Reply to comments from Referee #2

Dear Authors-

As handling editor of this manuscript, I am passing on an anonymous review submitted via email. The two specific comments were as follows:

1. Were the genotypes of the seedlings and saplings the same? Croteau (Mentha) and Lorio (Pinus) showed more than 30 years ago that genotype has a huge impact on the particular monoterpenes emitted, even from the same species of plant.

Reply: Thank you very much for pointing this out. We agree that genotype can drive large within-species variation in monoterpenes emissions from Mentha, Pinus (Bäck et al., 2012), and Oka trees (Welter et al., 2012; Staudt and Visnadi, 2023; Staudt et al., 2025). To minimize this source of variability, both cohorts were from the same clonal line documented by the nursery/plantation manager, so genotype/chemotype differences are unlikely to confound our age comparison. We have clarified this point in Section 2.1. (Lines 84-86):

"*Both seedlings and saplings were sourced from the same clonal line (documented by the nursery/plantation) and exhibited a uniform terpene chemotype, minimizing genotype/chemotype variability.*"

References:

Bäck, J., Aalto, J., Henriksson, M., Hakola, H., He, Q., and Boy, M.: Chemodiversity of a Scots pine stand and implications for terpene air concentrations, Biogeosciences, 9, 689-702, https://doi.org/10.5194/bg-9-689-2012, 2012.

Welter, S., Bracho-Nunez, A., Mir, C., Zimmer, I., Kesselmeier, J., Lumaret, R., Schnitzler, J. P., and Staudt, M.: The diversification of terpene emissions in Mediterranean oaks: lessons from a study of *Quercus suber*, *Quercus canariensis* and its hybrid *Quercus afares*, Tree Physiol., 32, 1082-1091, https://doi.org/10.1093/treephys/tps069, 2012.

Staudt, M., and Visnadi, I.: High chemodiversity in the structural and enantiomeric composition of volatiles emitted by Kermes oak populations in Southern France, Elem. Sci. Anth., 11, https://doi.org/10.1525/elementa.2023.00043, 2023.

Staudt, M., Rivet, C., and Erdogan, M.: Diversity of volatile emissions from Cork Oak: Quantity and quality vary independently across its range, Ecol. Evol., 15, https://doi.org/10.1002/ece3.72093, 2025.

2. Were the light, temperature, and nitrogen conditions similar for the seedling and sapling leaf development? This is NOT a question about light and temperature during measurement but during the growth of the leaf. Fall, Funk, Guenther, Harley, Monson, and several others have shown the importance of growth environment, especially for isoprene emission.

Building on these comments, though you describe ontogenetic effects, which does suggest some attention to environment, the major conclusions of the paper (outside sampling strategy) focus on age-dependent emissions, with no controls for environmental conditions. It is not clear how these two can be differentiated, or what the implications are for the results and conclusions.

Reply: We fully agree that growth environment (both "air" environment and "soil" environment) significantly influences BVOC emissions, especially isoprene as reported in previous studies (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). As for soil environment, nitrogen content does influence BVOC emissions, but previous studies suggested the response of BVOC emission from different tree species to nitrogen addition and amount is inconsistent. Some plant species are positive to nitrogen addition, some are negative, while others are insensitive (Peñuelas and Staudt, 2010). Among these investigated plant species, fast-growing tree species like Eucalyptus (Funk et al., 2006) and Poplar (Yuan et al., 2024) showed insignificant response to nitrogen addition, indicating soil nitrogen may not be an important factor in present study. Moreover, effects of nitrogen addition are plant-specific and the comprehensive effect might be small (Peñuelas and Staudt, 2010), therefore currently widely used BVOC emission models like MEGAN do not incorporate nitrogen content into their estimates, instead growth temperature and light are the dominant drivers. As noted in our response to Reviewer #1 (Comment 4), the growth-period temperature and light were comparable between campaigns. A sensitivity test using the MEGAN/Guenther acclimation functions indicates that plausible differences in these drivers would change standardized E_s by only ~8 % for a ±10 % change in PAR and ~11 % for a +1.1 °C change in temperature, small relative to the observed age effects (e.g., ~6-fold higher total monoterpenes and a >40-fold rise in β-ocimenes in saplings).

Nevertheless, beyond the revisions made for Reviewer #1 (Comment 4), we acknowledge the limitation that soil nutrient/moisture status was not resolved and therefore call for parallel, co-located growth condition experiments to fully isolate ontogeny from environment. We have added the following sentence to the Conclusions:

"*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.*" (Lines 238-241)

References:

Monson, R. K., Harley, P. C., Litvak, M. E., Wildermuth, M., Guenther, A. B., Zimmerman, P. R., and Fall, R.: Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves, Oecologia, 99, 260-270, https://doi.org/10.1007/bf00627738, 1994.

Harley, P. C., Litvak, M. E., Sharkey, T. D., and Monson, R. K.: Isoprene emission from Velvet bean leaves - Interactions among nitrogen availability, growth photo flux density, and leaf development, Plant Physiol., 105, 279-285, https://doi.org/10.1104/pp.105.1.279, 1994.

Harley, P., Guenther, A., and Zimmerman, P.: Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves, Tree Physiol., 16, 25-32, https://doi.org/10.1093/treephys/16.1-2.25, 1996.

Harley, P., Guenther, A., and Zimmerman, P.: Environmental controls over isoprene emission in deciduous oak canopies, Tree Physiol., 17, 705-714, https://doi.org/10.1093/treephys/17.11.705, 1997.

Fall, R., and Wildermuth, M. C.: Isoprene synthase: From biochemical mechanism to emission algorithm, J. Geophys. Res., 103, 25599-25609, https://doi.org/10.1029/98jd00808, 1998.

Funk, J. L., Giardina, C. P., Knohl, A., and Lerdau, M. T.: Influence of nutrient availability, stand age, and canopy structure on isoprene flux in a *Eucalyptus saligna* experimental forest, J. Geophys. Res., 111, G02012, https://doi.org/10.1029/2005jg000085, 2006.

Guenther, A., Karl, T., Harley, P., Wiedinmyer, C., Palmer, P. I., and Geron, C.: Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature), Atmos. Chem. Phys., 6, 3181-3210, https://doi.org/10.5194/acp-6-3181-2006, 2006.

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.

Yuan, X., Feng, Z., Shang, B., Calatayud, V., and Paoletti, E.: Ozone exposure, nitrogen addition and moderate drought dynamically interact to affect isoprene emission in poplar, Sci. Total Environ., 734, https://doi.org/10.1016/j.scitotenv.2020.139368, 2020.

Peñuelas, J., and Staudt, M.: BVOCs and global change, Trends Plant Sci., 15, 133-144, https://doi.org/10.1016/j.tplants.2009.12.005, 2010.

Paraphrasing a final author comment: If the authors did not control for genotype (not just species), AND if they did not control for the growth conditions, then I suggest rejection.

Reply: As stated above, we controlled for chemotype and documented similar growth-period temperature and light across campaigns; MEGAN-based sensitivities are small relative to the large age signals we report. We now explicitly acknowledge unresolved soil status and call for co-located, parallel growth condition studies in the Conclusions.