

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

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11

12 **Abstract**

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

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32 **1 Introduction**

33 Terrestrial vegetation release on the order of $\sim 1,000 \text{ Tg yr}^{-1}$ for biogenic volatile organic compounds
34 (BVOCs), representing $\sim 90\%$ of global non-methane VOCs injected to the troposphere (Guenther et al., 1995,
35 2012). Owing to their high reactivity with atmospheric lifetimes of only minutes to hours (Atkinson and Arey,
36 2003), BVOCs can strongly modulate the oxidative capacity of the troposphere and drive the production of
37 ozone and secondary organic aerosols (Di Carlo et al., 2004; Peñuelas and Staudt, 2010; Scott et al., 2017;
38 Pfannerstill et al., 2024; Weber et al., 2024). Reliable quantification of these fluxes is therefore essential for
39 assessing their impacts on air quality and climate.

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

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

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

59 Addressing these gaps requires large-sample, method-controlled measurements that span contrasting
60 developmental stages. Here we report parallel cuvette and branch chamber determination of BVOC emissions
61 from *Eucalyptus urophylla* seedlings grown under laboratory conditions (2-month-old) and saplings (2-year-
62 old) measured in situ at a managed plantation in subtropical China. The objectives are to 1) quantitatively
63 test the equivalence of the two chamber types for isoprene and, where detection limits permit, for terpenes;
64 2) disentangle how tree age influences both emission factors and chemical composition; and 3) assess the

65 implications for constructing representative E_s databases and for simulating ozone and secondary organic
66 aerosol formation in rapidly expanding plantation forests. By combining method inter-comparison with
67 statistically robust sampling across ontogeny, our work provides critical benchmark for future BVOC
68 inventories and modeling frameworks. It should be noted that we only focus on the seedling to sapling
69 transition, using 2-month-old seedlings and 2-year-old field grown saplings as contrasting stages; we do not
70 attempt to represent fully mature trees.

71 2 Materials and methods

72 2.1 Study sites and plant materials

73 Laboratory measurements were performed at the Guangzhou Institute of Geochemistry (GIG, 23.145° N,
74 113.364° E). Leaf experiments were conducted on 1-3 June 2023, and branch-chamber measurements were
75 conducted on 10-13 June 2023 (7-day separation). Field measurements were carried out in a managed *E.*
76 *urophylla* plantation at Heshan (22.649° N, 112.904° E), Guangdong province, China. Leaf and branch
77 measurements were performed concurrently on the same days during two campaigns: 11-13 July and 26-31
78 July 2022. The study region experiences a humid subtropical monsoon climate, with a 30-year mean
79 temperature of 22 °C and annual precipitation of ~1,700 mm (Mu et al., 2023; Zeng et al., 2024). As shown
80 in Table 1, two age classes, 2-month-old and 2-year-old, were investigated, with seedlings measured in
81 laboratory and 2-year-old ~~trees-saplings~~ measured *in situ* at the plantation. Seedlings were purchased from a
82 local nursery and measured by both leaf cuvette and dynamic branch chamber. These trees were placed in an
83 open area of GIG two weeks before measurements; no greenhouse or climate-chamber conditions were used.
84 Field trees were randomly chosen from >8 ha of homogeneous plantation to ensure spatial representativeness.
85 Both seedlings and saplings were sourced from the same clonal line (documented by the nursery/plantation)
86 and exhibited a uniform terpene chemotype, minimizing genotype/chemotype variability. All measurements,
87 both in the laboratory and in the field, were conducted between 9:00 and 17:00 local time under sunny
88 conditions. Midday maximum PAR exceeded 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during both campaigns (Figs. S2 and S3).
89 Mean daily air temperatures during the campaigns were 31.4 °C (seedling campaign at GIG) and 30.3 °C
90 (Field campaign; Fig. S1).

91 2.2 Enclosure measurements

92 2.2.1 Leaf cuvette

93 Leaf cuvette fluxes were obtained with a LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE,
94 USA) fitted with a 6800-01A fluorometer head (aperture 6 cm², air flow 500 $\mu\text{mol s}^{-1}$). We imposed standard
95 conditions for emission factors (E_g): leaf temperature 30 °C, PAR 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CO₂ 400 $\mu\text{mol mol}^{-1}$,
96 and 55% RH. The LI-6800 maintains closed loop control and continuously records actual leaf temperature
97 and PAR. Because measurements were made at the standard reference conditions, cuvette fluxes equal E_g.

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98 ~~after correction to leaf dry mass. Environmental set points were 30 °C leaf temperature, 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$~~
99 ~~PAR, 400 $\mu\text{mol mol}^{-1}$ CO_2 and 55% relative humidity to produce emission factors (E_s).~~ After 5 min
100 stabilization of photosynthesis, 200 mL min^{-1} of outlet air was diverted for 2 min through
101 Tenax TA/Carbograph 5TD adsorbent cartridges (Markes International Ltd, Bridgend, UK) using a dual-
102 channel pump (ZC-QL, Zhejiang Hengda Instrumentation Ltd., Zhejiang, China). Detailed leaf handling, leaf
103 area and dry mass determination are provided in Text S1 and are also described in Zeng et al. (2024, 2025ab).

104 2.2.2 Dynamic branch chamber

105 A cylindrical PMMA chamber ($\text{Ø} 25 \text{ cm} \times 28 \text{ cm}$, 13.7 L) internally coated with FEP film was used for
106 branch-level measurements. The charcoal- and KI-scrubbed ambient air (9 L min^{-1}) was supplied by a mass-
107 flow controller (Alicat Scientific, Inc., Tucson, AZ, USA) coupled with an oil-free pump (MPU2134-N920-
108 2.08; KNF, Freiburg, Germany), then it was well mixed with PTFE-bladed fans in the chamber (Zeng et al.,
109 2022a). ~~For comparability with leaf cuvette measurements, sunlit branches (3-5 m above ground) with ~6-10~~
110 ~~leaves were selected, avoiding mutual overlap so that foliage formed a single layer. Branches showing visible~~
111 ~~self-shading were not sampled. Meteorological and radiometric variables were logged concurrently to~~
112 ~~document illumination during enclosure. These practices follow our goal of minimizing light heterogeneity~~
113 ~~within the chamber. When the selected –Sunlit-sunlit branches (3-5 m above ground) were enclosed; fluxes~~
114 were allowed to stabilize for 1-2 h before sampling. Outlet air (and inlet blanks) was drawn at 200 mL min^{-1}
115 for 10 min by an automatic sampler (JEC921; Jectec Science and Technology, Co., Ltd, Beijing, China) onto
116 the same adsorbent tubes as above. Concurrent meteorological and radiometric variables were logged
117 continuously (Rotronic HC2A-S RH/T probes; LI-1500 PAR sensor; OMEGA/ RKC thermocouples). More
118 details about the branch sampling are provided in Text S2 and Zeng et al. (2022a).

119 2.3 Thermal desorption-GC/MS analysis

120 Tubes were analyzed within 7 days with a TD-100 system (Markes) coupled to an Agilent 7890 GC-
121 5975 MSD. Primary cartridge desorption was 280 °C , cold-trapping at -10 °C , then desorption at 320 °C .
122 Separation employed an HP-5 MS ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) column. The GC oven temperature program
123 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}
124 to 260 °C (2 min). Identification used authentic standards, while quantification based on calibration curves.
125 More information about the analysis and quantification are given in Text S3 and Zeng et al. (2022a, 2022b).

126 2.4 Calculation of emission rates and emission factors

127 For branch data, emission rates (E , $\mu\text{g g}^{-1} \text{ h}^{-1}$) were calculated as

$$128 \quad E = \frac{F(C_{out} - C_{in})}{g_{dw}}$$

129 where F is chamber flow (L h^{-1}) and g_{dw} the dry leaf mass. ~~Branch chamber fluxes measured under ambient~~

130 conditions were standardized to 30 °C and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using the MEGAN/Guenther temperature and
131 light response functions, yielding E_g directly comparable to cuvette derived values. Real-world emission rates
132 were standardized to 30 °C and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR using the MEGAN algorithms for light dependent
133 compounds. The temperature- and light-dependent function was applied for light dependent compounds, while
134 and a temperature-only function was used for light-independent monoterpenes ($\beta = 0.10 \text{ K}^{-1}$) and
135 sesquiterpenes ($\beta = 0.17 \text{ K}^{-1}$). For cuvette data, the LI-6800 set points already represent standard conditions,
136 so E_g is equal to E_s after correcting for leaf dry weight. More details about the calculation of emission factors
137 are provided in Text S4 and in Zeng et al. (2023).

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

142 2.5 Statistical analysis

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

148 3. Results and discussion

149 3.1 Method inter-comparison for isoprene E_s

150 Side-by-side measurements of *E. urophylla* seedlings (2 months old) and 2-years-old trees showed that the
151 two dominant enclosure techniques yielded statistically indistinguishable isoprene E_s . As shown in Fig. 1, for
152 seedlings measured at GIG, cuvette-derived isoprene E_s averaged at $102.4 \pm 34.1 \mu\text{g g}^{-1} \text{h}^{-1}$ (N=50) versus
153 $107.7 \pm 34.9 \mu\text{g g}^{-1} \text{h}^{-1}$ from branch chambers (N=15), a non-significant 5% difference ($p=0.61$). In the field,
154 2-year-old trees exhibited similarly close agreement: $66.9 \pm 31.4 \mu\text{g g}^{-1} \text{h}^{-1}$ (N=114) from leaf cuvettes and
155 $69.8 \pm 21.2 \mu\text{g g}^{-1} \text{h}^{-1}$ (N=26) from branch chambers ($p=0.57$). A direct test in which the same branch was first
156 sub-sampled leaf-by-leaf and then enclosed intact confirmed parity within analytical uncertainty (Fig. S4).

157 Leaf cuvette and dynamic branch chamber are the two most widely employed techniques for measuring plant
158 BVOC emissions (Niinemets et al., 2011). The present study provides the first large-sample validation that
159 leaf cuvette and dynamic branch chamber protocols are interchangeable for isoprene, contradicting the ~75 %
160 bias inferred from the earlier literature meta-analysis (Guenther et al., 1994). The convergence arises despite
161 distinct air flow regimes (0.75 L min^{-1} vs. 9 L min^{-1}) and path lengths, implying that adsorptive/ozonolysis

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162 losses are negligible for this highly volatile compound. Because branch chambers integrate six to ten leaves
163 per branch, their representativeness is at least as good as that of cuvettes as previously demonstrated for
164 Eucalyptus by Zeng et al. (2024), and they remain advantageous when mature crowns are inaccessible.

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

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

178 **3.2 Ontogenetic controls on the magnitude and speciation of BVOC emissions**

179 **3.2.1 Speciation diversity**

180 Dynamic branch chamber measurements revealed 12 BVOC species in 2-month-old seedlings (isoprene, 8
181 monoterpenes, 3 sesquiterpenes; Fig. [S2S5](#)) versus 17 species in 2-year-old trees (isoprene, 11 monoterpenes,
182 5 sesquiterpenes; Table [S12](#)). Thus, chemical richness increased by ~40% with age, consistent with the view
183 that metabolic complexity develops as trees mature (Satake et al., 2024).

184 **3.2.2 Emission magnitude**

185 Isoprene dominated the flux from both age classes but its standardized E_s declined significantly ($p < 0.001$)
186 from $107.7 \pm 34.9 \mu\text{g g}^{-1} \text{h}^{-1}$ in seedlings to $69.8 \pm 21.2 \mu\text{g g}^{-1} \text{h}^{-1}$ in two-year-old trees. When normalized by
187 leaf area, however, the two groups were indistinguishable (Fig. [2AS6a](#)), indicating that the mass-based
188 contrast mainly reflects an increase in leaf mass per area (LMA) with tree age (Fig. [S7](#)) (Fig. [S3](#)). Seedlings
189 therefore allocated a larger fraction of assimilated carbon to isoprene (Fig. [S6c2e](#)) despite exhibiting lower
190 net photosynthesis (P_n , Fig. [S6b2b](#)), suggesting a typical growth-defense trade-off of early ontogeny.
191 Consistent with our findings, isoprene E_s for the 1-year-old *E. globulus* was 5-fold higher than that for the 7-
192 year-old individual (Street et al., 1997). Winters et al. (2009) also documented lower isoprene E_s in four 10-
193 year-old eucalyptus species compared to their seedlings measured by He et al. (2000).

194 3.2.3 Terpene speciation shift

195 Total monoterpene emissions rose nearly 6-fold with tree age (1.09 vs. 6.14 $\mu\text{g g}^{-1} \text{h}^{-1}$), but the increase was
196 almost entirely due to acyclic β -ocimenes, whose E_s leapt from 0.13 \pm 0.06 to 5.33 \pm 4.61 $\mu\text{g g}^{-1} \text{h}^{-1}$ (Fig. 2 and
197 Table S1). By contrast, E_s for cyclic α -pinene, limonene, and 1,8-cineole declined by 30-65% (Fig. 32).
198 Consequently, terpene composition shifted from 75% cyclic (seedlings) to 85% acyclic (2-year-old trees)
199 (Fig. 32). Sesquiterpenes doubled in absolute terms but remained a minor (<1%) proportion of total BVOCs.

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

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

215 Environmental factors, particularly growth temperature, light availability, and soil moisture/nutrients, can
216 modulate BVOC emissions (Monson et al., 1994; Harley et al., 1994, 1996, 1997; Fall and Wildermuth, 1998;
217 Funk et al., 2006; Guenther et al., 2006, 2012; Yuan et al., 2020). In our study, both campaigns were
218 conducted under clear-sky, peak-summer conditions with comparable ambient temperature and high midday
219 PAR (Figs. S1-S3). A simple sensitivity using the MEGAN/Guenther acclimation scheme (Guenther et al.,
220 2012) indicates that, a ~10% change in growth PAR would change standardized E_s by ~8%, and a +1.1 °C
221 change in growth temperature by ~11%. These effects are small relative to the age-related differences
222 reported here, such as ~6-fold increase in total monoterpenes and a >40-fold rise in β ocimenes from seedlings
223 to saplings (Fig. 2 and Table S1). Nevertheless, because we did not measure soil nutrient or moisture status,
224 parallel experiments under controlled and co located growth conditions are warranted to fully disentangle
225 ontogeny from environment.

226 It should be noted that the seedlings used here were cultivated trees, where soil properties could be parallel

227 with those in previous seedling-based studies. In contrast, the taller trees measured under field conditions
228 reflect realistic and natural growth conditions. The substantial differences in E_g and emission composition
229 between seedlings and saplings underscore that seedling measurements are inappropriate as generic
230 surrogates for natural forest emissions.

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

237 **4. Conclusions**

238 Parallel measurements on *E. wrophylla* seedlings (2 months old) and saplings (2 years old) demonstrate that
239 leaf level cuvettes and dynamic branch chambers yield statistically indistinguishable isoprene E_g . This large
240 sample validation may resolve a long-standing methodological debate and confirms that both enclosure types
241 can be used interchangeably for the most abundant BVOC when appropriate QA/QC protocols are followed
242 (Zeng et al., 2022a, 2025c). For monoterpenes and sesquiterpenes, assessment of method equivalence was
243 precluded by their lower fluxes and the limited air volumes sampled. Because terpene emissions are more
244 sensitive to wall losses and analytical detection limits than isoprene, systematic inter-comparison studies with
245 larger sample volumes and a broader range of plant species are still required.

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

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259 Emission profiles changed markedly among age classes. Seedlings emitted ~50% more mass-based isoprene

260 and were enriched in cyclic monoterpenes (α -pinene and 1,8-cineole), whereas saplings produced 6 fold
261 higher total monoterpenes dominated by highly reactive acyclic β -ocimenes and exhibited a double rise in
262 sesquiterpenes. This shift likely implies a developmental re-allocation of carbon from generalized antioxidant
263 protection (isoprene) towards compounds better suited to coping with the more intense and severe biotic and
264 abiotic stresses experienced by field-grown trees.

265 Roughly one-third of the global BVOC E_g entries now feeding chemistry-climate models originate from
266 greenhouse seedlings. Our results show that these data tend to overestimate canopy-scale isoprene fluxes and
267 under-represent atmospheric reactivity by excluding large β -ocimene emissions from mature canopies.
268 Seedling measurements are therefore inappropriate as generic surrogates for natural forests. Incorporating
269 age-resolved E_g and prioritizing statistically robust and in-situ sampling of branches from adult trees,
270 particularly in rapidly expanding subtropical plantations, will reduce inventory uncertainty and refine
271 predictions of ozone and secondary organic aerosol formation.

272 Future studies should extend age-stratified measurements to other high isoprene genera (e.g. *Quercus*,
273 *Populus*) and to tropical species that dominate global BVOC budgets, evaluate method comparability for
274 low-volatility terpenes using larger-volume or adsorption-minimized chambers, and couple physiological
275 measurements with transcriptomics to unravel the molecular basis of the observed metabolic shift from cyclic
276 to acyclic terpenes.

277

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

280 **Supplement.** The related supplement is published alongside this article.

281 **Author contributions.** JZ and XT designed and carried out the experiments with the support of WP, YL, HR,
282 ZM, HG, and WS. JZ and XT analyzed the samples in the lab. JZ and XT analyzed the data and prepared the
283 original manuscript. XW and YZ revised the manuscript.

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

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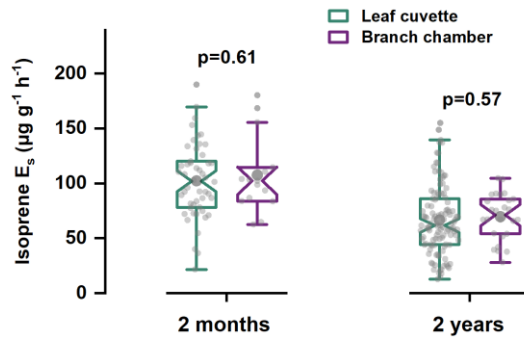
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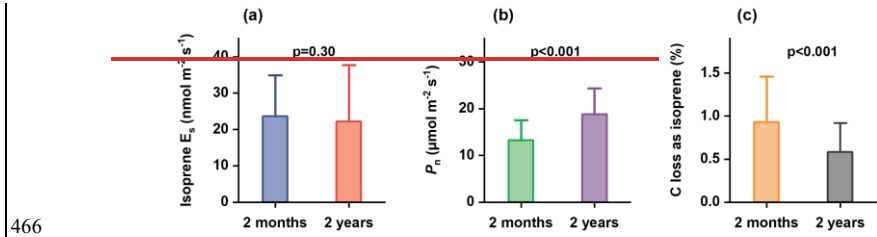
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462

463 **Figure 1.** Comparison of isoprene E_s from leaf cuvettes with those from dynamic branch chambers for both
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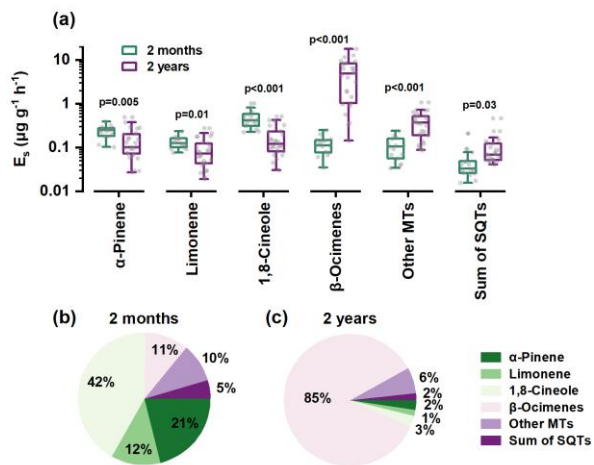
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467 **Figure 2.** Comparison of area-based isoprene E_s (a), net photosynthetic rate (P_n , b), and carbon loss fraction
 468 as isoprene emission (c) for 2-month-old trees with those for 2-year-old ones. These data were from the leaf
 469 cuvette measurements.

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471

472 **Figure 32.** Comparison of emission factors (a) and compositions (b,c) between 2-month-old and 2-year-old
 473 trees (a). Terpene composition for the 2-month-old trees (b) and 2-year-old trees (c).

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475

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

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477

Table 2. 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