Reply to comments from Referee #3

This manuscript presents parallel BVOC emission factor measurements for Eucalyptus urophylla using leaf cuvettes and dynamic branch chambers, comparing 2-month-old seedlings (laboratory campaign) with 2-year-old field saplings (plantation campaign). The dataset seems valuable and aims to address two important uncertainties for emission factor databases and model parameterizations: (i) method comparability and (ii) potential size/young-plant effects. I agree with the points raised by Reviewers #1 and #2, and I summarize my additional detailed concerns below, based on the authors' responses and the manuscript.

1) Provide more detail on field measurement conditions and within-chamber microclimate

Seedlings were measured in a laboratory campaign, while saplings were measured in situ at a plantation. Even if emissions are standardized to reference conditions (30 °C, 1000 PPFD), long-term growth environment (light history, temperature history, VPD), as well as unmeasured stressors (soil moisture/nutrients), can influence emission capacities and enzyme expression. Referees #1 and #2 already flagged this as a critical issue.

Given that field measurements were performed in summer and largely under sunny conditions (per your response to Referee #1), potential heat and water stress may also influence emissions and speciation. Therefore, I strongly recommend adding more quantitative information, including:

- How many trees were selected and how selection was randomized (or otherwise controlled).
- Distribution of measurement times (e.g., as you mentioned period '09:00-17:00'): what fraction of observations were collected during 12:00-15:00 vs morning/late afternoon?
- Within-chamber air temperature and RH during measurements (especially midday), and their distributions.
- Variability of observed emissions by time-of-day bins (morning/midday/afternoon) as Supplement.

The above points will strengthen interpretation and help the reader evaluate whether stress conditions contributed to the observed differences.

Reply: We appreciate this opportunity to clarify these concerns raised by Referee #3. Following your suggestions, below is the point-to-point response:

➢ For sapling measurements in the field, 26 trees were measured by dynamic branch chamber and 114 trees were measured by leaf cuvette; For seedling measurements at GIG, 15 trees were measured by dynamic chamber while 50 trees were measured by leaf cuvette. In the field, only the 114 trees for leaf cuvette were randomly selected to cover the whole plantation, while sunlit, single-layer branches were selectively chosen for dynamic chamber measurements to ensure method comparability with the leaf cuvette.

We have clarified these in the revised manuscript.

"*As shown in Table 1, two age classes, 2-month-old and 2-year-old, were investigated, with seedlings (15 for branch chamber and 50 for leaf cuvette) measured in laboratory and 2-year-old saplings (26 for branch chamber and 114 for leaf cuvette) measured in situ at the plantation.*" (Lines 80-82)

"*Field trees for leaf cuvette were randomly chosen from >8 ha of homogeneous plantation to ensure spatial representativeness, while single-layer, sunlit branches were selectively chosen for dynamic chamber measurements.*" (Lines 85-87)

- In the field measurements, 33% of the samples were collected in the morning (9:00-12:00), 56% during the midday (12:00-15:00), and the rest 10% in the late afternoon (15:00-17:00). We clarified this in the latest text.

"*All measurements, both in the laboratory and in the field, were conducted between 9:00 and 17:00 local time under sunny conditions, with 33%, 56%, 10% of the samples collected in the morning (9:00-12:00), midday (12:00-15:00), and late afternoon (15:00-17:00), respectively, in the field.*" (Lines 92-94)

- Within-chamber air temperature and RH were provided in Fig. R1.

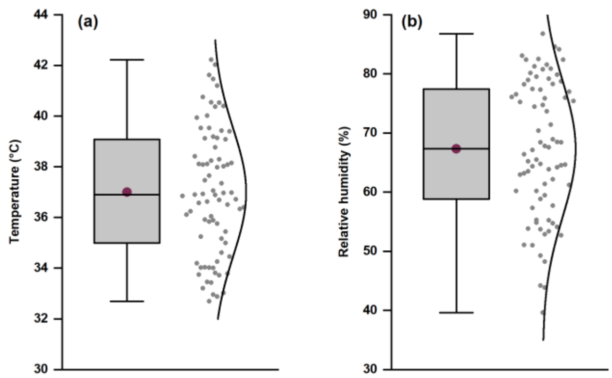

Figure R1. Within-chamber air temperature and RH during the field measurement

- Variability of observed real-world (non-normalized) emission rates of BVOCs by time-of-day bins was provided in Fig. S1 and cited in the latest manuscript.

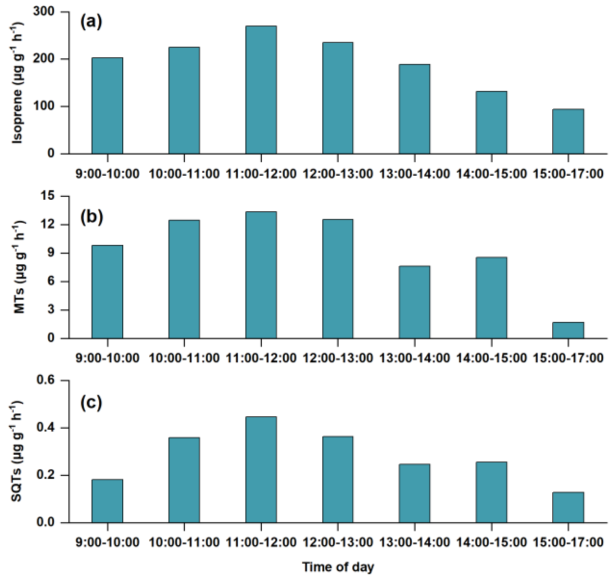

Figure S1. Mean isoprene (a), MT (b), and SQT (c) emission rates over time of the day for field measurements

2) Genotype/chemotype control: Your response indicates seedlings and saplings came from the same clonal line, reducing genotype/chemotype variability. This addresses a key concern raised by Referee #2. The authors did explained in their previous reply, but statement is not good enough to your audiences. Could you add one sentence describing how this was verified (nursery documentation and/or plantation records)? And if possible, report terpene composition variability within each cohort (e.g., dominant compounds and variability ranges) to demonstrate chemotype consistency.

Reply: We thank the reviewer for this helpful suggestion to improve clarity. In the revised manuscript, we have added a sentence in Sect. 2.1 "*Both seedlings and saplings were sourced from the same clonal line and exhibited a uniform terpene chemotype, minimizing genotype/chemotype variability. The clonal identity of all seedlings and saplings was confirmed using nursery propagation records and plantation establishment documents.*" (Lines 89-90)

Actually, the clonal identity is also supported by the similar terpene composition between seedlings and saplings measured during wintertime, both of them are stressless before measurement and dominated by cyclic α-pinene and 1,8-cineole as shown in Fig. S11. Although during summer measurements both seedlings and saplings experienced high temperatures that could cause heat stress, the large emission of stress-relevant β-ocimenes in field saplings while little emission in seedlings indicate that stress-induced gene expression may require time to accumulate.

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

Reference:

Zeng, J., Zhang, Y., Pang, W., Ran, H., Mu, Z., Guo, H., Lu, Y., Song, W., and Wang, X.: Contrasting Emission seasonality between light-dependent and light-independent biogenic VOCs from subtropical Eucalyptus trees, J. Geophys. Res. Atmos., 130, e2025JD043387, https://doi.org/10.1029/2025jd043387, 2025d.

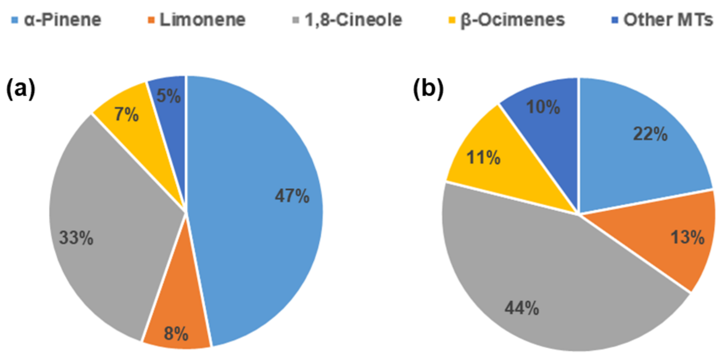

Figure S11. Monoterpene composition of saplings (a) in winter and seedlings (b) in summer. Both of them are stressless before measurements.

3) Standardization and "leaf temperature" should be unambiguous

The standardization approach is consistent with established EF protocols. However, the manuscript should clearly state whether "temperature" in branch chambers refers to leaf temperature or chamber air temperature, and how PAR at the leaf surface was assessed.

So, please consider:

- Specify whether leaf temperature was directly measured (e.g., thermocouple on leaf) or inferred from air temperature.
- If leaf temperature was not directly measured, report chamber air temperature during measurements (especially midday for field saplings) and discuss implications for standardization uncertainty.
- For lab seedlings, briefly describe any pre-treatment/conditioning (e.g., watering schedule, light acclimation, duration before measurements), since this can influence emission capacity.

Reply: As stated in Text S2, both leaf temperature and air temperature were measured, and the leaf temperature was used to calculate the E_s. PAR sensor was placed on the top of the chamber. Our chamber has a light transmittance of ~92%, so we didn't do any calibration and used the raw PAR data to calculate the E_s.

We clarified this in the revised manuscript: "*Specifically, leaf temperature was measured by thermocouples attached on the leaf, while PAR was measured by a LI-1500 PAR sensor placed on the top of the chamber, these parameters were used to calculate the standard emission factors (E_s).*" (Lines 124-126)

"*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 with the measured leaf temperature and PAR, yielding E_s directly comparable to cuvette derived values.*" (Lines 142-144)

For lab seedlings, these trees were not pre-treated/conditioned after purchasing from the local nursery, we only placed then in the open area of GIG and let them acclimate the ambient for two weeks; these is no greenhouse or climate-chamber conditions.

We have added this information in the following: "*These trees were placed in an open area of GIG and acclimated to the ambient condition for two weeks before measurements; no greenhouse or climate-chamber but outdoor conditions were used.*" (Lines 83-85)

4) Terpene speciation (β-ocimene dominance) requires stronger QA/QC reporting

The finding that saplings emit higher total monoterpenes dominated by β-ocimenes is interesting, but ocimene isomer identification can be challenging and potentially sensitive to analytical artifacts. Also, beta-ocimene can be a stress compounds, especially from wound tree, e.g. Yuan et al., 2025 (https://doi.org/10.3390/plants14050821), Fäldt et al., 2003 (DOI: 10.1007/s00425-002-0924-0).

I would like to know more details from the authors:

- Provide compound-class LOD/LOQ (or at minimum detection frequency and blank levels).
- Clarify identification approach for ocimenes: standards used? qualifier ions? Retention criteria?

Reply: In this study, the LOD of β-ocimenes was ~8 ng m^{-3} based on a sample volume of 2 L. Fig. S5 shows the chromatograms of a representative BVOC sample and its corresponding inlet blank sample during the field study. It shows that signals of β-ocimenes (especially trans-β-ocimene) are significantly higher than other compounds and the blank sample. Moreover, the identification was based on authentic standard of mixture of cis- and trans-β-ocimene. Quantification was based on calibration curve with ion of m/z 93, which was shown in Fig. S6.

We identified cis- and trans- isomers based on three steps: 1) we first searched the NIST library, the match similarity was >95%; 2) we used a nonpolar HP-5 chromatographic column, and cis- isomer has a slightly lower boiling point, so it will theoretically peak former than the trans- isomer; 3) we finally used a standard mixture of cis-β-ocimene and limonene, which explicitly determined the peak position for cis- and trans- isomers. In addition, 3,6-Dimethyl-1,3,7-octatriene, 3,4-Dimethyl-2,4,6-octatriene, and Alloocimene have a similar acyclic structure like β-ocimenes, which could be the byproducts of β-ocimene synthesis (Li et al., 2017). We identified them based on the NIST library with match similarity >95%, while their quantifications were based on β-ocimene.

We clarified this in the latest manuscript:

"*...onto the same adsorbent tubes as above. The chromatograms of a representative BVOC sample and its corresponding inlet blank sample during the field study was shown in Fig. S5. Concurrent meteorological and radiometric variables...*" (Lines 121-122)

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

Reference:

Li, M., Xu, J., Algarra Alarcon, A., Carlin, S., Barbaro, E., Cappellin, L., Velikova, V., Vrhovsek, U., Loreto, F., and Varotto, C.: In Planta Recapitulation of Isoprene Synthase Evolution from Ocimene Synthases, Mol Biol Evol, 34, 2583-2599, 10.1093/molbev/msx178, 2017.

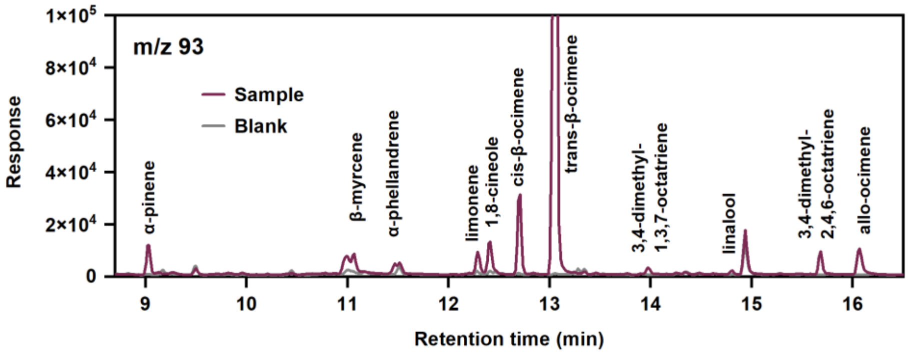

Figure S5. Chromatograms of a representative BVOC sample and its corresponding inlet blank sample

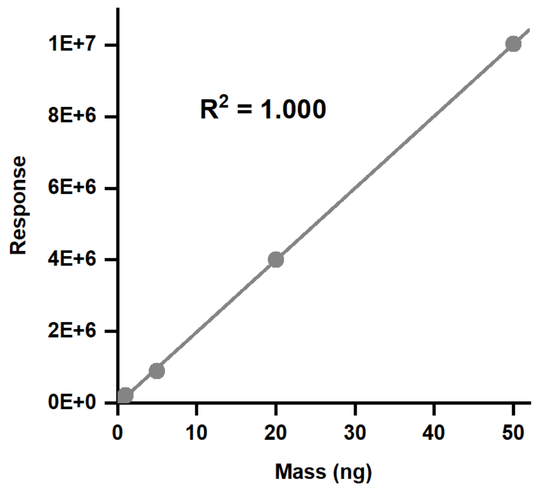

Figure S6. Calibration curve for β-ocimene

5) The "age effect" is coupled with growth environment; conclusions should be softened or supported with additional constraints

The manuscript states: "*Roughly one-third of the global BVOC Es entries now feeding chemistry-climate models originated from greenhouse seedlings*." Please provide one or more references that support this quantitative statement (including how "seedlings" are defined if not same like your measurement stated, and which EF database/model compilation is being referred to).

Reply: Here we refer to the UCAR E_s database that used by the MEGAN model. This database is the most complete and continuously updated one. We counted the references therein, and found that 1/3 of them are derived from greenhouse seedlings. We revised this sentence and cite related references:

"*Notably, we counted the most complete E_s database currently used by the MEGAN model (Wiedinmyer et al., 2004; Guenther et al., 2012), showing that one-third of the publications in this database were based on greenhouse seedlings.*" (Lines 250-252)

References:

Wiedinmyer, C., Guenther, A., Harley, P., Hewitt, C.N., Geron, C., Artaxo, P., Steinbrecher, R., Rasmussen, R.: Global organic emissions from vegetation. In: Granier, C., et al. (Eds.), Emissions of Atmospheric Trace Compounds. Kluwer Publishing Co, Dordrecht, The Netherlands, pp. 115-170, 2004.

Guenther, A. B., Jiang, X., Heald, C. L., Sakulyanontvittaya, T., Duhl, T., Emmons, L. K., and Wang, X.: The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions, Geosci. Model Dev., 5, 1471-1492, https://doi.org/10.5194/gmd-5-1471-2012, 2012.

The manuscript further concludes that current models may "*overestimate canopy-scale isoprene fluxes and under-represent atmospheric reactivity … from mature canopies*." This statement is too strong as written for two main reasons: 1) BVOC emission patterns are highly species-specific, and many tree species are monoterpene emitters with negligible isoprene (e.g., *Pinus pinea*, *Pinus sylvestris*). Therefore, the present measurements primarily constrain the tested species (*E. urophylla*) and should not be generalized broadly without careful qualification. 2) The two age classes studied (2 months and 2 years) are both relatively young; therefore, statements about "mature canopies" should be avoided unless supported by external evidence for older age classes of the same species or closely related taxa.

We sincerely thank the referee for this critical and constructive feedback regarding the framing of our conclusion. The referee is absolutely correct that our original statement was overly broad. Following your suggestion, we have revised the original sentence to:

"*Our results show that these data tend to overestimate canopy-scale isoprene fluxes of subtropical eucalyptus plantation and under-represent atmospheric reactivity by excluding large β-ocimene emissions from these tree canopies.*" (Lines 252-254)

"These ontogenetic shifts imply that one-third of the entries in global E_s compilations, which are derived from seedling studies, *are likely inappropriate as generic surrogates for natural forest emissions* ~~overestimate local isoprene fluxes while under-representing the atmospheric reactivity of mature canopies.~~" (Lines 23-25)

Others:

1) Nice modify on the exact sampling timeline (dates and gaps) clearly visible in Methods (as requested by Referee #1). And state clear how many seedlings and how many saplings were measured, and the selection criteria.

Reply: For sapling measurements in the field, 26 trees were measured by dynamic branch chamber and 114 trees were measured by leaf cuvette; For seedling measurements at GIG, 15 trees were measured by dynamic chamber while 50 trees were measured by leaf cuvette.

We have clarified these in the revised manuscript.

"*As shown in Table 1, two age classes, 2-month-old and 2-year-old, were investigated, with seedlings (15 for branch chamber and 50 for leaf cuvette) measured in laboratory and 2-year-old saplings (26 for branch chamber and 114 for leaf cuvette) measured in situ at the plantation.*" (Lines 80-82)

2) Use consistent terminology: seedlings and saplings/young trees, and avoid "mature trees/canopies" unless you provide supporting data/literature.

Reply: Thank you for the constructive suggestion. We have revised them in the latest manuscript.