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

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Abstract

Biogenic volatile organic compound (BVOC) emission factors (E_s) underpin air quality and climate models, yet current databases intermingle data from both seedlings and mature trees and from two enclosure techniques, leaf cuvettes and dynamic branch chambers, whose comparability has rarely been rigorously tested. Here we quantified BVOC emissions from *Eucalyptus urophylla* by pairing the two methods on a statistically representative number of 2-month-old seedlings in the laboratory and 2-year-old in-situ saplings measured at a managed plantations in subtropical China. Leaf-cuvette and branch-chamber determination of isoprene E_s matched within 5% for both age classes, demonstrating method equivalence. In contrast, tree age exerted a significant impact on both the magnitude and speciation of emissions. Seedlings emitted ~50% more isoprene and were enriched in cyclic monoterpenes like α-pinene and 1,8-cineole, whereas field-grown trees shifted toward highly reactive acyclic monoterpenes, with β-ocimenes accounted for over 85% of the terpene flux and a double rise in sesquiterpenes. These ontogenetic shifts imply that one-third of the entries in global E_s compilations, which are derived from seedling studies, likely overestimate local isoprene fluxes while under-representing the atmospheric reactivity of mature canopies. Our results validate the use of either chamber type for measuring isoprene E_s, highlight the need for improved analytical sensitivity before extending this equivalence to terpenes, and call for systematic, large-sample, branch-level measurements of adult trees to produce representative E_s values. Incorporating age-resolved emission factors into models will refine estimates of ozone and secondary organic aerosol formation in fast-growing subtropical plantations and other managed forests worldwide.

1 Introduction

Terrestrial vegetation release on the order of ~1,000 Tg yr^{-1} for biogenic volatile organic compounds (BVOCs), representing ~90% of global non-methane VOCs injected to the troposphere (Guenther et al., 1995, 2012). Owing to their high reactivity with atmospheric lifetimes of only minutes to hours (Atkinson and Arey, 2003), BVOCs can strongly modulate the oxidative capacity of the troposphere and drive the production of ozone and secondary organic aerosols (Di Carlo et al., 2004; Peñuelas and Staudt, 2010; Scott et al., 2017; Pfannerstill et al., 2024; Weber et al., 2024). Reliable quantification of these fluxes is therefore essential for assessing their impacts on air quality and climate.

Current bottom-up BVOC emission inventories rely on species-specific emission factors (E_s) that are applied within models such as the Model of Emissions of Gases and Aerosols from Nature (MEGAN; Guenther et al., 2012, 2020). Inaccurate E_s are now recognized as a leading source of uncertainty in regional and global estimates of BVOC budgets (Guenther et al., 2012; Zhang et al., 2020; Wang et al., 2023). Decades of enclosure studies produced several widely used databases, like the Sheffield (Hewitt and Street, 1992), UCAR (Wiedinmyer et al., 2004), and most recently, the tropical plant compilation of Mu et al. (2022). However, two methodological and biological issues remain unresolved.

Both leaf cuvettes and dynamic branch chambers have been used for measuring E_s. Guenther et al. (1994) suggested, from a literature survey, that isoprene E_s with leaf cuvette are approximately 75% higher than branch-based values, but a rigorous, side-by-side validation on the same trees is still lacking. Whether the two techniques can be used interchangeably is therefore uncertain.

As for the tree age, approximately one-third of database entries originate from greenhouse or growth-chamber seedlings (Guenther, 2013), yet seedling's physiology differs markedly from that of mature trees. Limited case studies on *Eucalyptus spp.* point to pronounced ontogenetic shifts in both the magnitude and speciation of BVOC emissions (Street et al., 1997; He et al., 2000; Winters et al., 2009). Meanwhile, considerable uncertainty may result from the potential intraspecific variability and chemo-diversity of BVOC emissions (Loreto et al., 2009; Staudt and Visnadi, 2023; Zeng et al., 2024). Previous investigations, however, employed small sample sizes and heterogenous protocols, leaving the statistical representativeness of age effects largely unconstrained.

Addressing these gaps requires large-sample, method-controlled measurements that span contrasting developmental stages. Here we report parallel cuvette and branch chamber determination of BVOC emissions from *Eucalyptus urophylla* seedings grown under laboratory conditions (2-month-old) and saplings (2-year-old) measured in situ at a managed plantation in subtropical China. The objectives are to 1) quantitatively test the equivalence of the two chamber types for isoprene and, where detection limits permit, for terpenes; 2) disentangle how tree age influences both emission factors and chemical composition; and 3) assess the implications for constructing representative E_s databases and for simulating ozone and secondary organic aerosol formation in rapidly expanding plantation forests. By combining method inter-comparison with statistically robust sampling across ontogeny, our work provides critical benchmark for future BVOC inventories and modeling frameworks. It should be noted that we only focus on the seedling to sapling transition, using 2-month-old seedlings and 2-year-old field grown saplings as contrasting stages; we do not attempt to represent fully mature trees.

2 Materials and methods

2.1 Study sites and plant materials

Laboratory measurements were performed at the Guangzhou Institute of Geochemistry (GIG, 23.145° N, 113.364° E). Leaf experiments were conducted on 1-3 June 2023, and branch-chamber measurements were conducted on 10-13 June 2023 (7-day separation). Field measurements were carried out in a managed *E. urophylla* plantation at Heshan (22.649° N, 112.904° E), Guangdong province, China. Leaf and branch measurements were performed concurrently on the same days during two campaigns: 11-13 July and 26-31 July 2022. The study region experiences a humid subtropical monsoon climate, with a 30-year mean temperature of 22 °C and annual precipitation of ~1,700 mm (Mu et al., 2023; Zeng et al., 2024). As shown in Table 1, two age classes, 2-month-old and 2-year-old, were investigated, with seedlings measured in laboratory and 2-year-old saplings measured in situ at the plantation. Seedlings were purchased from a local nursery and measured by both leaf cuvette and dynamic branch chamber. These trees were placed in an open area of GIG two weeks before measurements; no greenhouse or climate-chamber conditions were used. Field trees were randomly chosen from >8 ha of homogeneous plantation to ensure spatial representativeness. Both seedlings and saplings were sourced from the same clonal line (documented by the nursery/plantation) and exhibited a uniform terpene chemotype, minimizing genotype/chemotype variability. All measurements, both in the laboratory and in the field, were conducted between 9:00 and 17:00 local time under sunny conditions. Midday maximum PAR exceeded 1200 μmol m^{-2} s^{-1} during both campaigns (Figs. S2 and S3). Mean daily air temperatures during the campaigns were 31.4 °C (seedling campaign at GIG) and 30.3 °C (Field campaign; Fig. S1).

2.2 Enclosure measurements

2.2.1 Leaf cuvette

Leaf cuvette fluxes were obtained with a LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE, USA) fitted with a 6800-01A fluorometer head (aperture 6 cm^2, air flow 500 μmol s^{-1}). We imposed standard conditions for emission factors (E_s): leaf temperature 30 °C, PAR 1000 μmol m^{-2} s^{-1}, CO_2 400 μmol mol^{-1}, and 55% RH. The LI-6800 maintains closed loop control and continuously records actual leaf temperature and PAR. Because measurements were made at the standard reference conditions, cuvette fluxes equal E_s

after correction to leaf dry mass. After 5 min stabilization of photosynthesis, 200 mL min^{-1} of outlet air was diverted for 2 min through Tenax TA/Carbograph 5TD adsorbent cartridges (Markes International Ltd, Bridgend, UK) using a dual-channel pump (ZC-QL, Zhejiang Hengda Instrumentation Ltd., Zhejiang, China). Detailed leaf handling, leaf area and dry mass determination are provided in Text S1 and are also described in Zeng et al. (2024, 2025ab).

2.2.2 Dynamic branch chamber

A cylindrical PMMA chamber (Ø 25 cm × 28 cm, 13.7 L) internally coated with FEP film was used for branch-level measurements. The charcoal- and KI-scrubbed ambient air (9 L min^{-1}) was supplied by a mass-flow controller (Alicat Scientific, Inc., Tucson, AZ, USA) coupled with an oil-free pump (MPU2134-N920-2.08; KNF, Freiburg, Germany), then it was well mixed with PTFE-bladed fans in the chamber (Zeng et al., 2022a). For comparability with leaf cuvette measurements, sunlit branches (3-5 m above ground) with ~6-10 leaves were selected, avoiding mutual overlap so that foliage formed a single layer. Branches showing visible self-shading were not sampled. These practices follow our goal of minimizing light heterogeneity within the chamber. When the selected sunlit branches were enclosed; fluxes were allowed to stabilize for 1-2 h before sampling. Outlet air (and inlet blanks) was drawn at 200 mL min^{-1} for 10 min by an automatic sampler (JEC921; Jectec Science and Technology, Co., Ltd, Beijing, China) onto the same adsorbent tubes as above. Concurrent meteorological and radiometric variables were logged continuously (Rotronic HC2A-S RH/T probes; LI-1500 PAR sensor; OMEGA/ RKC thermocouples). More details about the branch sampling are provided in Text S2 and Zeng et al. (2022a).

2.3 Thermal desorption-GC/MS analysis

Tubes were analyzed within 7 days with a TD-100 system (Markes) coupled to an Agilent 7890 GC-5975 MSD. Primary cartridge desorption was 280 °C, cold-trapping at -10 °C, then desorption at 320 °C. Separation employed an HP-5 MS (30 m × 0.25 mm × 0.25 µm) column. The GC oven temperature program was started at 35 °C (3 min), 5 °C min^{-1} to 100 °C (1 min), 10 °C min^{-1} to 120 °C (12 min), and 20 °C min^{-1} to 260 °C (2 min). Identification used authentic standards, while quantification based on calibration curves. More information about the analysis and quantification are given in Text S3 and Zeng et al. (2022a, 2022b).

2.4 Calculation of emission rates and emission factors

For branch data, emission rates (E, µg g^{-1} h^{-1}) were calculated as

$$\mathrm{E} = \frac{F\,(C_{out} - C_{in})}{g_{dw}}$$

where F is chamber flow (L h^{-1}) and g_{dw} the dry leaf mass. Branch chamber fluxes measured under ambient conditions were standardized to 30 °C and 1000 µmol m^{-2} s^{-1} using the MEGAN/Guenther temperature and light response functions, yielding $\mathrm{E_s}$ directly comparable to cuvette derived values. The temperature- and light-dependent function was applied for light dependent compounds, while a temperature-only function was used for light-independent monoterpenes ($\beta = 0.10\ K^{-1}$) and sesquiterpenes ($\beta = 0.17\ K^{-1}$). More details about the calculation of emission factors are provided in Text S4 and in Zeng et al. (2023).

Notably, E_s can be normalized either to leaf dry mass ($\mu g\ g^{-1}\ h^{-1}$) or to projected leaf area ($\mu g\ m^{-2}\ h^{-1}$). Because leaf mass per area (LMA, $g\ m^{-2}$) increases with age in *E. urophylla* (Table 1), the two normalizations emphasize different aspects of physiology and scaling (E_s-area = E_s-mass × LMA). To remove duplication and prevent unit confusion, we only report mass-based E_s in the main text and present all area-based values in the Supplement.

2.5 Statistical analysis

All statistics were performed in Excel 2019. Isoprene and terpene E_s were log-normal, and they were log-transformed before testing. Equality of means between age classes or methodologies was evaluated with Welch's t-test; distributions that failed Shapiro-Wilk normality ($p < 0.05$) were compared with Mann-Whitney U. Significance was accepted at $p < 0.05$. Results are reported as mean ± 1 SD unless otherwise specified.

3. Results and discussion

3.1 Method inter-comparison for isoprene E_s

Side-by-side measurements of *E. urophylla* seedlings (2 months old) and 2-years-old trees showed that the two dominant enclosure techniques yielded statistically indistinguishable isoprene E_s. As shown in Fig. 1, for seedlings measured at GIG, cuvette-derived isoprene E_s averaged at $102.4 \pm 34.1\ \mu g\ g^{-1}\ h^{-1}$ (N=50) versus $107.7 \pm 34.9\ \mu g\ g^{-1}\ h^{-1}$ from branch chambers (N=15), a non-significant 5% difference (p=0.61). In the field, 2-year-old trees exhibited similarly close agreement: $66.9 \pm 31.4\ \mu g\ g^{-1}\ h^{-1}$ (N=114) from leaf cuvettes and $69.8 \pm 21.2\ \mu g\ g^{-1}\ h^{-1}$ (N=26) from branch chambers (p=0.57). A direct test in which the same branch was first sub-sampled leaf-by-leaf and then enclosed intact confirmed parity within analytical uncertainty (Fig. S4).

Leaf cuvette and dynamic branch chamber are the two most widely employed techniques for measuring plant BVOC emissions (Niinemets et al., 2011). The present study provides the first large-sample validation that leaf cuvette and dynamic branch chamber protocols are interchangeable for isoprene, contradicting the ~75 % bias inferred from the earlier literature meta-analysis (Guenther et al., 1994). The convergence arises despite distinct air flow regimes ($0.75\ L\ min^{-1}$ vs. $9\ L\ min^{-1}$) and path lengths, implying that adsorptive/ozonolysis losses are negligible for this highly volatile compound. Because branch chambers integrate six to ten leaves per branch, their representativeness is at least as good as that of cuvettes as previously demonstrated for Eucalyptus by Zeng et al. (2024), and they remain advantageous when mature crowns are inaccessible.

Establishing methodological equivalence for isoprene enables age effects to be probed with confidence using the more logistically efficient branch chamber dataset (Sect. 3.2). Whether the same holds for lower-volatility monoterpenes and sesquiterpenes remains an open question due to the assessment of method equivalence was precluded by their lower fluxes and the limited air volumes sampled. Because terpene emissions are more sensitive to wall losses and analytical detection limits than isoprene, systematic inter-comparison studies with larger sample volumes and a broader range of plant species are still required.

Moreover, the equivalence we observed for isoprene was obtained under sunlit, low LAI branches (~6-10 leaves) chosen to limit mutual shading. Larger or denser branches that capture multi-layer foliage can depress within chamber PAR for a subset of leaves and thus bias light dependent fluxes low relative to single leaf cuvettes; in such cases, method differences may not remain negligible. We therefore recommend selecting unshaded branches, documenting within enclosure PAR (or its ratio to ambient), and reporting the number of enclosed leaves and leaf layering as part of QA/QC.

3.2 Ontogenetic controls on the magnitude and speciation of BVOC emissions

3.2.1 Speciation diversity

Dynamic branch chamber measurements revealed 12 BVOC species in 2-month-old seedlings (isoprene, 8 monoterpenes, 3 sesquiterpenes; Fig. S5) versus 17 species in 2-year-old trees (isoprene, 11 monoterpenes, 5 sesquiterpenes; Table S1). Thus, chemical richness increased by ~40% with age, consistent with the view that metabolic complexity develops as trees mature (Satake et al., 2024).

3.2.2 Emission magnitude

Isoprene dominated the flux from both age classes but its standardized E_s declined significantly ($p<0.001$) from 107.7±34.9 $\mu g\ g^{-1}\ h^{-1}$ in seedlings to 69.8±21.2 $\mu g\ g^{-1}\ h^{-1}$ in two-year-old trees. When normalized by leaf area, however, the two groups were indistinguishable (Fig. S6a), indicating that the mass-based contrast mainly reflects an increase in leaf mass per area (LMA) with tree age (Fig. S7). Seedlings therefore allocated a larger fraction of assimilated carbon to isoprene (Fig. S6c) despite exhibiting lower net photosynthesis (P_n, Fig. S6b), suggesting a typical growth-defense trade-off of early ontogeny. Consistent with our findings, isoprene E_s for the 1-year-old *E. globulus* was 5-fold higher than that for the 7-year-old individual (Street et al., 1997). Winters et al. (2009) also documented lower isoprene E_s in four 10-year-old eucalyptus species compared to their seedlings measured by He et al. (2000).

3.2.3 Terpene speciation shift

Total monoterpene emissions rose nearly 6-fold with tree age (1.09 vs. 6.14 $\mu g\ g^{-1}\ h^{-1}$), but the increase was almost entirely due to acyclic β-ocimenes, whose E_s leapt from 0.13±0.06 to 5.33±4.61 $\mu g\ g^{-1}\ h^{-1}$ (Fig. 2 and Table S1). By contrast, E_s for cyclic α-pinene, limonene, and 1,8-cineole declined by 30-65% (Fig. 2).

Consequently, terpene composition shifted from 75% cyclic (seedlings) to 85% acyclic (2-year-old trees) (Fig. 2). Sesquiterpenes doubled in absolute terms but remained a minor (<1%) proportion of total BVOCs.

Such a cyclic-to-acyclic transition has been observed in other *Eucalyptus* species and might be attributed to age-dependent expression of terpene synthesis and to selectively pressures from biotic/abiotic stress in the field (Monson et al., 2021; Pollastri et al., 2021). For example, most previous studies reported that cyclic α-pinene and 1,8-cineole were the dominant MTs in stressless lab-grown seedlings (Evans et al., 1982; Guenther et al., 1991; He et al., 2000; Tsui et al., 2009; Malik et al., 2019), whereas acyclic MTs like β-ocimenes were generally low or even undetectable. In contrast, most stress-rich field-grown trees could emit large amounts of acyclic β-ocimenes (Street et al., 1997; Nunes and Pio, 2001; Sørensen et al., 2020; Purser et al., 2020, 2021; Nagalingam et al., 2023).

Both isoprene and β-ocimenes are known to play key roles in plant antioxidant defense. Isoprene helps scavenge reactive oxygen species (ROS) (Jardine et al., 2014), maintain membrane stability (Sharkey and Singsaas, 1995; Pollastri et al., 2019, 2021), and regulate antioxidative processes (Zuo et al., 2019; Monson et al., 2021), whereas the highly reactive β-ocimene may act as an even more efficient, direct antioxidant against ROS. The highly reactive β-ocimenes can quench ROS more efficiently than isoprene (Pollastri et al., 2021), providing a plausible advantage for field-grown trees exposed to stronger light, heat, drought, and herbivory.

Environmental factors, particularly growth temperature, light availability, and soil moisture/nutrients, can modulate BVOC emissions (Monson et al., 1994; Harley et al., 1994, 1996, 1997; Fall and Wildermuth, 1998; Funk et al., 2006; Guenther et al., 2006, 2012; Yuan et al., 2020). In our study, both campaigns were conducted under clear-sky, peak-summer conditions with comparable ambient temperature and high midday PAR (Figs. S1-S3). A simple sensitivity using the MEGAN/Guenther acclimation scheme (Guenther et al., 2012) indicates that, a ~10% change in growth PAR would change standardized E_s by ~8%, and a +1.1 °C change in growth temperature by ~11%. These effects are small relative to the age-related differences reported here, such as ~6-fold increase in total monoterpenes and a >40-fold rise in β ocimenes from seedlings to saplings (Fig. 2 and Table S1). Nevertheless, because we did not measure soil nutrient or moisture status, parallel experiments under controlled and co-located growth conditions are warranted to fully disentangle ontogeny from environment.

It should be noted that the seedlings used here were cultivated trees, where soil properties could be parallel with those in previous seedling-based studies. In contrast, the taller trees measured under field conditions reflect realistic and natural growth conditions. The substantial differences in E_s and emission composition between seedlings and saplings underscore that seedling measurements are inappropriate as generic surrogates for natural forest emissions. Despite this, all age contrasts presented here are seedlings vs. saplings; extrapolation to mature trees requires additional in-situ measurements.

Notably, roughly one-third of the global BVOC E_s entries now feeding chemistry-climate models originate from seedlings. Our results show that these data tend to overestimate canopy-scale isoprene fluxes and under-represent atmospheric reactivity by excluding large β-ocimene emissions from natural tree canopies. Incorporating age-resolved E_s and prioritizing statistically robust and in-situ sampling of branches from adult trees, particularly in rapidly expanding subtropical plantations, will reduce inventory uncertainty and refine predictions of ozone and secondary organic aerosol formation.

4. Conclusions

Parallel measurements on *E. urophylla* seedlings (2 months) and saplings (2 years) show that leaf cuvettes and dynamic branch chambers yield indistinguishable isoprene E_s when applied to sunlit, single-layer branches. Age exerts a strong control on speciation: seedlings have higher mass-based isoprene and are enriched in cyclic monoterpenes, whereas saplings exhibit ~6-fold higher total monoterpenes dominated by β-ocimenes and increased sesquiterpenes. These findings indicate that laboratory-grown seedlings are not reliable proxies for field-grown saplings, and likely not for mature trees either. Despite this, other drivers, particularly soil properties (e.g., nitrogen availability, moisture) were not resolved here and may also influence emissions. This therefore motivates age-stratified and in-situ branch measurements on mature trees under parallel growth conditions to comprehensively probe age effects. Future studies should extend such measurements to other high isoprene genera (e.g. Quercus, Populus) and to tropical species that dominate global BVOC budgets, evaluate method comparability for low-volatility terpenes using larger-volume or adsorption-minimized chambers, and couple physiological measurements with transcriptomics to unravel the molecular basis of the observed metabolic shift from cyclic to acyclic terpenes.

Data availability. The measurement data used in this study can be available at https://doi.org/10.17632/jw8g8gkm5t.1 (Zeng, 2025).

Supplement. The related supplement is published alongside this article.

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Conflict of interest. The authors declare no conflicts of interest relevant to this study.

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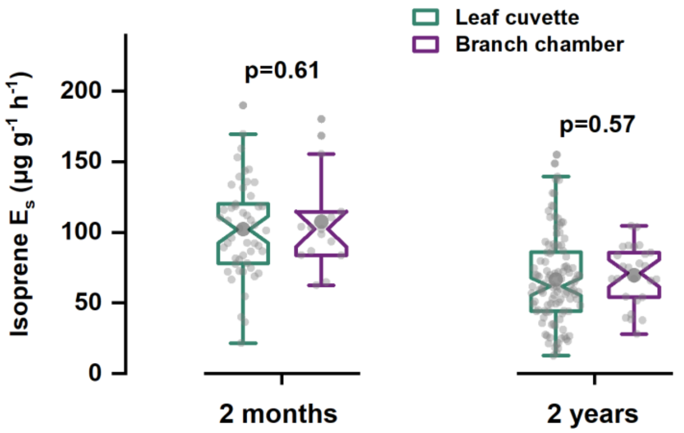

Figure 1. Comparison of isoprene E_s from leaf cuvettes with those from dynamic branch chambers for both 2-month-old and 2-year-old trees.

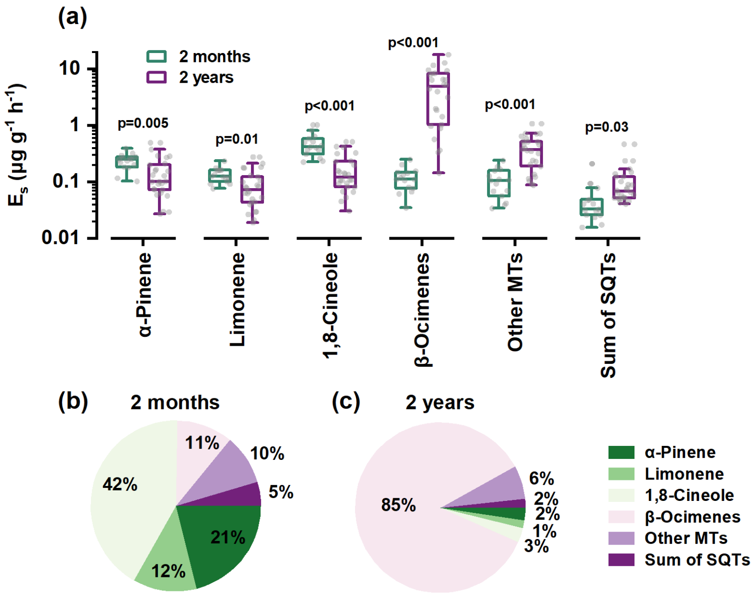

Figure 2. Comparison of emission factors (a) and compositions (b,c) between 2-month-old and 2-year-old trees.

Table 1. Two age classes of *Eucalyptus urophylla* investigated in this study

Site	Laboratory	Field
Age class	2 months	2 years
Height (m)	0.3-0.4	10-12
Cuvette (no. of replicate)	50	114
Branch chamber (no. of replicate)	15	26
Cuvette LMA (g m^{-2})	57±7	82±13
Branch-chamber LMA (g m^{-2})	55±8	78±11