

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

31

32 **1 Introduction**

33 Terrestrial vegetation release on the order of $\sim 1,000$ 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 emission factors are now recognized as a leading source of uncertainty in regional
43 and global estimates of BVOC budgets (Guenther et al., 2012; Zhang et al., 2020; Wang et al., 2023). Decades
44 of 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 (15 for branch
81 chamber and 50 for leaf cuvette) measured in laboratory and 2-year-old saplings (26 for branch chamber and
82 114 for leaf cuvette) measured in situ at the plantation. Seedlings were purchased from a local nursery and
83 measured by both leaf cuvette and dynamic branch chamber. These trees were placed in an open area of GIG
84 and acclimated to the ambient condition for two weeks before measurements; no greenhouse or climate-
85 chamber but outdoor conditions were used. Field trees for leaf cuvette were randomly chosen from >8 ha of
86 homogeneous plantation to ensure spatial representativeness, while sunlit, single-layer branches were
87 selectively chosen for dynamic chamber measurements. Both seedlings and saplings were sourced from the
88 same clonal line and exhibited a uniform terpene chemotype, minimizing genotype/chemotype variability.
89 The clonal identity of all seedlings and saplings was confirmed using nursery propagation records and
90 plantation establishment documents. This is also supported by the similar monoterpene composition between
91 stressless saplings and seedlings (detailed discussions see Sect. 3.2.3). All measurements, both in the
92 laboratory and in the field, were conducted between 9:00 and 17:00 local time under sunny conditions, with
93 33%, 56%, 10% of the samples collected in the morning (9:00-12:00), midday (12:00-15:00), and late
94 afternoon (15:00-17:00), respectively, in the field. The real-world (non-normalized) emission rates of BVOCs
95 by time-of-day bins was provided in Fig. S1. Midday maximum PAR exceeded 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during both
96 campaigns (Figs. S2 and S3). Mean daily air temperatures during the campaigns were 31.4 °C (seedling
97 campaign at GIG) and 30.3 °C (Field campaign; Fig. S4).

98 **2.2 Enclosure measurements**

99 **2.2.1 Leaf cuvette**

100 Leaf cuvette fluxes were obtained with a LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE,
101 USA) fitted with a 6800-01A fluorometer head (aperture 6 cm², air flow 500 μmol s⁻¹). We imposed standard
102 conditions for emission factors (E_s): leaf temperature 30 °C, PAR 1000 μmol m⁻² s⁻¹, CO₂ 400 μmol mol⁻¹,
103 and 55% RH. The LI-6800 maintains closed loop control and continuously records actual leaf temperature
104 and PAR. Because measurements were made at the standard reference conditions, cuvette fluxes equal E_s
105 after correction to leaf dry mass. After 5 min stabilization of photosynthesis, 200 mL min⁻¹ of outlet air was
106 diverted for 2 min through Tenax TA/Carbograph 5TD adsorbent cartridges (Markes International Ltd,
107 Bridgend, UK) using a dual-channel pump (ZC-QL, Zhejiang Hengda Instrumentation Ltd., Zhejiang, China).
108 Detailed leaf handling, leaf area and dry mass determination are provided in Text S1 and are also described
109 in Zeng et al. (2024, 2025ab).

110 **2.2.2 Dynamic branch chamber**

111 A cylindrical PMMA chamber (Ø 25 cm × 28 cm, 13.7 L) internally coated with FEP film was used for
112 branch-level measurements. The charcoal- and KI-scrubbed ambient air (9 L min⁻¹) was supplied by a mass-
113 flow controller (Alicat Scientific, Inc., Tucson, AZ, USA) coupled with an oil-free pump (MPU2134-N920-
114 2.08; KNF, Freiburg, Germany), then it was well mixed with PTFE-bladed fans in the chamber (Zeng et al.,
115 2022a). For comparability with leaf cuvette measurements, sunlit branches (3-5 m above ground) with ~6-10
116 leaves were selected, avoiding mutual overlap so that foliage formed a single layer. Branches showing visible
117 self-shading were not sampled. These practices follow our goal of minimizing light heterogeneity within the
118 chamber. When the selected sunlit branches were enclosed; fluxes were allowed to stabilize for 1-2 h before
119 sampling. Outlet air (and inlet blanks) was drawn at 200 mL min⁻¹ for 10 min by an automatic sampler
120 (JEC921; Jectec Science and Technology, Co., Ltd, Beijing, China) onto the same adsorbent tubes as above.
121 The chromatograms of a representative BVOC sample and its corresponding blank sample of inlet air during
122 the field study was shown in Fig. S5. Concurrent meteorological and radiometric variables were logged
123 continuously (Rotronic HC2A-S RH/T probes; LI-1500 PAR sensor; OMEGA/RKC thermocouples).
124 Specifically, leaf temperature was measured by thermocouples attached on the leaf, while PAR was measured
125 by a LI-1500 PAR sensor placed on the top of the chamber, these parameters were used to calculate the
126 standard emission factors (E_s). More details about the branch sampling are provided in Text S2 and Zeng et
127 al. (2022a).

128 **2.3 Thermal desorption-GC/MS analysis**

129 Tubes were analyzed within 7 days with a TD-100 system (Markes) coupled to an Agilent 7890 GC-
130 5975 MSD. Primary cartridge desorption was 280 °C, cold-trapping at -10 °C, then desorption at 320 °C.
131 Separation employed an HP-5 MS (30 m × 0.25 mm × 0.25 μm) column. The GC oven temperature program
132 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

133 to 260 °C (2 min). Identification used authentic standards, while quantification based on calibration curves.
134 Fig. S6 shows the calibration curve for β -ocimene (cis- and trans-) as an example. Ocimene-like compounds
135 such as 3,6-dimethyl-1,3,7-octatriene, 3,4-dimethyl-2,4,6-octatriene, and alloocimene were identified by the
136 NIST library and quantified based on β -ocimene. More information about the analysis and quantification of
137 other MTs are given in Text S3 and Zeng et al. (2022a, 2022b).

138 2.4 Calculation of emission rates and emission factors

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

$$140 \quad E = \frac{F(C_{out} - C_{in})}{g_{dw}}$$

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

148 Notably, E_s 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
149 leaf mass per area (LMA, g m^{-2}) increases with age in *E. urophylla* (Table 1), the two normalizations
150 emphasize different aspects of physiology and scaling ($E_s\text{-area} = E_s\text{-mass} \times \text{LMA}$). To remove duplication
151 and prevent unit confusion, we only report mass-based E_s in the main text and present all area-based values
152 in the Supplement.

153 2.5 Statistical analysis

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

159 3. Results and discussion

160 3.1 Method inter-comparison for isoprene E_s

161 Side-by-side measurements of *E. urophylla* seedlings (2 months old) and 2-years-old trees showed that the
162 two dominant enclosure techniques yielded statistically indistinguishable isoprene E_s . As shown in Fig. 1, for

163 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
164 $107.7 \pm 34.9 \mu\text{g g}^{-1} \text{h}^{-1}$ from branch chambers (N=15), a non-significant 5% difference ($p=0.61$). In the field,
165 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
166 $69.8 \pm 21.2 \mu\text{g g}^{-1} \text{h}^{-1}$ (N=26) from branch chambers ($p=0.57$). A direct test in which the same branch was first
167 sub-sampled leaf-by-leaf and then enclosed intact confirmed parity within analytical uncertainty (Fig. S7).

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

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

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

188 **3.2 Ontogenetic controls on the magnitude and speciation of BVOC emissions**

189 **3.2.1 Speciation diversity**

190 Dynamic branch chamber measurements revealed 12 BVOC species in 2-month-old seedlings (isoprene, 8
191 monoterpenes, 3 sesquiterpenes; Fig. S8) versus 17 species in 2-year-old trees (isoprene, 11 monoterpenes,
192 5 sesquiterpenes; Table S1). Thus, chemical richness increased by ~40% with age, consistent with the view
193 that metabolic complexity develops as trees mature (Satake et al., 2024).

194 **3.2.2 Emission magnitude**

195 Isoprene dominated the flux from both age classes but its standardized E_s declined significantly ($p < 0.001$)
196 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
197 leaf area, however, the two groups were indistinguishable (Fig. S9a), indicating that the mass-based contrast
198 mainly reflects an increase in leaf mass per area (LMA) with tree age (Fig. S10). Seedlings therefore allocated
199 a larger fraction of assimilated carbon to isoprene (Fig. S9c) despite exhibiting lower net photosynthesis (P_n ,
200 Fig. S9b), suggesting a typical growth-defense trade-off of early ontogeny. Consistent with our findings,
201 isoprene E_s for the 1-year-old *E. globulus* was 5-fold higher than that for the 7-year-old individual (Street et
202 al., 1997). Winters et al. (2009) also documented lower isoprene E_s in four 10-year-old eucalyptus species
203 compared to their seedlings measured by He et al. (2000).

204 3.2.3 Terpene speciation shift

205 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
206 almost entirely due to acyclic β -ocimenes, whose E_s leapt from 0.13 ± 0.06 to $5.33 \pm 4.61 \mu\text{g g}^{-1} \text{h}^{-1}$ (Fig. 2 and
207 Table S1). By contrast, E_s for cyclic α -pinene, limonene, and 1,8-cineole declined by 30-65% (Fig. 2).
208 Consequently, terpene composition shifted from 75% cyclic (seedlings) to 85% acyclic (2-year-old trees)
209 (Fig. 2). Sesquiterpenes doubled in absolute terms but remained a minor (<1%) proportion of total BVOCs.

210 During the branch experiments, both seedlings and saplings were exposed to high growth temperature
211 conditions, which could lead to significant stress on plant physiology as discussed by Zeng et al. (2025d).
212 This was evidenced by the substantial emissions of typical stress-induced β -ocimene from saplings. However,
213 although seedlings were also subjected to heat stress, they did not exhibit similarly high β -ocimene emissions
214 but dominated by α -pinene and 1,8-cineole, closely resembling those under non-stress saplings measured
215 during the warm dry season (Fig. S11). This indicates that stress-induced gene expression may require time
216 to accumulate, and that seedlings grown in greenhouses, having not experienced environmental stresses, may
217 not adequately represent the emission characteristics of trees in natural field conditions.

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

226 Both isoprene and β -ocimenes are known to play key roles in plant antioxidant defense. Isoprene helps
227 scavenge reactive oxygen species (ROS) (Jardine et al., 2014), maintain membrane stability (Sharkey and

228 Singsaas, 1995; Pollastri et al., 2019, 2021), and regulate antioxidative processes (Zuo et al., 2019; Monson
229 et al., 2021), whereas the highly reactive β -ocimene may act as an even more efficient, direct antioxidant
230 against ROS. The highly reactive β -ocimenes can quench ROS more efficiently than isoprene
231 (Pollastri et al., 2021), providing a plausible advantage for field-grown trees exposed to stronger light, heat,
232 drought, and herbivory.

233 Environmental factors, particularly growth temperature, light availability, and soil moisture/nutrients, can
234 modulate BVOC emissions (Monson et al., 1994; Harley et al., 1994, 1996, 1997; Fall and Wildermuth, 1998;
235 Funk et al., 2006; Guenther et al., 2006, 2012; Yuan et al., 2020). In our study, both campaigns were
236 conducted under clear-sky, peak-summer conditions with comparable ambient temperature and high midday
237 PAR (Figs. S1-S3). A simple sensitivity using the MEGAN/Guenther acclimation scheme (Guenther et al.,
238 2012) indicates that, a $\sim 10\%$ change in growth PAR would change standardized E_s by $\sim 8\%$, and a $+1.1^\circ\text{C}$
239 change in growth temperature by $\sim 11\%$. These effects are small relative to the age-related differences
240 reported here, such as ~ 6 -fold increase in total monoterpenes and a >40 -fold rise in β ocimenes from seedlings
241 to saplings (Fig. 2 and Table S1). Nevertheless, because we did not measure soil nutrient or moisture status,
242 parallel experiments under controlled and co-located growth conditions are warranted to fully disentangle
243 ontogeny from environment.

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

250 Notably, we counted the most complete E_s database currently used by the MEGAN model (Wiedinmyer et
251 al., 2004; Guenther et al., 2012), showing that one-third of the publications in this database were based on
252 greenhouse seedlings. Our results show that these data tend to overestimate canopy-scale isoprene fluxes of
253 subtropical eucalyptus plantation and under-represent atmospheric reactivity by excluding large β -ocimene
254 emissions from these tree canopies. Incorporating age-resolved E_s and prioritizing statistically robust and in-
255 situ sampling of branches from adult trees, particularly in rapidly expanding subtropical plantations, will
256 reduce inventory uncertainty and refine predictions of ozone and secondary organic aerosol formation.

257 **4. Conclusions**

258 Parallel measurements on *E. urophylla* seedlings (2 months) and saplings (2 years) show that leaf cuvettes
259 and dynamic branch chambers yield indistinguishable isoprene E_s when applied to sunlit, single-layer
260 branches. Age exerts a strong control on speciation: seedlings have higher mass-based isoprene and are

261 enriched in cyclic monoterpenes, whereas saplings exhibit ~6-fold higher total monoterpenes dominated by
262 β -ocimenes and increased sesquiterpenes. These findings indicate that laboratory-grown seedlings are not
263 reliable proxies for field-grown saplings, and likely not for mature trees either. Despite this, other drivers,
264 particularly soil properties (e.g., nitrogen availability, moisture) were not resolved here and may also
265 influence emissions. This therefore motivates age-stratified and in-situ branch measurements on mature trees
266 under parallel growth conditions to comprehensively probe age effects. Future studies should extend such
267 measurements to other high isoprene genera (e.g. Quercus, Populus) and to tropical species that dominate
268 global BVOC budgets, evaluate method comparability for low-volatility terpenes using larger-volume or
269 adsorption-minimized chambers, and couple physiological measurements with transcriptomics to unravel the
270 molecular basis of the observed metabolic shift from cyclic to acyclic terpenes.

271

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

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

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

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

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285 **References**

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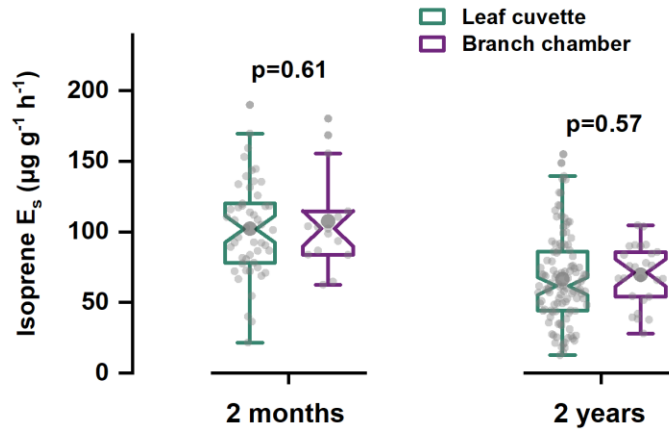
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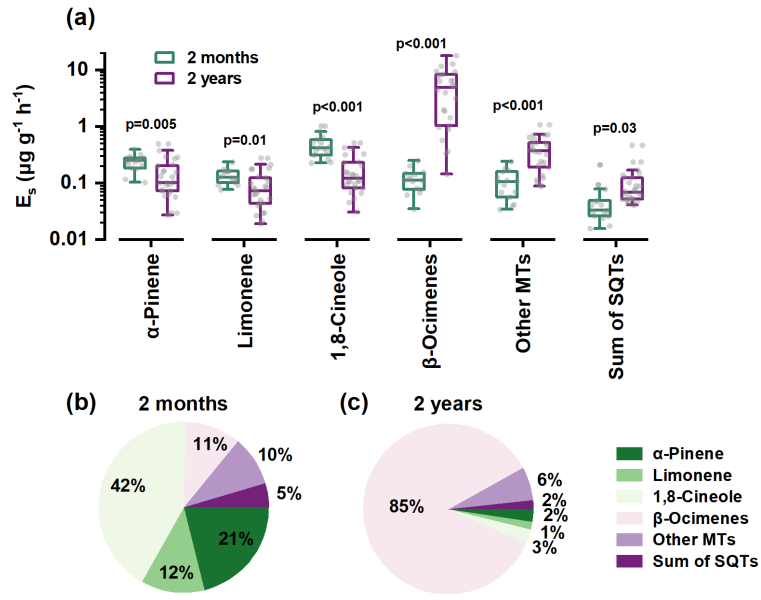
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461 **Figure 1.** Comparison of isoprene E_s from leaf cuvettes with those from dynamic branch chambers for both
 462 2-month-old and 2-year-old trees.

463



464

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

467

468

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

469