA). The chamber's design and characterization have been detailed in previous study (Zeng et al., 2022a, 2025c). With a total volume of 13.7 L, the chamber has a diameter of 25 cm and a height of 28 cm, providing sufficient space for the enclosed plant materials. To ensure proper air circulation, the chamber operated at an optimized flow rate of 9 L min^{-1}, maintained by a mass flow controller (Alicat Scientific, Inc., Tucson, AZ, USA) followed by an air pump (MPU2134-N920-2.08; KNF, Freiburg, Germany). Before entering the chamber, the circulating air was purified using activated charcoal and KI scrubber to scavenge VOCs and ozone. Homogeneous conditions inside the chamber were provided by a Teflon fan (Shenzhen Shuangmu Plastic Material Co. Ltd, Shenzhen, China), which continuously mixed the air. When measuring, healthy and sunlit branches located 3-5 meters above the ground were enclosed into the chamber. To prevent artificial disturbances from affecting leaf physiological states, ambient air was introduced into the chamber for a duration of 1 to 2 hours prior to sampling, allowing the stabilization of emissions.

Once stabilized, air from the chamber was directed through an automatic sampler (JEC921; Jectec Science and Technology, Co., Ltd, Beijing, China) fitted with adsorbent cartridges, maintaining a consistent flow rate of 200 mL min^{-1}, and capturing sample air for 10 minutes. Simultaneously, a background sample of the filtered inlet air was collected in the same way for comparison. After sampling, the adsorbent cartridges were securely sealed with copper caps and temporarily stored at 4 °C in a portable refrigerator during field activities. They were then transported to the laboratory and preserved at -20 °C. Environmental conditions were continuously monitored during the measurements. Temperature and relative humidity, both inside and outside the chamber, were measured using two identical temperature and humidity sensors (HC2A-S; Rotronic, Bassersdorf, Switzerland). Leaf temperature was recorded using two thermocouples (ST-50; RKC Instrument Inc., Tokyo, Japan), while four additional thermocouples (HTK305000; OMEGA Engineering Inc., CT, USA) were used to monitor the air temperature inside the chamber (Zeng et al., 2022a). Photosynthetically active radiation (PAR) was monitored by a light sensor (LI-1500; Li-Cor Inc., Lincoln, NE, USA) positioned on top of the chamber. Once measurements were completed, the sampled branches were cut and transported to the laboratory. They were subsequently dried in an oven at 60 °C for 48 hours to obtain their dry weight (g).

S3. Lab analysis

A thermal desorption system (TD-100, Markes International Ltd, Bridgend, UK) integrated with a 7890 gas chromatograph (GC) and a 5975 mass selective detector (MSD) (Agilent Technologies, Inc., CA, USA) was used to analyze the collected adsorbent cartridges. The TD-100 thermally desorbed the adsorbent cartridges at 280 °C for 10 minutes. These analytes were transported via pure helium into a cryogenic trap (U-T11PGC-2S, Markes International Ltd, Bridgend, UK), maintained at -10 °C. After trapping, the system heated the trap rapidly to 320 °C, releasing the compounds for GC/MSD analysis. The GC system employed an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Inc., CA, USA). The GC oven was programmed to start at 35 °C (held for 3 minutes), then increased at 5 °C min^{-1} to 100 °C (held for 1 minute), followed by a rise of 10 °C min^{-1} to 120 °C (held for 12 minutes), and finally to 260 °C with 2-minute hold. The MSD operated in both scan mode and selected ion monitoring mode (SIM), utilizing electron impact ionization at 70 eV. Identification of target compounds was achieved by comparing retention times with standards, while calibration curves were used for quantification. More information about the identification and quantification are available in previously published studies (Zeng et al., 2022a, 2022b).

S4. Calculation of emission factors for branch chamber measurements

To determine the emission factors (E_s), the real-world emission rates were standardized using Equation 1 for isoprene and light-dependent MTs (Guenther et al., 1993). The algorithm is expressed as:

$$E = E_s \cdot C_T \cdot C_L \quad \text{(S1)}$$

where E (μg g^{-1} h^{-1}) represents the real-world emission rate at actual leaf temperature and light, E_s (μg g^{-1} h^{-1}) denotes emission rate under 30 °C leaf temperature and 1,000 μmol m^{-2} s^{-1} PAR. The C_T and C_L are the light- and temperature-dependent algorithms, respectively, which can be calculated by Equations 2 and 3, respectively. C_T is expressed as:

$$C_T = \frac{exp\frac{C_{T1}(T-T_s)}{RT_sT}}{1 + exp\frac{C_{T2}(T-T_M)}{RT_sT}} \quad \text{(S2)}$$

where T is the leaf temperature, T_s represents standard condition for the leaf temperature (303.15 K), R is the ideal gas constant (8.314 J K^{-1} mol^{-1}). The empirical coefficients C_{T1}, C_{T2}, and T_M are set at 95000 J mol^{-1}, 230000 J mol^{-1}, and 314 K, respectively. The light-dependent algorithm C_L is expressed as:

$$C_L = \frac{\alpha C_{L1} \text{PAR}}{\sqrt{1 + \alpha^2 \text{PAR}^2}} \quad \text{(S3)}$$

where PAR (μmol m^{-2} s^{-1}) represents the photosynthetic active radiation. Both α (0.0027) and C_{L1} (1.066) are empirical coefficients. For compounds that do not depend on light, such as some MTs and SQTs, the E_s is determined by:

$$E = E_s \cdot \exp(\beta (T-T_s)) \quad \text{(S4)}$$

where β is an empirical coefficient that reflects the exponential relationship between emission rates and temperature.

Table S1. Comparison of BVOC emission factors among two age groups

Compounds	**2 months old**	**2 years old**
Isoprene	107.72±34.93	69.75±21.15
α-Pinene	0.24±0.07	0.15±0.12
β-Pinene	0.01±0.01	n.d.
β-Myrcene	0.01±0.01	0.17±0.13
α-Phellandrene	n.d.	0.04±0.03
Limonene	0.14±0.03	0.09±0.07
1,8-Cineole	0.48±0.22	0.17±0.13
cis-β-Ocimene	0.07±0.04	0.37±0.30
trans-β-Ocimene	0.06±0.02	4.96±4.31
3,6-Dimethyl-1,3,7-octatriene	n.d.	0.06±0.04
Linalool	0.08±0.06	0.07±0.05
3,4-Dimethyl-2,4,6-octatriene	n.d.	0.05±0.04
Alloocimene	n.d.	0.02±0.02
Sum of MTs	1.09±0.35	6.14±5.23
α-Longipinene	0.02±0.01	0.01±0.01
α-Copaene	0.03±0.03	0.03±0.02
β-Caryophyllene	0.01±0.01	0.04±0.05
α-Humulene	n.d.	0.01±0.01
Alloaromadendrene	n.d.	0.01±0.01
Sum of SQTs	0.05±0.05	0.10±0.10

n.d.: not detected

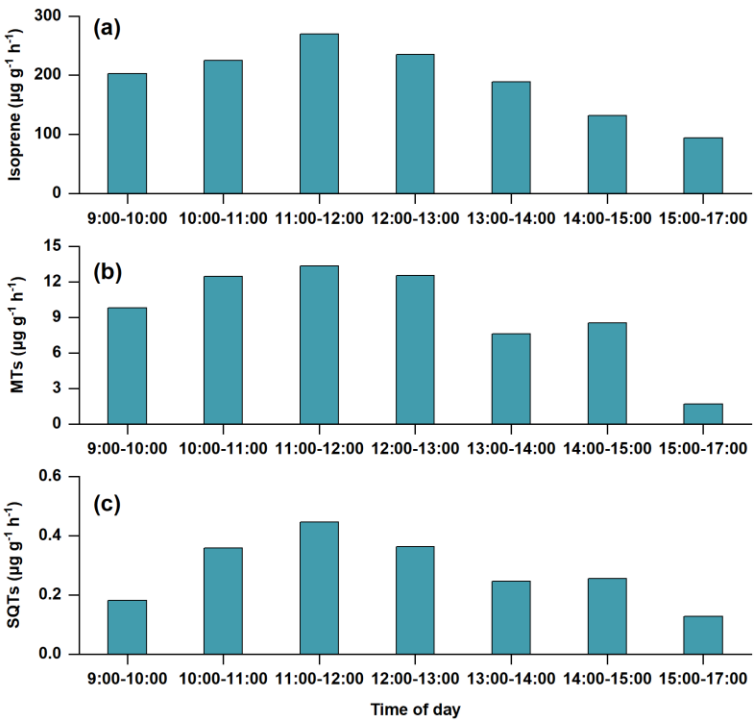

Figure S1. Mean isoprene (a), MT (b), and SQT (c) emission rates over time of the day for field measurements

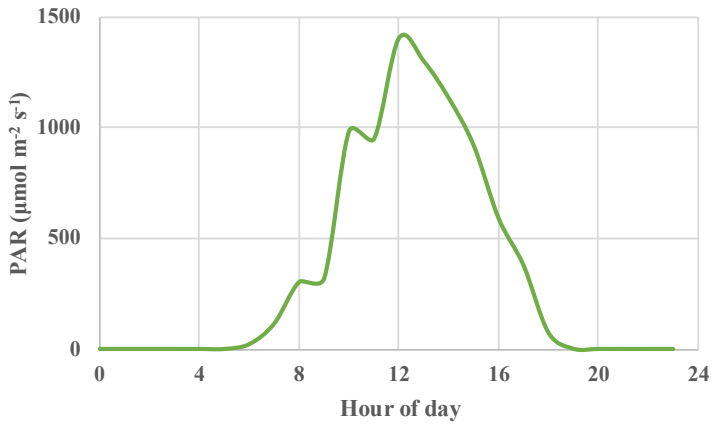

Figure S2. Diurnal variations of PAR recorded during field measurements

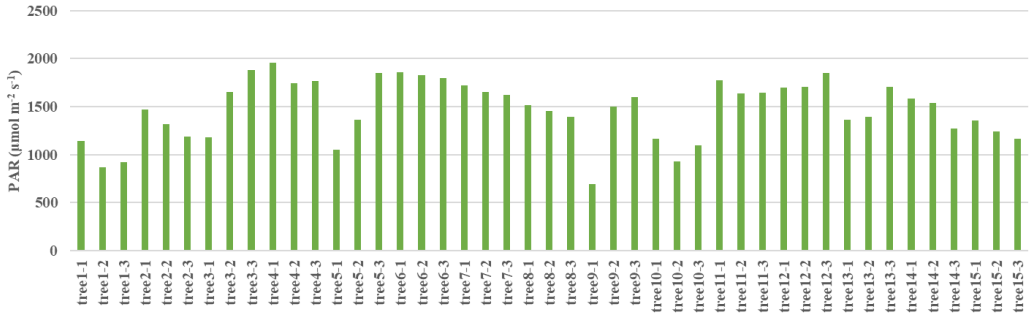

Figure S3. PAR values recorded for each BVOC sample during laboratory branch measurements

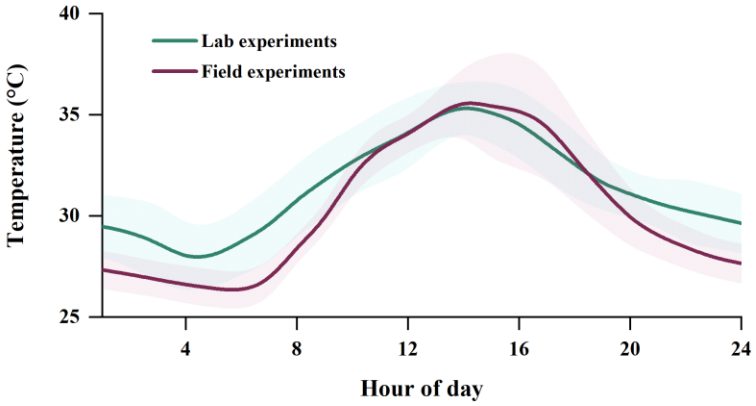

Figure S4. Comparison of temperatures between laboratory and field measurements

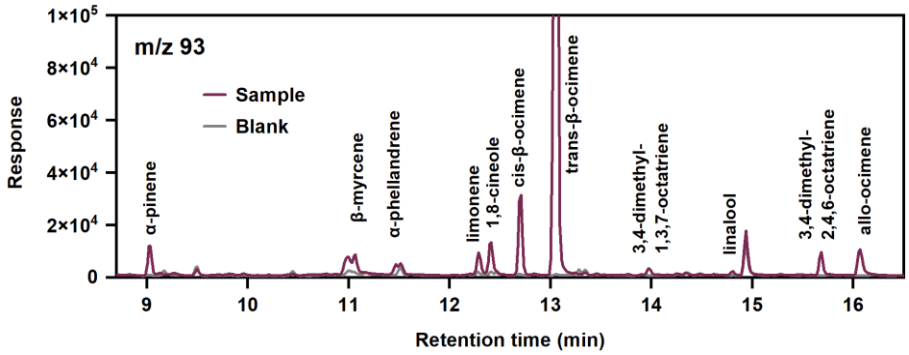

Figure S5. Chromatograms of a representative BVOC sample and its corresponding inlet blank sample

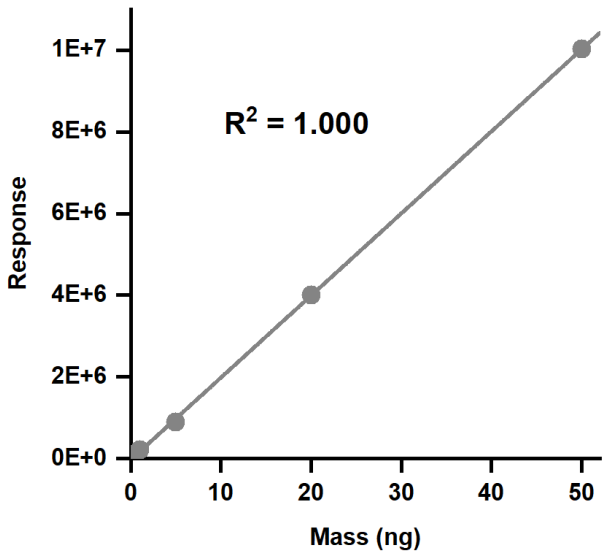

Figure S6. Calibration curve for β-ocimene

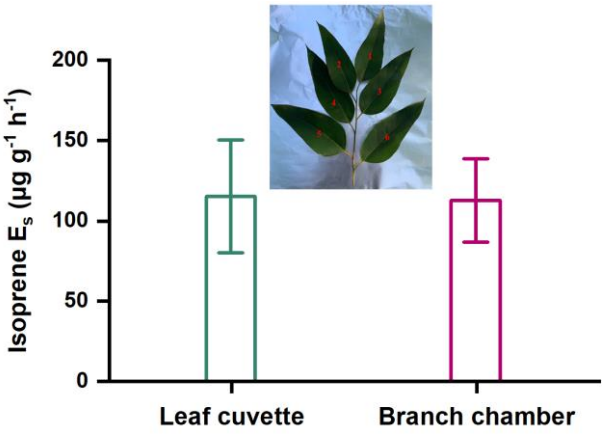

Figure S7. Comparison of isoprene E_s for the same branch measured by both leaf cuvette and branch chamber. The leaf cuvette results represent mean of the six leaves, while the branch chamber results indicate mean of three samples.

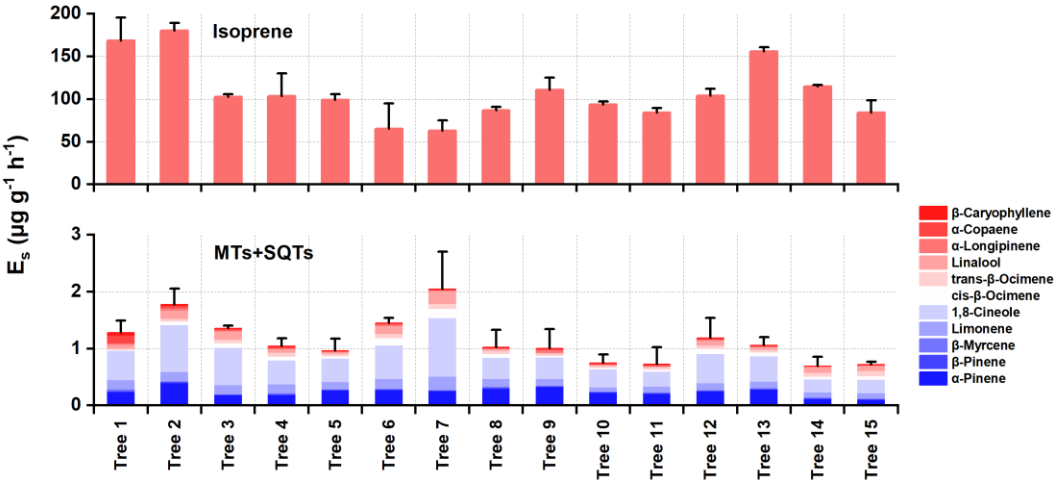

Figure S8. Compound-specific emission factors of BVOCs for the 15 seedlings.

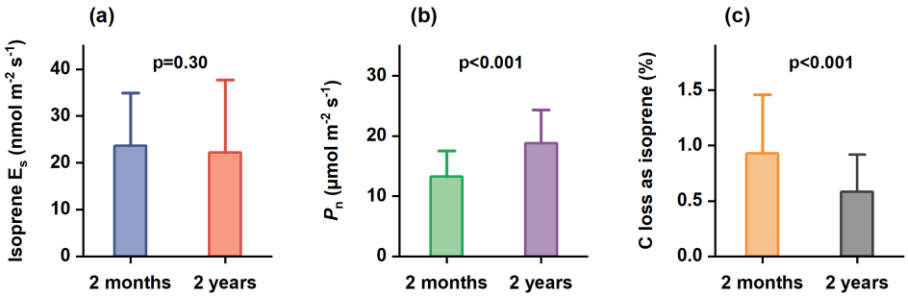

Figure S9. Comparison of area-based isoprene E_s (a), net photosynthetic rate (P_n, b), and carbon loss fraction as isoprene emission (c) for 2-month-old trees with those for 2-year-old ones. These data were from the leaf cuvette measurements.

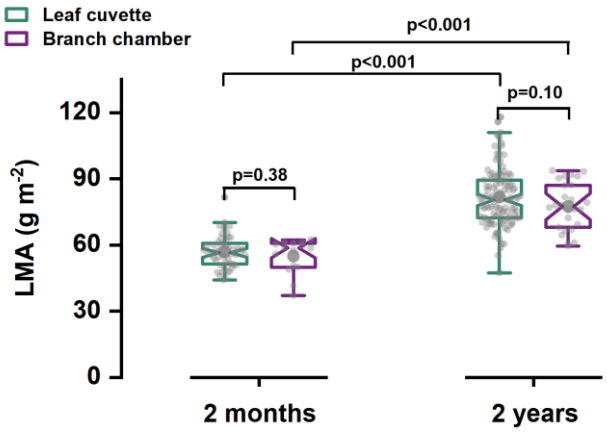

Figure S10. Comparison of leaf mass per aera (LMA, g m^{-2}) between 2-month-old and 2-year-old trees for both leaf cuvette and branch chamber measurements.

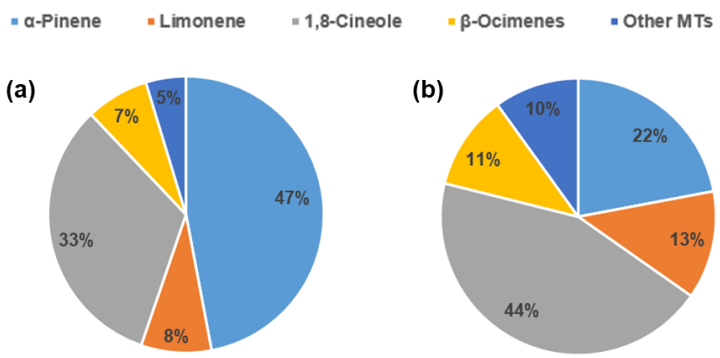

Figure S11. Monoterpene composition of saplings (a) in winter and seedlings (b) in summer. Both of them are stressless before measurements.

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