Reply to comments from Referee #1

This research is a fundamental study in the field of BVOCs, highlighting the previously overlooked emission factors and potential variations in the composition of BVOCs with tree age in previous studies. It also clarifies to a certain extent issues such as the comparability of different sampling methods. Overall, the research has good innovation, especially for model users and developers, it has very good enlightenment and guidance significance. However, the manuscript still requires some revision to enhance its reliability, make the manuscript's logic more complete, and enrich the content to reach the publication standard.

1. The manuscript lacks a description of the specific sampling times. When were the leaf samples and branch samples collected? How many days apart were this two methods?

Reply: We thank the reviewer for this comment. We now report the exact dates for each campaign and clarify the interval between methods. **Laboratory**: leaf cuvette measurements were conducted on 1-3 June 2023 and branch chamber measurements one week later (10-13 June 2023; 7-day gap). **Field**: leaf and branch experiments were performed concurrently on the same days during two campaigns on 11-13 July and 26-31 July 2022. These details have been added to Section 2.1 of the revised manuscript:

"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.*" (Lines 73-78)

2. When using the Li-6800 to take samples at the leaf level, the environmental temperature was set at 30°C and 1000 mol m-2 s-1. If the growth environment of the seedlings was not strictly 30°C and 1000 mol m-2 s-1, then the actual leaf temperature would definitely not be equal to the set value, and thus the set value could not be used as a substitute. Unless the 2-month-old seedlings were completely grown in a greenhouse or an artificial climate chamber in the laboratory. However, if the 2-month-old seedlings were completely grown in an artificial climate chamber, it would not be fully consistent with the basic assumption stated in the manuscript that the emission factor of the seedlings does not represent that of mature trees. Because most studies based on seedlings are not conducted in artificial climate chambers or greenhouses. That is to say, the 2-month-old seedlings in this study are not in the traditional sense of seedlings.

Reply: We appreciate this opportunity to clarify.

(i) **Leaf cuvette (LI-6800):** For each sample we imposed **standard reference conditions** for emission factors (E_s): leaf temperature **30 °C** and PAR (or PPFD) **1000 µmol m^{-2} s^{-1}**, using the LI-6800's closed-loop control of leaf temperature and irradiance at the leaf chamber interface. The instrument continuously records **actual** leaf temperature and PAR; after a short stabilization (≥5 min) these matched the set points during sampling. Because the

cuvette imposes the micro-environment, the flux measured under these conditions is, by definition, **E_s** (after dry-mass normalization). We now state this explicitly in Methods 2.2.1 (lines 93-98):

"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.*"

(ii) **Branch chamber:** Branch-level measurements are made under ambient temperature and light. To compare directly with cuvette-derived E_s, we **standardized** branch fluxes to **30 °C** and **1000 µmol m^{-2} s^{-1}** using the MEGAN/Guenther temperature and light response functions (Text S4). This removes the influence of deviations between ambient and reference conditions and places both methods on the same E_s basis. We have clarified this in Section 2.4 (Lines 127-129):

"*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 E_s directly comparable to cuvette derived values.*"

(iii) **What we mean by "seedlings":** Our **2-month-old** plants were **not** grown in a climate chamber. They were purchased from a local nursery and **acclimated outdoors** (open area at GIG, full sun, ambient meteorology) for two weeks before measurement. This is consistent with the common use of nursery stock in seedling-based BVOC studies; we therefore consider them representative of "traditional seedlings" rather than climate-chamber plants. We have added this clarification to Section 2.1 (Lines 81-83):

"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.*"

3. Similarly, the manuscript mentions several times that the emission factors for young seedlings and mature trees are different. However, in this paper, *Eucalyptus urophylla* was only 2-year-old saplings under field conditions. Can these represent mature trees?

Reply: We agree that the 2-year-old individuals are not fully mature trees; they are best described as field-grown saplings. We have therefore replaced "mature trees" with "2-year-old saplings (field-grown)" throughout and adjust with the framing accordingly. Our objective was to compare seedlings with saplings to probe early ontogenetic shifts under natural conditions, not to provide definitive mature-canopy E_s. For context, the saplings we measured reached ~**10-12 m** in height (Table 1), i.e., tall but still juvenile for *E. urophylla*. We now state this limitation explicitly in the

Discussion/Conclusions and emphasize the need for measurements on fully mature trees. These edits are implemented in the revised manuscript.

Abstract (Line 17):

"…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 plantation in subtropical China."

Introduction (Lines 68-70):

"*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.*"

Methods, Section 2.1 (Lines 79-81):

"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."

Results/Discussion (Lines 224-225):

"*All age contrasts presented here are seedlings vs. saplings; extrapolation to mature trees requires additional measurements.*"

Conclusions (Lines 237-238)

"*These findings indicate that laboratory-grown seedlings are not reliable proxies for field-grown saplings, and likely not for mature trees either.*"

4. Based on the second and the third comment, I have another concern. Are there other variables besides tree age and sampling method that affect the emission factor of *Eucalyptus urophylla* in this research? Are there also influences/interferences from the growth environment (indoor and outdoor), soil conditions, and water conditions? Please clarify and add necessary discussions.

Reply: We agree that growth environment and edaphic/water status can influence BVOC emissions. We address the most influential short- to mid-term drives, temperature and light, in both the study design and the analysis. All measurements were made on sunny days during peak-summer campaigns and between 09:00-17:00 local time. Ambient temperatures during the two campaigns were similar (mean daily **31.4 °C** for the seedling campaign at GIG vs **30.3 °C** for the field campaign; Fig. S1). Midday PAR exceeded 1200-1500 µmol m^{-2} s^{-1} in both campaigns (Figs. S2 and S3). Using the MEGAN/Guenther acclimation formulations (Guenther et al., 2012), a **±10 %** change in growth PAR alters standardized E_s by **≈ 8 %**, and a **+1.1 °C** change in growth temperature by **≈ 11 %**, indicating that reasonable differences in light/temperature during growth would exert **minor** effects

relative to the **large age contrasts** we observe (e.g., ~6-fold increase in total monoterpenes and >40-fold rise in β-ocimenes; Fig. 2, Table S1). We have added these clarifications and sensitivity tests to the revised manuscript.

We did not quantify soil nutrients or soil moisture in this study, and we acknowledge this as a limitation. Prior work shows that responses to N and water can be species- and compound-specific; therefore, parallel growth-environment experiments within a single plantation will be needed to fully isolate ontogenetic from environmental controls. We now state this explicitly in the Discussion and Conclusions.

Finally, to avoid any ambiguity about "indoor vs outdoor" growth, we clarify that the 2-month-old seedlings were nursery stock acclimated outdoors at GIG for two weeks prior to measurement, whereas 2-year-old saplings were measured in situ at the plantation. These additions are included in the point-by-point response and incorporated into Section 2.1.

The following modifications have been made in the revised manuscript:

We added the following sentences in Methods Section 2.1 (Lines 86-90):

"*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).*"

The following discussions were added in the Section 3.2.3 (Lines 209-219):

"*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.*"

The following text were added in the Conclusion section:

"*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.*" (Lines 238-241)

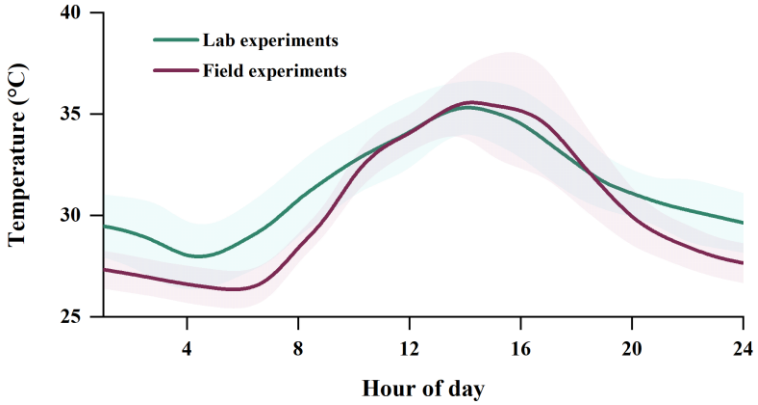

Figure S1. Comparison of temperatures between laboratory and field measurements

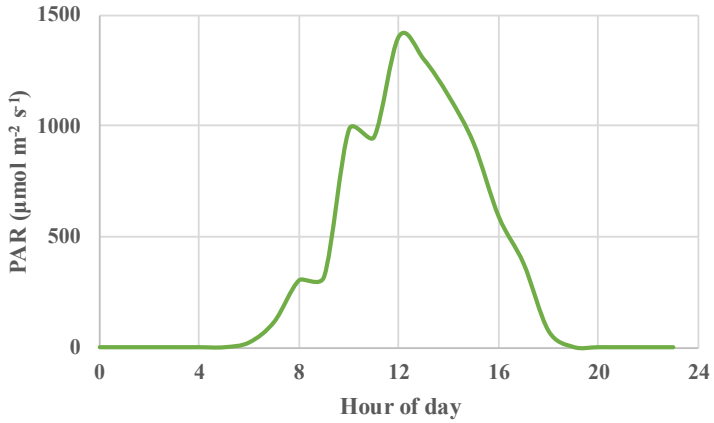

Figure S2. Diurnal variations of PAR recorded during field measurements

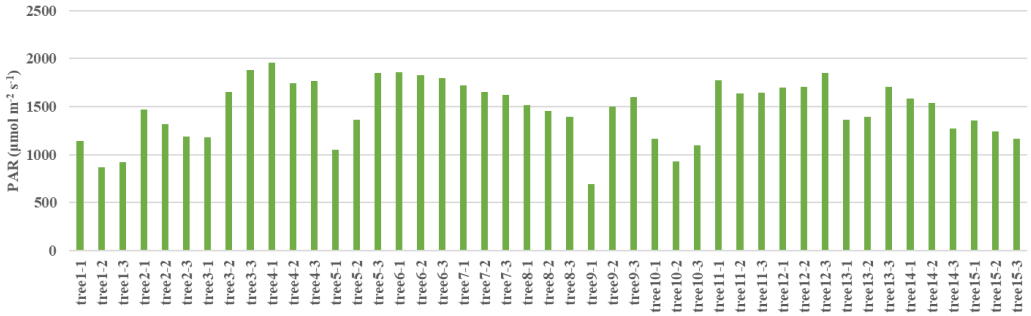

Figure S3. PAR values recorded for each BVOC sample during laboratory branch measurements

5. The manuscript mentioned that in the sampling chamber used in this paper, there are only 6 to 10 leaves. It can be anticipated that the shading effect within each leaf might be relatively small. However, when the sampling cover is larger (with the number of BVOCs sampling leaves within the chamber far exceeding 10, which should be quite common), will there be significant shading effects between different leaves under these conditions? Will the differences in different sampling methods still be negligible under such circumstances? This should be discussed more.

Reply: We agree. In our study, we intentionally enclosed sunlit, sparse branches (6-10 leaves) to minimize mutual shading, which helps explain the close agreement with leaf-cuvette isoprene E_s

(Sect. 3.1). As pointed by the reviewer, under larger or denser enclosures that capture many leaves (e.g., >10) and multiple layers, leaf-leaf shading can lower within-chamber PAR for a fraction of leaves, biasing instantaneous light-dependent emissions (e.g., isoprene and de novo monoterpenes) downward relative to single-leaf cuvette values. In such situations, method differences may re-emerge unless irradiance within the enclosure is verified and shading is minimized. We now state this explicitly, recommend that sunlit, single-layer branches be selected for inter-comparison work, and note that studies enclosing tens of leaves should (i) document the within-enclosure PAR (or its ratio to ambient), and (ii) avoid multi-layer foliage where possible. These clarifications are added to the Methods and Discussion in the revision.

We have added sentences in the revised manuscript Section 2.2.2 (Lines 108-111):

"*For comparability with leaf cuvette measurements, sunlit branches 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.*"

We have replaced/expanded the inter-comparison discussion in Section 3.1 (lines 166-171):

"*The equivalence we observed 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.*"

6. Is there excessive inference in the conclusion section? (line 197) The trees used in the text were not mature ones, did they have canopies? Additionally, the conclusion section is not concise enough; many parts should be placed in the discussion section instead.

Reply: We agree. Our field cohort comprises 2-year-old, field-grown saplings with established crowns (typical height 10-12 m; Table 1), but they are not mature trees. We have therefore replaced "mature trees/canopies" with "saplings" or "natural tree canopies" where appropriate, and we moved the broader inventory/modeling implications from the Conclusions to the end of Sect. 3.2 (Discussion). We also streamlined the Conclusions to report only what is directly supported by our data (method equivalence for isoprene under sunlit, single-layer branches; seedling-to-sapling shifts in magnitude/speciation; key limitations and next steps).

The part in the Conclusions section was replaced with a concise one (Lines 233-241):

"*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."

The following text was transferred to the end part of Sect. 3.2. (Lines 226-231):

"*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.*"

7. Problems with the figures and tables in the article: Firstly, the author separately reported Es based on quality and Es based on area. I understand the reason for the author's actions, but the relevant background was not well introduced in the manuscript. Additionally, it is suggested that Isoprene's Es placed in the appendix to avoid confusion regarding the units of Es in the main text. Secondly, there is a suspicion of duplicate reporting of Es for Isoprene in Figures 1, 3, and Table 2. The author is requested to reorganize and present it in a more concise form.

Reply: Thank you for your helpful suggestion. Following your suggestion, we have transferred Figure 2 and table 2 to the Supplement. Species-level emission factors can be reported per leaf dry mass or per projected leaf area. These two forms are inter-convertible through leaf mass per area (LMA) already reported in Table 1, and can emphasize different biology (biosynthetic capacity per biomass vs. per area for canopy-scaling). To avoid confusion, the main text and figures now use mass-based E_s (μg g^{-1} h^{-1}). Area-normalized values and the mass-area conversions are provided only in the Supplement with explicit cross-references in the text. We retain a single isoprene figure, the method inter-comparison (leaf cuvette vs. branch chamber), as in Figure 1. We remove isoprene bars/panels from the age-contrast figure and move the former figure/table that re-listed isoprene E_s to the Supplement. Figure 2 now focuses on monoterpene/sesquiterpene magnitudes and composition by age (no second isoprene plot).

We have added sentences after first paragraph in Sect. 2.4 (lines 133-136):

"*Notably, E_s can be normalized either to leaf dry mass (μg g^{-1} h^{-1}) or to projected leaf area (μg 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.*"

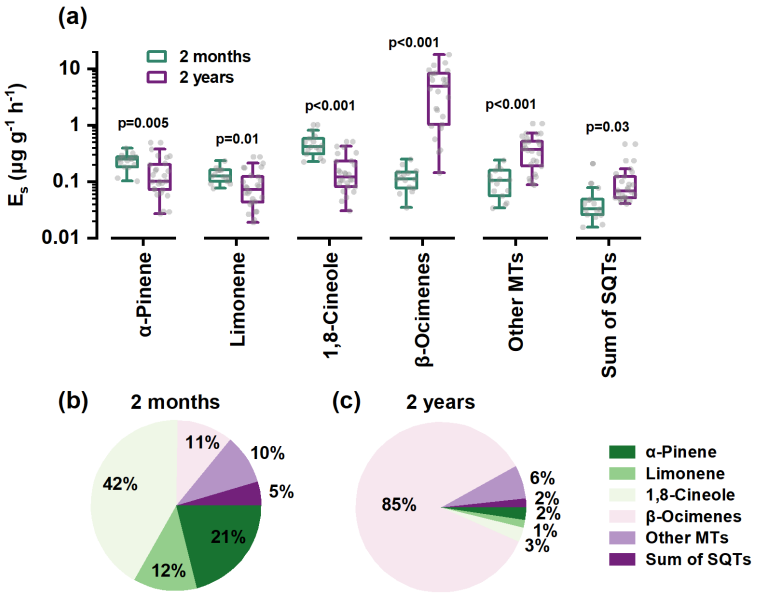

Figure 2. Comparison of emission factors (a) and compositions (bc) between 2-month-old and 2-year-old trees (a).