Author's response for "**Chiral Volatile Organic Compound Fluxes from Soil in the Amazon Rainforest across seasons**", Schüttler et al.

Dear Editor, dear Reviewer,

I, along with my co-authors, thank you for the careful evaluation of our manuscript "Chiral Volatile Organic Compound Fluxes from Soil in the Amazon Rainforest across seasons" and for the constructive comments that helped us improve the work.

We have revised the manuscript accordingly. In brief, we:

- Changed the used statistical models to linear mixed-effect models to account for pseudo-replication
- Further discuss the treatment of the blank chamber results
- Detailed the discussion in regards to all the helpful feedback

We appreciate your consideration and hope the revisions meet your expectations. Please let us know if further clarifications are needed.

Best wishes,
Johanna Schüttler (on behalf of all co-authors)

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*The reviewer and editor comments are included in **black**, author responses are in **blue**, the original manuscript texts are in **purple**, while modifications to the manuscript are underlined and in **red**. Line numbers in our response relate to the original submitted document (preprint).*

Content:

Reply to comment from the Editor:

Dear authors,

I agree with the reviewers that your study presents interesting data on a relevant and understudied subject. However, I also agree with Reviewers #2 and #3 that there are major issues regarding insufficient and/or pseudo replication that require careful attention. There are also other issues that have been raised, e.g., regarding background measurements and their treatment. Please prepare a revised version that addresses the reviewers' concerns.

Kind regards,
Nicolas Brüggemann
Associate Editor

Response:

Dear Dr. Brüggemann,

First, we would like to thank you for taking the time editing our manuscript. We appreciate that the relevance of the manuscript is seen.

Following the comments from the reviewers, we adapted the statistical models used throughout the study to linear-mixed effect models accounting for the time-series and pseudo-replication in our dataset. The fixed-effects structure captures season and diurnal variation, and the random-effects structure (random intercepts for spot and sampling date) addresses non-independence and pseudo-replication. We agree that it would be ideal to have more biological replicates, however in this work, the intention was to monitor the same soil spots across time of day and seasons, at the disadvantage of additional soil spot locations. The re-analysis of the data using linear mixed-effect models did not change the general interpretation and discussion of the presented results, as most p-values and trends changed only slightly and mostly remained statistically significant.

Regarding the background (blank chamber) measurements, we still present the primary results as non-subtracted fluxes alongside the corresponding blank chamber measurements to ensure transparency and to avoid over- or under-correction. Blanks might not have been fully representative, as most blank measurements were not taken at the same time as sample chamber measurements, blank values varied across seasons, and the number of blanks taken in each campaign is different (Table A2).

We also include a sensitivity analysis now in the Appendix showing how subtracting chamber blanks affects the most important compounds, but does not alter general trends.

Reply to comments from Reviewer 1:

The paper by Schüttler et al. offer some interesting data on BVOC exchanges between the soil and atmosphere and extends our understanding of these fluxes in tropical systems. As a Biologist, not a Chemist, I found the methods descriptions commendably clear, logical, and easy to follow. I do NOT know enough to comment critically on these methods, but the descriptions made perfect sense to me.

The importance of BVOC fluxes in these systems has been known for over thirty years, but we still have very few data sets of soil fluxes, per se; this study, even though it is based on few chambers in one site, offers some tantalizing insights. The authors need to bear in mind in their statistical models when their samples are truly independent, when they are time series, and when they are pseudo-replicated. I suspect they will want to either re-structure their models or, at the very least, acknowledge when their data violate assumptions of independence. I do not see this as a major issue because the results are so striking.

Response: First, we would like to thank you for taking the time reviewing our work and for the encouraging words. By pointing us to additional aspects of our work, especially from a biologist's point of view, we think we can add substantial improvements to the manuscript.

We thank you for putting our attention to our statistical model. We acknowledge that our data is not independent due to repeated measurement over time from the same soil spots when we compare seasons or soil spots. To address this, we have re-analyzed the data using linear mixed-effects models, which account for the structure of our time-series data by including random intercepts for each measurement date. We describe the now used statistical model in the methods, changed the corresponding results for soil spot, seasonal, and chiral ratio differences, and adjusted Figure 6 and 8. While some p-values changed slightly under the new statistical model, the overall trends and conclusions were not affected when comparing fluxes and chiral ratios per season and soil spots.

We also adjusted the order of ambient parameters in Figure 6 to the same order as was used in Figure 2, added ozone to Figure 2 (f), and adjusted the unit for PAR from µmol m^{-2} s^{-1} to mmol m^{-2} s^{-1}.

2.6 Statistical analysis

Line 195: Statistical analyses were performed using python (version 3.12.4) with the following packages: numpy (v.2.0.0), pandas (v.2.2.2), matplotlib (v.3.9.1), seaborn (v.0.13.2), statsmodel (v.0.14.5 ~~2~~), and scipy (v 1.16.0). Data visualization was conducted using matplotlib and seaborn. Statistical differences were assessed using linear mixed-effect models because the dataset contains repeated measurements over time from the same soil chambers and ambient sampling points, which violates assumptions of independence of simpler tests. Local time (hour-of-day as a categorical factor), was included as a fixed effect in all models, because we expected a diurnal pattern for the measured VOC fluxes and mixing ratios. ~~between soil fluxes measured in different seasons and from different soil plots~~ were ~~determined using the Tukey HSD (Honestly significant difference) test following a significant result from ANOVA~~ To assess seasonal differences in fluxes, a linear mixed-effects model was implemented with season, chamber spot location, and local time as fixed effects and the sampling date spot as random effects. Differences between soil spots within a single season were assessed with chamber spot location and local time as fixed effects and the sampling date as a random effect. Using the Holm–Bonferroni method, p-values were adjusted for multiple comparisons afterwards in both cases.

For comparisons of enantiomeric ratios between atmospheric and soil chambers and between seasons, a linear mixed-effect model with fixed effect for local time and a random effect for the sampling date and chamber spot or ambient air sampling location was used. Ratios in both cases were log-transformed

prior to analysis to stabilize variance and improve residual normality. To quantify associations of environmental parameters on fluxes, mixed-effects models were fitted with fixed effect of local time and adjusting for random effects of measurement date and chamber spot location. Effect sizes (β coefficients) represent the change in flux per unit increase in the predictor. ~~, the non-parametric Mann-Whitney U test was used due to non-normality of the data. For correlations between the fluxes and environmental parameter Pearson coefficients were calculated.~~ Statistical significance was accepted for $p < 0.05$.

3.2 Meteorological conditions

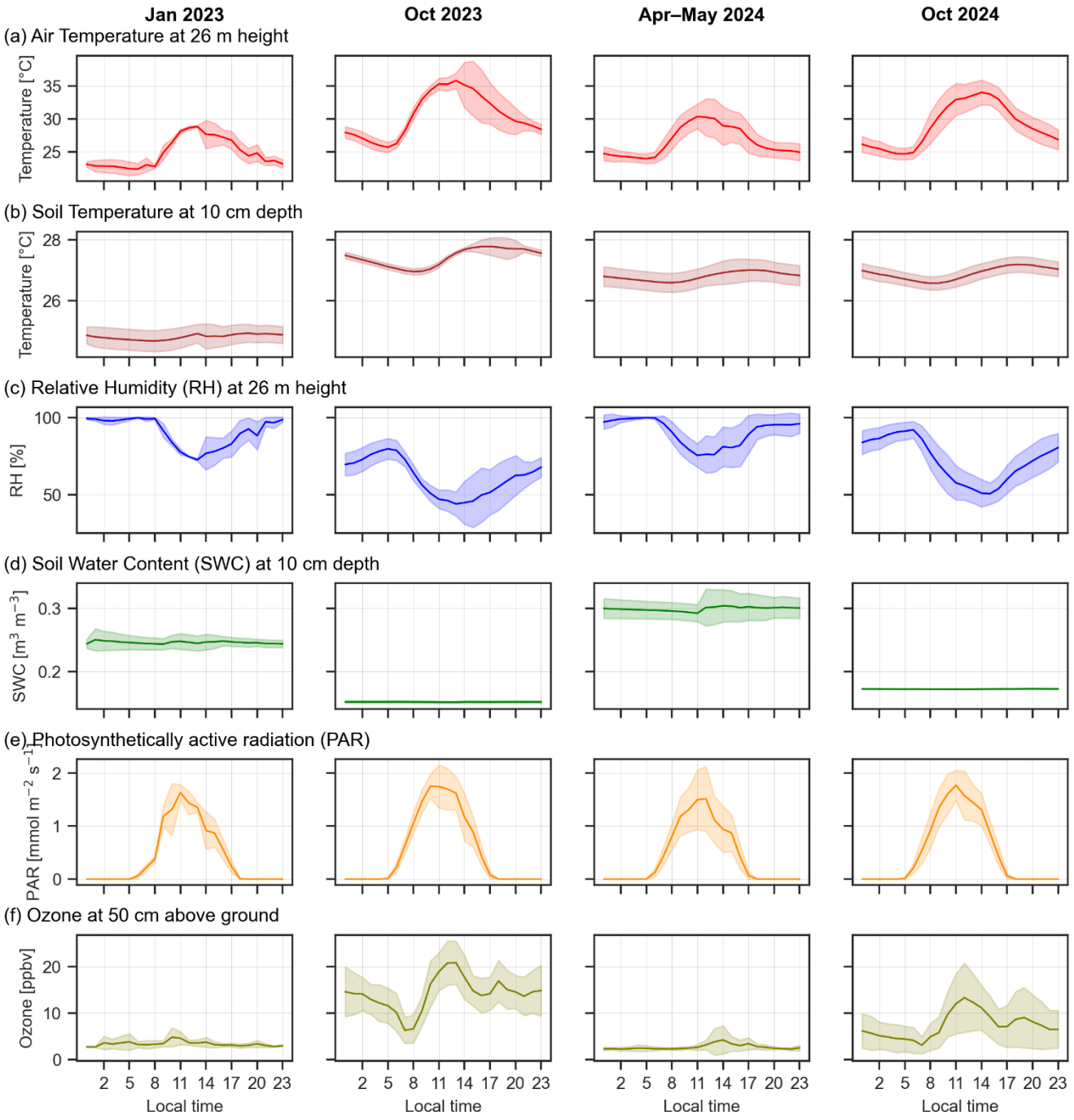

Figure 2 Meteorological data during the measured seasons with (a) temperature (red) and (c) relative humidity (blue) measured at 26 m at the Instant tower, (b) soil temperature (orange) and (d) soil water-content (green) measured at 10 cm depth and (e) photosynthetically active radiation (PAR) incoming at 81m at the Instant tower and (f) ozone

measured 50 cm above the ground at the instant tower across the four measurement periods in the different seasons. The line represents the mean and the shaded area is the standard deviation.

3.3 Diurnal and seasonal dynamics of soil terpenoid exchanges

Line 272: The fluxes showed strong seasonal variation, with higher uptake fluxes in the dry seasons compared to the dry-to-wet and wet seasons (~~Tukey test~~ Holm–Bonferroni adjusted $p < 0.001$ for comparing October 2024 and $p < 0.01$ for comparing with October 2023).

3.4 Effects of the soil properties on terpenoid soil fluxes

Line 289: For isoprene, in the dry-to-wet season 2023 and wet season 2024, no significant differences in fluxes were found ($p > 0.05$) (Fig. 4a) between the measured soil chambers. However, in the ~~other three~~ two dry seasons there was a significantly higher isoprene uptake by spot 1 than spot 5 (Holm–Bonferroni adjusted $p < 0.01$ for ~~dry season 2023 and $p < 0.001$ for dry season 2024 all~~), and in the dry season 2024, also in spot 5 than spot 4 (Holm–Bonferroni adjusted $p < 0.001$).

Comparing fluxes of MTs from different soil spots, we note clear monoterpene speciation differences (Fig. 4b). The highest emission rates were observed for soil spot 1 in the dry-to-wet transition season 2023. Here, the flux was significantly higher compared to the other two spots (Holm–Bonferroni adjusted $p < 0.0001$).

3.4.1 Effect of litter removal

Line 289: When litter was removed from the soil plot, in the two seasons dry-to-wet season 2023 and dry season 2024, no significant difference was found in the fluxes for isoprene and total MTs ($p > 0.05$).

3.4.2 Soil fluxes VS environmental conditions

Line 322: Isoprene, MVK, and MACR ~~have a~~ were strongly negatively ~~correlation~~ associated ~~(Fig. 6)~~ with their ambient atmospheric ~~concentration~~ mixing ratios (β= -8.7, -10, and -12 nmol m^{-2} h^{-1} per 1 ppbv increase respectively; $p < 0.001$; Fig. 6), after accounting for repeated chamber spot location measurements and dates in the linear mixed-effect model (Fig. 6). This indicat~~ing~~es the uptake rates were higher (so flux values became more negative) when the available concentrations in the air above the soil were higher. Most MTs and SQTs show the same pattern, with flux rates decreasing as ambient mixing ratios increased. Exceptions were β-myrcene and tricyclene, which exhibited a positive association with ambient mixing ratios (β = +27 and +24 nmol m^{-2} h^{-1} per 1 ppbv; $p < 0.001$ and $p < 0.01$, respectively). While effect sizes differed between enantiomers, the direction of the associations was generally similar.

Soil and air temperature were negatively associated with isoprene, MACR, MVK, and total MT fluxes (flux decreased with warming), whereas SQT soil flux increased with temperature. In contrast, soil water content and relative humidity were positively associated with flux. Total MTs showed the strongest positive association with PAR (β = 16 nmol m^{-2} h^{-1} per mmol m^{-2} s^{-1}; $p < 0.001$).

~~Different MT species like α phellandrene, 3-carene, γ terpinene, limonene, and β-ocimene, also show negative correlation with their mixing ratios (-0.21 to -0.82; p <0.001), while β-myrcene had positive correlation with the ambient concentration (0.54; p <0.001). In general, the correlations with the environmental conditions like air and soil temperature and soil water content were stronger for isoprene and its oxidation products. The correlation with the photosynthetic active radiation (PAR) was highest for ocimene (0.29; p <0.01) and the total SQTs, as well as α-copaene (0.26 and 0.24; p <0.001).~~

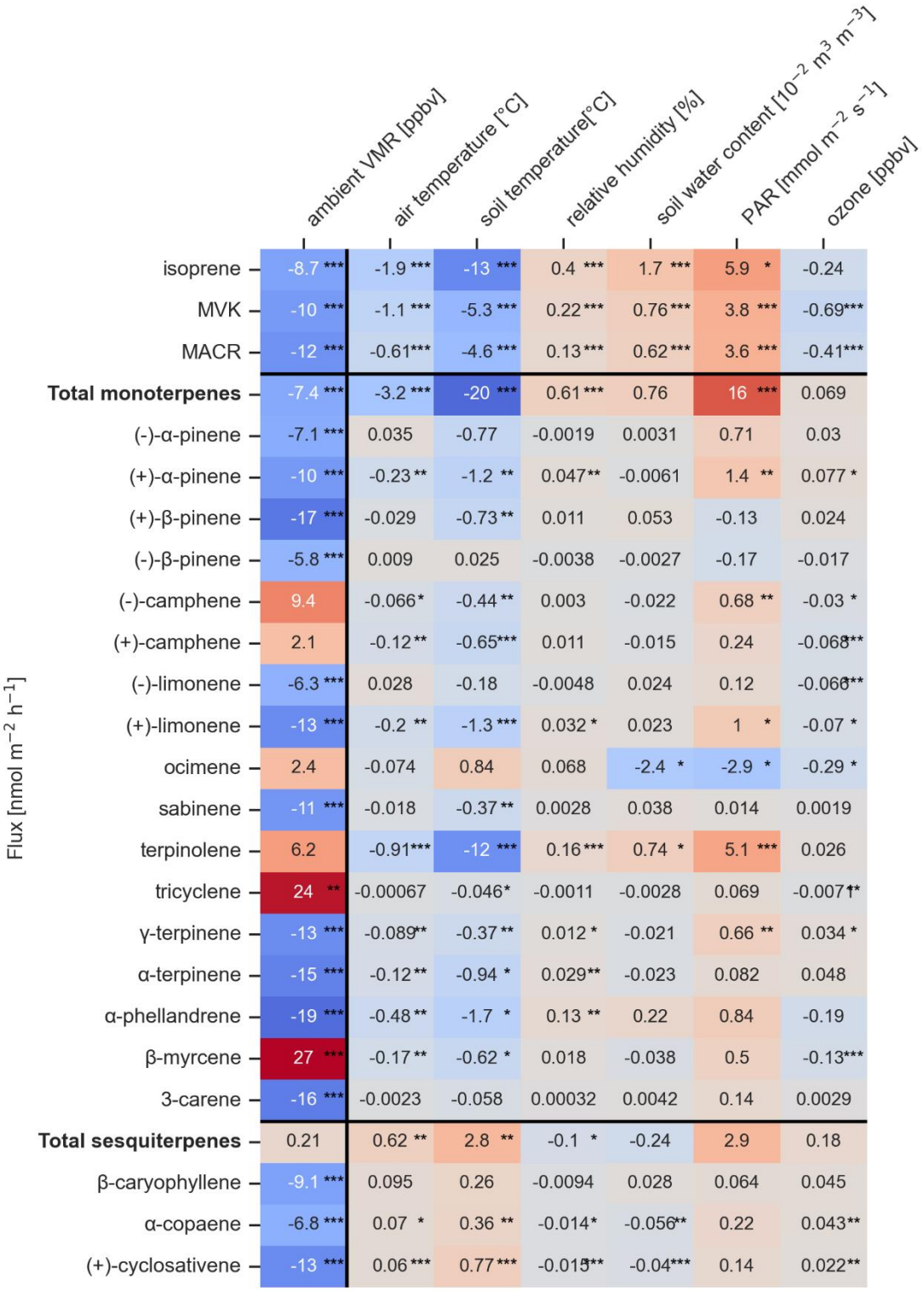

Figure 6 Heatmap of ~~correlations between the~~ β coefficients from linear mixed-effects models quantifying the change of flux in nmol m^{-2} h^{-1} of measured compounds ~~and~~ per unit change in environmental variables, after adjusting for the fixed effect of chamber spot location and random effect for measurement date. ~~various e~~ Environmental variables are: ambient mixing ratio (VMR) of each compound in ppbv, air temperature at 26 m in °C, incoming photosynthetically active radiation (PAR) at 81 m in mmol m^{-2} s^{-1}, soil temperature in °C and soil water content (scaled by 10^{-2}) at 10 cm depth in 10^{-2} m^3 m^{-3}. Statistical significance of the ~~Pearson correlation~~ β coefficients is indicated by asterisks: (*) for p <0.05, (**) for p<0.01, and (***) for p< 0.001.

3.6 Chirality

Line 352: The (−)-α-pinene was significantly more enriched ~~(p < 0.05)~~ inside the soil chamber than in the ambient air in the dry-to-wet season 2023 (p < 0.001), dry season 2023 (p < 0.05), and in the wet season 2024 (p < 0.01) (Fig. 8).

Line 357: When only comparing the ambient enantiomeric ratios across the season, they are in most cases significantly different from each other (Fig. 8 e)-h)), especially when comparing both dry ~~wet~~ season 2024 ~~with the dry season~~ and 2023, it was significantly different for all chiral MTs, except for camphene.

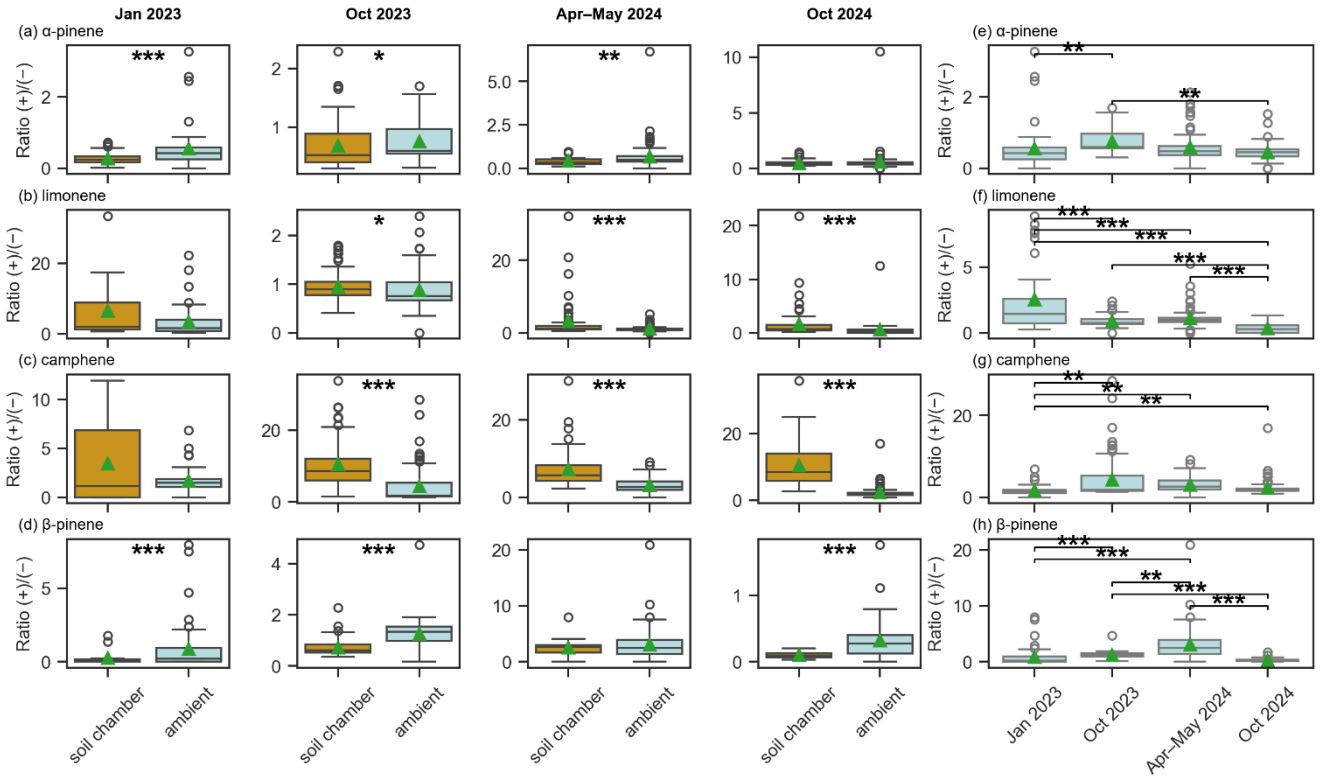

Figure 8 Boxplots show the ratio of the (+)- to (−)-enantiomers for (a) α-pinene, (b) limonene, (c) camphene and (d) β-pinene in both ambient air (light blue) and soil chamber air (dark orange) across different seasons. The rightmost panels display the seasonal distribution of ambient air enantiomeric ratios for (e) α-pinene, (f) limonene, (g) camphene and (h) β-pinene. The boxes represent 25% to 75% of the dataset. Mean values are indicated by green triangles, while the median values are indicated by the central lines. Whiskers indicate the minimum and maximum data points at 1.5 times the interquartile range. Circles represent the outliers. Significance was assessed using linear mixed-effect model accounting for local time as fixed effect; sampling date and chamber spot as random effect. A single asterisk (*) denotes statistically significant differences between groups ($p < 0.05$), double asterisks (**) indicate highly significant differences ($p < 0.01$), and (***) indicate very high significant differences ($p < 0.001$). For improved visualization, in plot (e), two outliers with a ratio of (+)/(−) greater than 5 and in plot (f) one outlier with the ratio greater than 10 are not displayed. They are still included in the statistical analysis.

4.1.1 Isoprene and the oxidation products MACR and MVK

Line 380: For isoprene, MACR, and MVK, ~~strong correlations between soil fluxes and both ambient mixing ratios (-0.74 to -0.81; $p < 0.001$) and key environmental parameters such as soil water content (0.54 to 0.63; $p < 0.001$) and temperature (-0.48 to -0.56; $p < 0.001$) (Fig. 7) are found.~~ increasing soil fluxes were associated with increasing ambient mixing ratios (β = -8.7, -10, and -12 nmol m-2 h-1 per 1 ppbv respectively; $p < 0.001$) and key environmental parameters as soil temperature (β = -13, -5.3, and -4.6 nmol m-2 h-1 per 1 °C; $p < 0.001$), while decreased with increasing soil water content (β = 1.7, 0.76, and 0.62 nmol m-2 h-1 per 0.01 m3 m-3; $p < 0.001$) (Fig. 6). ~~The correlation with the environmental parameters like temperature hint either to the higher ambient concentrations of isoprene at higher temperatures (Alves et al., 2016) or to more efficient uptake rates at higher temperatures.~~ The pattern with more uptake at higher temperatures and lower soil water content likely reflects co-variation between meteorology and ambient isoprene: warmer and drier periods tend to higher ambient isoprene (Alves et al., 2016). Also, soil microbial uptake rates could be more efficient at higher temperatures.

4.1.2 Monoterpenes and sesquiterpenes

Line 400: ~~Different MTs correlated negatively or positively with ambient concentrations (Fig. 7), indicating there are different processes responsible for the soil fluxes of each MT.~~ The lack of a

consistent pattern in the association between soil fluxes of individual MTs and ambient mixing ratios, PAR, temperature, and soil water content suggests that these exchanges are not governed by a single overarching mechanism. Instead, soil MT fluxes appear to result from compound-specific abiotic and biotic processes within the soil.

Line 408: SQTs were mostly associated with temperature (Fig. 6), reflecting the observed seasonal trends. Microbial activity and/or abiotic release could be increased with temperature.

4.2 Effect of litter

Line 420: Although this result was not statistically significant (~~ANOVA t-test~~ $p>0.05$, sample size with litter n=23, without litter n=8) it gives directional evidence towards the role of litter on total soil terpenoid fluxes.

A few results stood to me, with respect to the underlying Biology. I hope the authors find these useful as they revise.

1. the lack of isoprene uptake in the litter layer suggests that litter microbes have not adapted to this carbon source. Given the earlier described role of litter microbes in methanol and terpene consumption, this result is worth highlighting.

Response: Thank you for pointing out this additional aspect of our results. We will highlight this result in our discussion and conclusion:

4.2 Effect of litter

Line 418: This suggests that microorganisms residing in the soil layer beneath the litter are primarily responsible for metabolizing these compounds, while at the same time indicating that litter microbes here have not adapted to isoprene as a carbon source.

5. Conclusion

Line 538: For the uptake of isoprene, MACR, and MVK ambient concentrations and temperature seem to be the primary drivers, while the litter layer here did not have an effect on the consumption of these compounds.

2. the difference in enantiomeric variability between the monoterpenes and the sesquiterpenes is congruent with earlier ideas that the sesquis come from the MVA pathway while the monoterps are made by the DOX-P pathway.

Response: We thank the reviewer for this insightful comment. While there is a tendency that in plants monoterpenes (MT) are made by the 2-C-methyl-d-erythritol 4-phosphate (MEP/DOX-P) pathway in plastids and sesquiterpenes (SQT) derive from the mevalonate (MVA) pathway in the cytosol, this classical apportionment has been shown to be an oversimplification (Hemmerlin et al., 2012). Isotope-labeling and inhibitor studies show substantial cross-contribution of MEP and MVA to both MTs and SQTs in several species (Dudareva et al., 2005; Yu and Utsumi, 2009; Opitz et al., 2014; Bergman et al., 2020). In general, finding both enantiomers of an SQT is rarer than for MTs, several SQTs occur in both (+) and (−) forms, sometimes within the same genus or even the same species (Finefield et al., 2012). The enantiomeric outcome of a terpenoid arises at the level of terpene synthases (stereoselective enzymes) downstream from the pathway supplying precursors (Schwab et al., 2002). It was shown by Byron et al. (2022) that MT enantiomers can be produced de novo, while the other enantiomer can be attributed to storage emissions and therefore decoupled from the time of biosynthesis and therefore partly decoupled from precursor pathways.
Thus, observing both enantiomers for some MTs but only one for SQTs in our dataset reflects more on

the differences in expressed synthase isoforms and tissue/temporal regulation, rather than indicating distinct precursor pathways.

We would therefore choose not to include discussing the pathways of the precursors in this manuscript, as our study in its design does not give additional insights towards understanding MT and SQT pathways in plants.

3. the idea of terpene emission from leaf litter, *per se*, rather than microbial activity during decomposition, could be developed a bit. The reason for pursuing this distinction is that a fair bit is known about the distribution of different terpenes in different plant taxa, so the direct leaf-contribution to BVOC fluxes should be predictable if the plant species composition is known.

Response: We appreciate this suggestion and agree that distinguishing direct (abiotic) volatilization from leaf litter and microbially mediated production and consumption is important. In our study site, the plant community is highly diverse (Andreae et al., 2015), and the leaf litter in each soil spot consists of a mixture of species whose individual terpene profiles are unknown. This makes it difficult to predict the contribution of direct litter emissions based on species composition alone.
Many studies on litter BVOCs have focused on temperate forests with coniferous trees that are known to emit terpenes in higher quantities and for longer time periods from their resin ducts as they decompose more slowly (Greenberg et al., 2012; Tang et al., 2019; Viros et al., 2021; Isidorov et al., 2024). Most tropical leaf litter is from deciduous plants and has more labile storage pools. However, we lack information on the residence time of isoprene, MTs, and SQTs in leaf litter (Tang et al., 2019). We expect abiotic emissions from leaf litter at our site to be transient and likely minor compared to microbial activity, especially over the time scale of our measurements, with (visibly) limited fresh litter additions during the measurement period.

So, we would expect abiotic emissions from leaf litter at our measurement site to be less prominent, emitted only in a short period after leaf fall, and not reliably predictable.

4.2 Effect of litter
Line 422: The alteration could be attributed to the abiotic degradation of storage pools within the litter material and/ or to the ~~activity of~~ production and consumption by microorganisms inhabiting the litter surfaces. Abiotic litter emission varies according to decomposition stages as terpenes volatilize from storage pools. As in a tropical forest most plant species are deciduous, storage pool emissions are expected to a lower degree than in litter from coniferous trees (Greenberg et al., 2012; Viros et al., 2021; Isidorov et al., 2024). Previous studies have shown that biotic VOC emissions from litter in California can be 5 to 10 times higher ~~compared~~ to those from abiotic processes (Gray et al., 2010), indicating that the microbial community on ~~the~~ litter surfaces plays a significant role in VOC fluxes. Similar findings have been reported in soil terpenoid VOC flux shifts following litter removal in other ecosystems, such as an eucalyptus plantation (Mu et al., 2023), in Boreal forests (Mäki et al., 2017, 2019), and in a Mediterranean forest (Yang et al., 2024). However, very limited information is available on the contribution of leaf litter volatiles over time (Tang et al., 2019), especially in tropical ecosystems. Litter VOCs were found to have an influence in microbial community structures (McBride et al., 2020) and are known to mediate many microbe–microbe, microbe–plant, and microbe–animal interactions (Bitas et al., 2013; Schmidt et al., 2015; Schulz-Bohm et al., 2017). So, the very local litter structure could be responsible for the different MT and SQTs in the studied soil spots. The highest litter fall rates are usually observed at the end of the dry season and can be increased during an El Niño dry season (Martius et al., 2004; Barlow et al., 2007; Brando et al., 2008), so ~~also~~ the seasonal differences observed for MTs and SQTs could be partly attributed to the litter layer.

4. For over 30 years, thanks to Carleton White, we've been aware of the importance of soil microbes as consumers of BVOCs. When the flux is net upward, as Schüttler et al. found here

for the 10 and 15 C terpenes, then the consumption processes are smaller than the production ones. The results of this manuscript suggest that it is time for some more process-based studies of soil BVOC fluxes so that we can begin to estimate gross fluxes also.

Response: We thank the reviewer for highlighting the long-standing evidence that soils can act as a microbial sink for monoterpenes (White, 1991, 1994). We have revised the Introduction and Discussion to acknowledge this work and clarified in the manuscript that our measurements report net fluxes as production minus consumption.

1 Introduction
Line 50: MTs can also be metabolized by soil microbes as a carbon source and can have an effect on pathways such as methanotrophy, nitrification, and denitrification in soil microbes (White, 1991, 1994).

4.1.2 Monoterpenes and sesquiterpenes
Line 407: So, the roots, as well as the microbiome, could have contributed to the different MT species net fluxes.

5. Conclusion
Line 539: MT and SQT net emissions and uptake ~~showe~~were found to be governed ~~on~~by the litter layer and season, as well as showing very local differences from spot to spot in the composition of the total flux.
Line 552: By taking up isoprene and the net emission of MTs and SQTs the soil will exert partial control over near-surface ambient atmospheric ozone and OH.

5. I applaud the authors for their wet & dry season measurements, but I think the link to El Nino is rather tenuous, and, imo, they should de-emphasize this point. But I leave this to the editor's discretion.

Response: We appreciate the reviewer's point of view and agree that the link to El Niño could be de-emphasized, as we cannot prove that the observed higher rates of sesquiterpene (SQT) emission by the soil spots in the dry season 2023 were caused by the El Niño event.

4.1.2 Monoterpenes and sesquiterpenes
Line 411: In ~~Only i~~n the El Niño-influenced dry season 2023 the emission of SQT was more pronounced ~~was an emission pattern of SQT evident.~~

5. Conclusion
Line 541: ~~An El Niño drought period caused~~ Enhanced SQT emissions from soil were observed during the El Niño-influenced dry season 2023.

6. the authors make the important point that soil fluxes could result from microbes and/or roots. There have been similar observations on volatile sulfur fluxes in tropical systems. Distinguishing plant from microbial sources of production and consumption is crucial for developing predictive modeling. If the authors choose to discuss this, they should bear in mind McDonald and Falls's older work showing microbial consumption on the leaf surface, which could look like leaf consumption.

Response: We thank the reviewer for raising this important point. Distinguishing between root and microbial contributions to soil VOC fluxes is indeed critical for improving predictive models of biogenic emissions. However, our current dataset does not allow us to partition these sources, as both roots and rhizosphere microbes can produce VOCs, and their activities may be tightly coupled. However, there is no evidence that roots consume VOCs. Future studies could distinguish roots and free-living/rhizosphere microbes' VOC emissions via stable isotope labeling e.g., by pulse-labeling

plants with $^{13}CO_2$ or adding ^{13}C-labeled substrates to the soil (Gkarmiri et al., 2017; Cabugao et al., 2022; Pugliese et al., 2023; Meischner et al., 2025). Another approach could be sterilization in microcosms, root exclusion, having root-free controls or depth-resolved studies (Raza et al., 2021; Wannenmacher et al., 2025). However, such manipulations are difficult to implement without disturbing natural conditions.

While our study focused on terpenoid soil VOCs, not specifically on volatile sulfur compounds, we recognize the relevance of prior work on volatile sulfur fluxes in tropical ecosystems (Andreae and Andreae, 1988; Kesselmeier et al., 1993; Jardine et al., 2015; Pugliese et al., 2023), where differentiating plant and microbial contributions is also a major challenge (Kesselmeier and Hubert, 2002).

We agree with the reviewer's caution regarding microbial consumption potentially masking root-derived emissions analogous to observations of methanol uptake by leaf-surface microbes (MacDonald and Fall, 1993; Fall and Benson, 1996). While our study did not measure methanol or leaf processes, we focused on net soil-atmosphere fluxes, integrating both root and microbial activity. This approach provides insights into overall soil VOC dynamics, but underscores the need for targeted experiments to resolve source contributions in future work.

4.1.1 Isoprene and the oxidation products MACR and MVK

Line 396: Therefore, a consumption of these compounds by roots and/or microorganisms in the soil is a possible explanation for the observed net uptake rates.

Line 404: Roots have also been implicated as sources of MTs from soils (Asensio et al., 2008a), and both plant roots and microbial communities are responsive to climatic variation and drought stress (Bourtsoukidis et al., 2014; Byron et al., 2021; Honeker et al., 2023; Pugliese et al., 2023). So, the roots, as well as the microbiome, could have contributed to the different MT species fluxes. With our measurement, we can only make assumptions about the net exchange between the soil-sphere compartments and the atmosphere.

4.6 Limitations of this study and future directions

Line 532: Additionally, isotopic labelling and online measurements with a greater time resolution between samples could further strengthen the understanding of soil BVOC exchange processes, especially regarding resolving roots and/ or microbiome sources.

In short, this is a fine contribution and one that I look forward to citing when it appears in the literature.

Thank you!

Reply to comments from Reviewer 2:

General Comments

This study examines fluxes (emissions and uptakes) of VOCs from soil in the Amazon rainforest. The authors present seasonal measurements spanning multiple wet and dry periods and investigate the chirality of VOC fluxes, timely and highly relevant research questions. The results show seasonal shifts in the emission and uptake of various VOCs (MTs, SQTs, isoprene, and two isoprene oxidation products) between soil and atmosphere. Dry conditions led to SQT soil emissions, but MTs and ISP uptake. Additionally, the enantiomeric compositions of VOCs emitted from soil differ from those present in ambient air, with further seasonal variation observed.

I consider this study on the rainforest ecosystem very important, given also the very limited existing knowledge. The methodologies, particularly regarding VOC flux measurements, are generally robust, and the manuscript is nicely written and well-organized. However, I have specific concerns about the number of replicates included in the study, which should be taken seriously.

Response: We thank the reviewer for their time and encouraging words. All comments have been addressed, as described in detail below. Concerning the replicates, more is always better. However, ultimately, there are always limits in personnel, equipment, time, access, instrument capacity, and funding that restrict replicates in fieldwork. We address the concern over the number replicates by examining the statistical methods used in detail, and by showing the main results are robust when alternative methods are applied. The Amazon Forest is enormous, and the soils diverse, so the representativeness of such studies can always be questioned. Our strategy was to examine soils with diverse respiration rates and to look for the role of litter in the gas exchange.

Specific Comments

My primary concern is about the number of replicates used in the study. The authors utilized three chambers placed in three separate soil plots, which appears to be the minimum acceptable number of biological replicates (i.e., three). However, two of these chambers represent soil emissions with natural litter abundance, while only one chamber represents a plot without litter. Additionally, the rationale for excluding (or separating) the “spot 1” near the termite nest from the analysis and treating it separately is unclear. Are these data considered outliers? Why can’t those measurements be considered part of the biological variability that exists in the rainforest? Also, the results on soil emissions following litter removal are based on only 2 replicates, which raises questions about the reliability of the findings. The results of figure 5 should be also tested for significance.

Response: We thank the reviewer for the constructive feedback regarding the number of biological replicates and the treatment of "spot 1" in our study. We appreciate the opportunity to clarify the methodological aspects.

We acknowledge that the study includes only three soil spots per seasons which is the minimum acceptable number for biological replication in field studies. The design was unbalanced in the January 2023 season, with two chambers representing natural conditions with intact litter layer and one spot with a removed litter layer. In the following three seasons, all three spots had intact litter layers as found and only in the last season of Oct 2024 for the last two days of measurement the litter layer was removed in “spot 5” (with a measurement pause of 24 hours prior to the first VOC sampling after removal of litter).

We now state this more explicitly in the methods and in Table 1.

Line 418: In January 2023 (dry-to-wet season 2023), the three chambers were installed on spot 1, spot 2, and spot 3 (Table 1). To investigate the effect of litter content on soil fluxes, the litter layer was

removed from spot 3. Spot 1 was located near a termite nest. In October 2023 (dry season 2023), the chambers in spots 2 and 3 were moved to spots 4 and 5. Spot 1 remained at the original site. In all three chambers the ~~where their~~ litter layers were kept intact. Additional soil flux measurements without litter were performed from spot 5 in October 2024 (Table 1). Spots 4 and 5 were located within 15 m of spots 2 and 3. For soil flux measurements, the chamber lids were kept closed for 15 minutes before collecting samples, following a method used for cartridge measurements from a steady-state chamber within a study by Pugliese et al., (2023).

Table 1 Overview of measurement campaigns with attributed season, start date, end date, measured chambers and the number of flux data points

Name in plots	Season	Start Date	End Date	Number of flux data points	Chambers Measured
Jan 2023	Dry-to-wet	2023-01-22	2023-01-26	20	Spot 1, Spot 2, Spot 3 without litter
Oct 2023	Dry	2023-10-01	2023-10-14	39	Spot 1, Spot 4, Spot 5
Apr-May 2024	Wet	2024-04-24	2024-05-02	35	Spot 1, Spot 4, Spot 5
Oct 2024	Dry	2024-10-11	2024-10-20	37	Spot 1, Spot 4, Spot 5
		2024-10-18	2024-10-20	6	Spot 5 without litter

While we are comparing “spot 1” with the other measured spots in section 3.4, we did not separate it from the analysis as an outlier or exclude it in any model or mean value shown in our graphs. We noted that it behaved differently from the other two spots in some seasons for some compounds, which could be attributed to the higher organic content. However, we cannot say how representative this spot location is across the Amazon rainforest compared to the other two soil spots measured. This information will come from future regional soil surveys, which can assist in more realistic upscaling of soil effects. Our study is more focused on soil-mediated impacts on atmospheric trace gases. We do think the effect exhibited by spot 1 is part of the biological variability that exists, and therefore did not treat it as an outlier, but rather we included it in all further analysis

We agree that the results on litter vs removed litter soil are only based on two replicates and might not be reliable. We were already performing an ANOVA t-test for the significance of the result of isoprene and total monoterpene difference between intact litter layer and removed litter that resulted to be not significant (see Line 420). Following the comments by reviewer 1, we adjusted our used statistical model to linear mixed-effect models, as the dataset contains repeated measures over time and does not fulfill the conditions of same sample size for a repeated measures ANOVA. This linear mixed-effect model still led to a non-significant result for flux differences between litter and no-litter. We consider it useful for future research to present our results, but with a transparent discussion of the limited reliability of our findings in the manuscript due to the limited biological replicates per season. However, our intention was investigating long-term BVOC trends from the same soil spots across seasons.

3.4.1 Effect of litter removal

When litter was removed from two ~~the~~ soil plots, in the two seasons dry-to-wet season 2023 and dry season 2024, no significant difference was found in the fluxes for isoprene and total MTs ($p > 0.05$) when compared to the spot 2 and spot 5, respectively, with the litter layer. However, there was a notable shift in the terpenoid speciation between the soil chambers with and without litter (Fig. 5). In the dry season 2024, there was an emission of β-pinene, α-copaene, and β-caryophyllene in spot 5 with litter and an uptake of those terpenoids when the litter was removed. In contrast, limonene was taken up in both cases, but less with the litter layer intact. Similarly in the dry-to-wet season 2023, β-pinene, α-terpinene, and 3-carene emissions declined with litter removal and α-pinene even shifted from emission to uptake.

4.2 Effect of litter

Line 417: The removal of litter during the dry-to-wet season 2023 and the dry season 2024 in one of the spots each did not significantly affect the flux of isoprene. This suggests that microorganisms residing in the soil layer beneath the litter are primarily responsible for metabolizing these compounds. In contrast, the removal of litter in those two spots did decrease the flux of certain compounds like α-pinene, β-pinene, limonene, camphene, α-copaene and β-caryophyllene. Although this result was not statistically significant (~~ANOVA t test~~ $p>0.05$ for all the aforementioned compounds in both seasons, sample size with litter n=23, without litter n=8), it gives directional evidence towards the role of litter on total soil terpenoid fluxes.

5. Conclusion

Line 539: MT and SQT emissions and uptake showed to be governed on the litter layer and season, as well as showing very local differences from spot to spot in the composition of the total flux. Because of the small replication size and the large differences between spots, the effect of the litter layer is exploratory.

Another point that is unclear is why the blank measurements were not subtracted from the measured data, but instead showed alongside it. The presented flux data might therefore be offset. In addition to performing a background correction, I suggest calculating and including the LOD (or LOQ) for these measurements in the graph to assess the technical limitation of the flux measurements. Was the blank measurement consistent throughout all seasons or years, or did it fluctuate?

Response: Thank you for raising the suggestion to subtract blank measurements. Indeed, our flux data could be offset by choosing not to subtract blank measurements. However, we chose to show the "raw" flux in the plots with the mean blank measurements as lines, as the blank measurements varied between seasons (see Table A2 in the appendix of the paper) and were not done in parallel to each of the samples. Also, in general, chamber measurements can be offset due to temperature and humidity increases within the chamber when compared to the outside air, and so can the blank values. We accounted for the temperature difference when calculating the fluxes. However, these values could still not be the true blank in a chamber. Possible effects include wall-sorption and desorption, material emissions or contamination of the used chamber and foil with litter and soil residue. Subtracting non-representative blanks would risk over- or under-correcting our fluxes, so we would prefer to show them as non-subtracted values alongside the blank measurements.

Line 260: The net soil fluxes and mean blank values are shown in Figure 3. Blank values varied across seasons, and the number of blanks taken in each campaign differed (Table A2, Figure A5). Therefore, blank values were not subtracted to avoid under- or over-correcting from possibly non-representative blank values.~~of~~ ~~I~~Isoprene and two of its oxidation products methacrolein (MACR) and methyl vinyl

ketone (MVK) (Fig. 3) were negative in all four measured seasons, indicating that these species were consistently taken up by the soil.

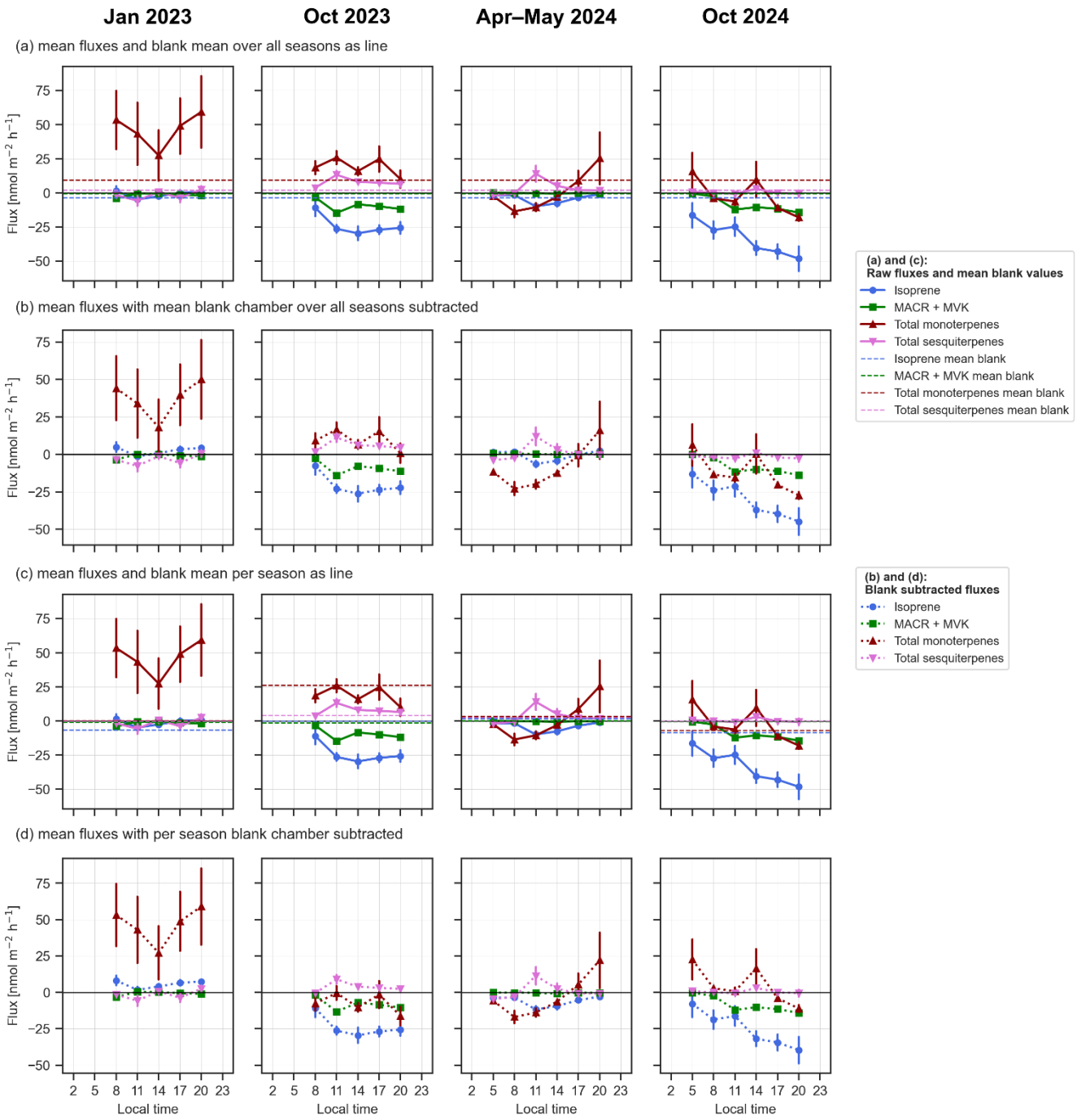

Figure A5 Diurnal curves of the mean calculated fluxes of isoprene (blue), isoprene oxidation products methacrolein (MACR) and methyl vinyl ketone (MVK), the total monoterpenes (red) and the total sesquiterpenes (pink) over the four measured seasons Jan 2023, Oct 2023, Apr-May 2024 and Oct 2024 in the course of a day from 5 am to 8 pm with blank values as dotted lines as mean (a) over all seasons (N=21) and (b) for each season (N=2 for Jan 2023, N=4 for Oct 2023, N=4 for Apr-May 2024, and N=11 for Oct 2025) and with subtracted blank values from the flux (b) over all seasons and (d) per season in nmol m^{-2} h^{-1}. Error bars indicate the standard error of the mean.

Thank you for suggesting to include the LOD for these measurements. The mean LOD across the four measurement campaigns for the vmr in pptv for each compound in the Table A1 is added. For the flux, we only considered it a flux different from 0 if the difference between the measured concentration inside and outside of the chamber was higher than the variability of the measurement. We consider this approach to be more reliable than calculating an LOD for the flux.

Table A1 Mean Retention times, mean measured flux in nmol m^{-2} h^{-1} with standard deviation, mean limit of detection (LOD) across the measurement campaigns in pptv with standard deviation, and mean ambient ratio in pptv with standard deviation of all measured terpenoid substances in each season. * Substance LOD and calibration performed using liquid calibration as described in the method section. ° Substance only tentatively identified and calibrated to (+)- and (-)-camphene.

		mean flux [nmol m^{-2} h^{-1}]					ambient vmr [pptv]			
Number of data points		31	70	58	55	Mean LOD [pptv]	33	81	58	54
Substance	**mean RT [min]**	Jan 2023	Oct 2023	Apr-May 2024	Oct 2024		Jan 2023	Oct 2023	Apr-May 2024	Oct 2024
isoprene	5.50	-1.46 ± 4.72	-27.18 ± 21.82	-5.00 ± 7.06	-35.33 ± 26.32	0.81 ± 1.68	220.66 ± 262.27	3829.13 ± 1462.75	356.48 ± 448.87	3977.21 ± 1922.10
MACR	8.04	-0.58 ± 1.25	-10.84 ± 7.43	-0.23 ± 0.92	-8.63 ± 7.07	0.58 ± 0.90	92.74 ± 78.31	974.02 ± 506.21	69.94 ± 41.23	820.60 ± 476.11
MVK	8.55	-2.94 ± 4.68	-11.48 ± 8.83	-0.33 ± 1.18	-11.34 ± 10.30	0.63 ± 0.98	303.22 ± 291.41	1168.65 ± 713.21	95.35 ± 53.64	1049.95 ± 688.54
sabinene	32.82	2.69 ± 6.70	-0.31 ± 0.14	-0.14 ± 0.21	-0.15 ± 0.42	0.51 ± 1.10	15.71 ± 17.54	30.86 ± 9.65	11.81 ± 8.42	25.48 ± 15.92
tricyclene	33.42	0.09 ± 0.21	0.14 ± 0.44	0.00 ± 0.06	-0.01 ± 0.02	0.03 ± 0.03	2.88 ± 3.10	2.74 ± 1.82	1.30 ± 0.69	2.24 ± 1.28
(−)-α-pinene	33.95	5.63 ± 9.86	3.35 ± 8.11	-0.30 ± 1.56	-1.46 ± 1.95	0.21 ± 0.68	42.20 ± 52.10	312.59 ± 144.49	62.43 ± 42.52	230.42 ± 124.94
(+)-α-pinene	34.44	3.50 ± 6.54	1.91 ± 5.15	0.05 ± 0.61	-0.60 ± 1.01	0.18 ± 0.58	38.61 ± 106.25	222.45 ± 168.90	21.48 ± 11.59	101.49 ± 74.42
3-carene	35.70	0.06 ± 0.32	-0.00 ± 0.14	-0.19 ± 0.64	-0.12 ± 0.38	0.18 ± 1.18	2.54 ± 4.57	2.11 ± 6.50	22.85 ± 41.13	10.87 ± 19.46
(+)-α-fenchene °	36.93	0.26 ± 0.35	0.88 ± 1.86	-0.07 ± 0.13	0.19 ± 0.19	0.32 ± 0.91	0.00 ± 0.00	15.07 ± 20.76	10.28 ± 4.33	2.22 ± 3.94
(−)-α-fenchene °	37.22	0.17 ± 0.13	0.45 ± 0.99	-0.01 ± 0.04	0.10 ± 0.13	0.24 ± 1.02	0.00 ± 0.00	3.42 ± 8.69	3.88 ± 1.89	0.85 ± 1.08
(+)-camphene	37.63	1.94 ± 3.01	0.77 ± 2.76	-0.22 ± 0.16	-0.35 ± 0.55	0.32 ± 0.91	10.24 ± 22.46	68.85 ± 24.63	19.55 ± 9.66	55.73 ± 23.17
(−)-camphene	38.28	1.49 ± 2.63	0.99 ± 2.27	-0.00 ± 0.08	0.07 ± 0.18	0.24 ± 1.02	9.86 ± 21.10	10.88 ± 15.89	3.41 ± 2.37	7.44 ± 4.91
(+)-β-pinene	39.61	3.33 ± 7.38	0.58 ± 0.93	0.22 ± 1.59	0.12 ± 0.24	0.21 ± 0.68	2.08 ± 4.78	27.08 ± 22.66	23.51 ± 15.27	14.18 ± 8.61
(−)-β-pinene	40.02	1.70 ± 2.07	0.41 ± 1.18	-0.00 ± 0.33	-0.47 ± 0.90	0.18 ± 0.58	3.95 ± 7.88	35.95 ± 14.49	11.78 ± 14.15	117.78 ± 50.05
ocimene *	40.91		6.62 ± 7.22		-0.36 ± 0.41	0.07 ± 0.06		105.92 ± 99.78		29.31 ± 25.42

(−)-limonene *	41.48	1.39 ± 2.31	0.27 ± 2.38	-0.25 ± 0.45	-0.30 ± 3.19	0.12 ± 0.05	7.07 ± 5.79	98.56 ± 110.33	27.36 ± 24.79	59.94 ± 67.64
(+)-limonene	41.87	1.36 ± 3.46	0.11 ± 1.31	-0.71 ± 1.15	-1.18 ± 3.98	0.28 ± 1.53	10.21 ± 8.20	84.53 ± 66.76	57.35 ± 54.25	91.84 ± 212.67
terpinolene	45.32	0.17 ± 0.29	2.36 ± 6.76	4.13 ± 27.40	0.68 ± 4.73	0.26 ± 0.26	8.72 ± 8.41	28.18 ± 29.79	91.87 ± 102.60	14.46 ± 9.11
α-copaene *	57.04	0.00 ± 0.00	1.54 ± 0.89	0.38 ± 1.18	-0.06 ± 1.59	0.04 ± 0.02	0.00 ± 0.00	63.81 ± 30.85	26.27 ± 22.84	115.55 ± 116.90
β-caryophyllene	60.78	0.04 ± 0.13	0.33 ± 0.75	1.90 ± 4.82	0.94 ± 4.29	1.04 ± 6.04	0.00 ± 0.00	42.06 ± 31.43	24.34 ± 51.74	136.66 ± 210.52
(+)-cyclosativene *	62.65		0.66 ± 0.50	-0.02 ± 0.39	0.00 ± 0.00	0.11 ± 0.10		3.41 ± 6.67	36.40 ± 24.52	0.00 ± 0.00
(−)-α-cedrene *	63.79	0.00 ± 0.01	0.04 ± 0.07	0.04 ± 0.45	0.00 ± 0.00	0.10 ± 0.05	0.00 ± 0.00	1.51 ± 2.20	27.88 ± 17.12	0.00 ± 0.00
(+)-δ-cadinene *	66.24	0.05 ± 0.14	0.33 ± 0.21	0.01 ± 0.05	-0.04 ± 0.15	1.00 ± 0.71	1.04 ± 3.20	14.26 ± 6.60	1.96 ± 1.31	6.77 ± 9.71

The study design for Section 3.4, 3.4.1, and Figure 4 is unclear. The analysis aimed to investigate the effects of soil properties on soil fluxes. However, it is based on recurrent measurements from the same chamber/spot, ie, it appears based on 1 biological replicate.

Response: Yes, we are comparing the different soil spots with diverse properties (respiration rate, organic content), so each subplot includes only one biological replicate. Our strategy is to examine how differently these soil type extremes affect atmospheric trace gases. We agree that we should phrase the section title differently, as we are not strictly investigating the effects of different soil properties, e.g., spot 4 and spot 5 had similar properties, while spot 1 had a higher organic content, but comparing different soil spots.

Line 286: 3.4 ~~Effects of the soil properties on~~ Comparison of the terpenoids ~~soil~~ fluxes from the different soil spots

Line 321: 3.5 ~~4.3~~ Soil fluxes VS environmental conditions

The correlation between soil fluxes and environmental conditions (fig.6) is interesting. Why was the chirality not considered here, being a central focus of the study? Also, the figure 6 shows the correlation between SWC and VOC emissions, but it is not mentioned anywhere in the text.

Response: We thank the reviewer for the interest in the correlation between the chirality and environmental conditions, we now added the enantiomers to Figure 6. Also, we agree that the discussion of the relationship between SWC and the soil fluxes should be extended in the text. As the SWC and temperatures co-vary, so interpretation has to be done carefully.

Following the comment from reviewer 1 we adjusted our statistical model as it was violating assumptions of independence. Instead of Pearson correlations, we now use a linear mixed-effect model

with fixed effect of local time (categorical value for hour of the day) and adjusting for random effects of measurement date and chamber spot location to assess β coefficients as effect sizes of environmental parameters on fluxes. This now modified statistical model are similar in direction to the results from Pearson correlations submitted in the preprint. This revised model, besides taking into account our data structure and limitations (repeated measurements over time), also has improved interpretability as the effect sizes show the change in flux per increase in the variable.

The chirality was omitted when Pearson correlations were used to make the plot look cleaner and as the difference between chiral pairs was not strong. We now present the heatmap with β coefficients from the linear mixed-effects models including both enantiomers. While the effect size differs between enantiomers, the direction (negative, positive or close to zero) of the β coefficients remains the same for the chiral pairs.

We adjusted the order of ambient parameters in Figure 6 to the same order as was used in Figure 2, added ozone to Figure 2 (f), and adjusted the unit for PAR from μmol m^{-2} s^{-1} to mmol m^{-2} s^{-1}.

2.6 Statistical analysis

Line 193: Statistical analyses were performed using python (version 3.12.4) with the following packages: numpy (v.2.0.0), pandas (v.2.2.2), matplotlib (v.3.9.1), seaborn (v.0.13.2), statsmodel (v.0.14.5 ~~2~~), and scipy (v 1.16.0). Data visualization was conducted using matplotlib and seaborn. Statistical differences were assessed using linear mixed-effect models, because the dataset contains repeated measurements over time from the same soil chambers and ambient sampling points, which violates assumptions of independence of simpler tests. Local time (hour-of-day as a categorical factor), was included as a fixed effect in all models, because we expected a diurnal pattern for the measured VOC fluxes and mixing ratios. ~~between soil fluxes measured in different seasons and from different soil plots~~ were ~~determined using the Tukey HSD (Honestly significant difference) test following a significant result from ANOVA~~

Line 198: We fitted linear mixed-effects models with fixed effects for environmental predictors and local time, and random intercepts for measurement date and chamber spot location, to quantify the association of predictors with fluxes. Regression slopes (β) represent the change in flux per unit increase in the predictor.~~For correlations between the fluxes and environmental parameter Pearson coefficients were calculated.~~ Statistical significance was accepted for $p < 0.05$.

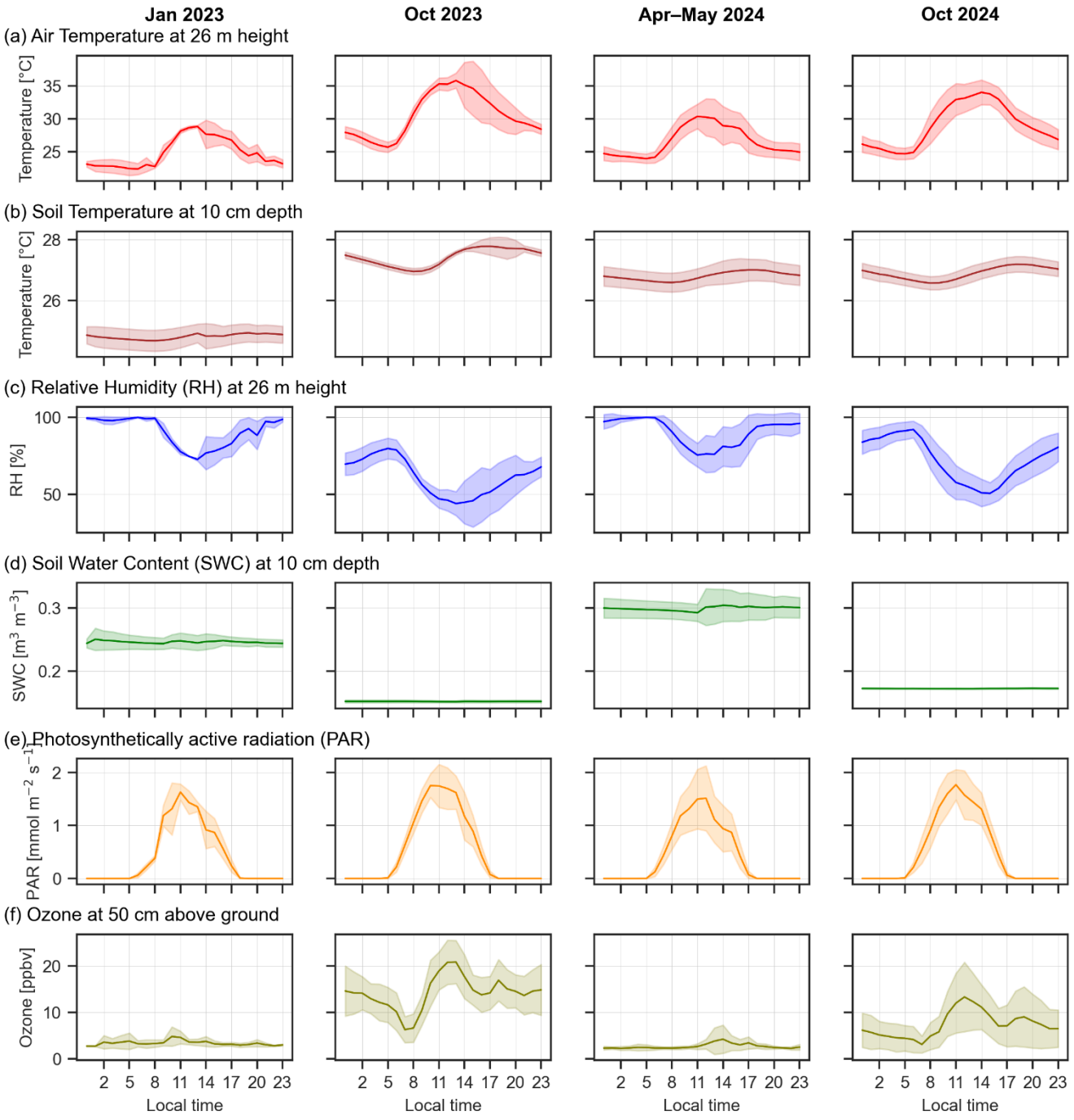

Figure 2 Meteorological data during the measured seasons with (a) temperature (red) and (c) relative humidity (blue) measured at 26 m at the Instant tower, (b) soil temperature (orange) and (d) soil water-content (green) measured at 10 cm depth and (e) photosynthetically active radiation (PAR) incoming at 81m at the Instant tower and (f) ozone measured 50 cm above the ground at the instant tower across the four measurement periods in the different seasons. The line represents the mean, and the shaded area is the standard deviation.

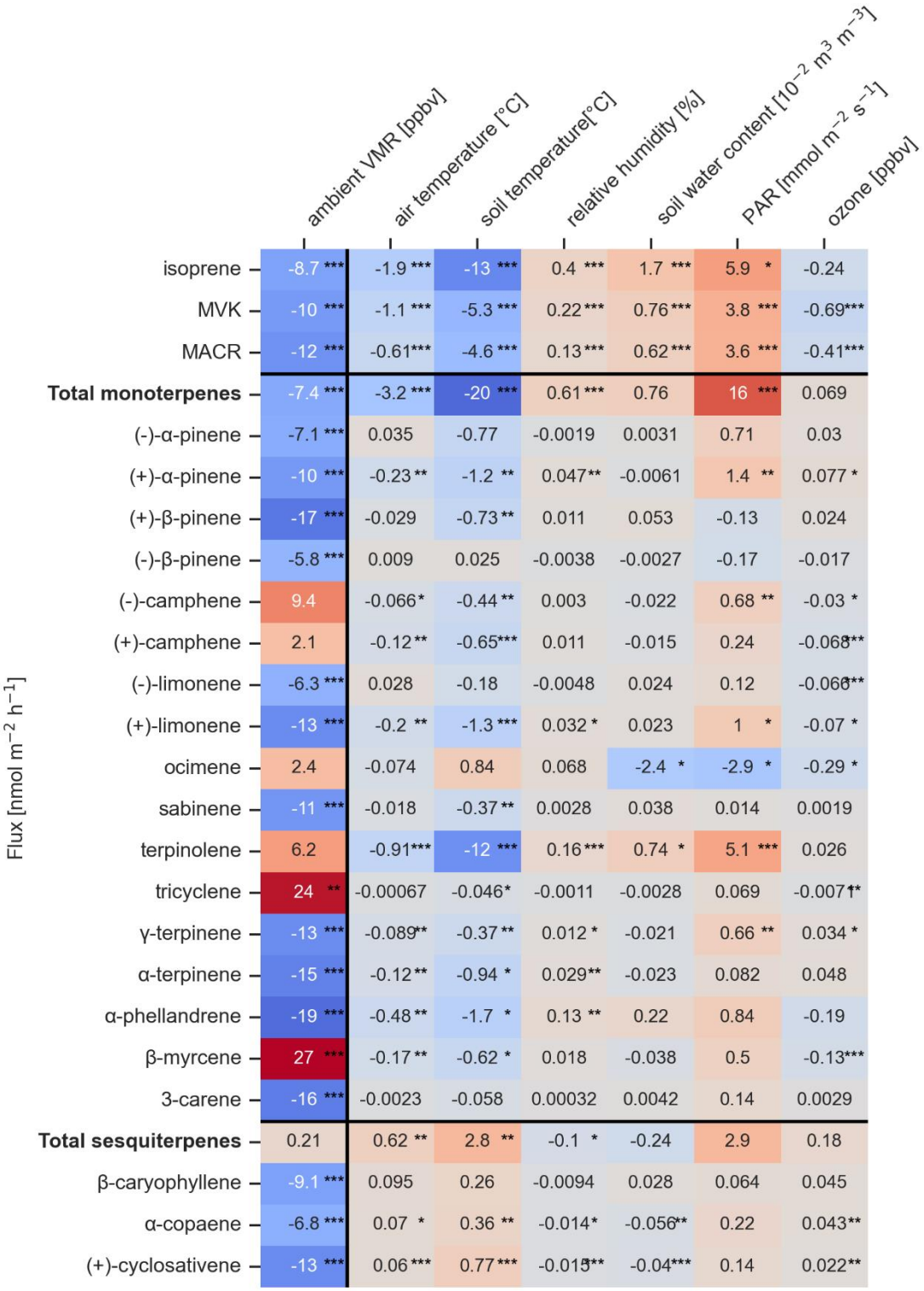

Figure 6 Heatmap of ~~correlations between the~~ β coefficients from linear mixed-effects models quantifying the change of flux in nmol m^{-2} h^{-1} of measured compounds ~~and~~ per unit change in environmental variables, after adjusting for the fixed effect of chamber spot location and random effect for measurement date. ~~various e~~ Environmental variables are: 545 **ambient mixing ratio (VMR) of each compound in ppbv, air temperature at 26 m in °C, incoming photosynthetically active radiation (PAR) at 81 m in mmol m^{-2} s^{-1}, soil temperature in °C and soil water content (scaled by 10^{-2}) at 10 cm depth in 10^{-2} m^3 m^{-3}. Statistical significance of the ~~Pearson correlation~~ β coefficients is indicated by asterisks: (*) for p <0.05, (**) for p<0.01, and (***) for p< 0.001.**

3.4.2 Soil fluxes VS environmental conditions

550 Line 322: Isoprene, MVK, and MACR ~~have a~~ were strongly negatively ~~correlation~~ associated ~~(Fig. 6)~~ with their ambient atmospheric ~~concentration~~ mixing ratios (β= -8.7, -10, and -12 nmol m^{-2} h^{-1} per 1 ppbv increase respectively; p < 0.001; Fig. 6), after accounting for repeated chamber spot location

measurements and dates in the linear mixed-effect model. This indicatinges the uptake rates were higher (so flux values became more negative) when the available concentrations in the air above the soil were higher. Most MTs and SQTs show the same pattern, with flux rates decreasing as ambient mixing ratios increased. Exceptions were β-myrcene and tricyclene, which exhibited a positive association with ambient mixing ratios (β = +27 and +24 nmol m^{-2} h^{-1} per 1 ppbv; $p < 0.001$ and $p < 0.01$, respectively). While effect sizes differed between enantiomers, the direction of the associations was generally similar. Soil and air temperature were negatively associated with isoprene, MACR, MVK, and total MT fluxes (flux decreased with warming), whereas SQT soil flux increased with temperature. In contrast, soil water content and relative humidity were positively associated with flux. Total MTs showed the strongest positive association with PAR (β = 16 nmol m^{-2} h^{-1} per mmol m^{-2} s^{-1}; $p < 0.001$).

~~Different MT species like α-phellandrene, 3-carene, γ-terpinene, limonene, and β-ocimene, also show negative correlation with their mixing ratios (-0.21 to -0.82; p <0.001), while β-myrcene had positive correlation with the ambient concentration (0.54; p <0.001). In general, the correlations with the environmental conditions like air and soil temperature and soil water content were stronger for isoprene and its oxidation products. The correlation with the photosynthetic active radiation (PAR) was highest for ocimene (0.29; p <0.01) and the total SQTs, as well as α-copaene (0.26 and 0.24; p <0.001).~~

4.1.1 Isoprene and the oxidation products MACR and MVK

Line 380: For isoprene, MACR, and MVK, ~~strong correlations between soil fluxes and both ambient mixing ratios (-0.74 to -0.81; p < 0.001) and key environmental parameters such as soil water content (0.54 to 0.63; p < 0.001) and temperature (-0.48 to -0.56; p < 0.001) (Fig. 7) are found.~~ increasing soil fluxes were associated with increasing ambient mixing ratios (β = -8.7, -10, and -12 nmol m^{-2} h^{-1} per 1 ppbv respectively; $p < 0.001$) and key environmental parameters as soil temperature (β = -13, -5.3, and -4.6 nmol m^{-2} h^{-1} per 1 °C; $p < 0.001$), while decreased with increasing soil water content (β = 1.7, 0.76, and 0.62 nmol m^{-2} h^{-1} per 0.01 m^3 m^{-3}; $p < 0.001$) (Fig. 6). ~~The correlation with the environmental parameters like temperature hint either to the higher ambient concentrations of isoprene at higher temperatures (Alves et al., 2016) or to more efficient uptake rates at higher temperatures.~~ The pattern of greater uptake at higher temperatures and lower soil water content likely reflects co-variation between meteorology and ambient isoprene: warmer, drier periods tend to have higher ambient isoprene (Alves et al., 2016). Also, soil microbial uptake rates could be more efficient at higher temperatures.

4.1.2 Monoterpenes and sesquiterpenes

Line 400: ~~Different MTs correlated negatively or positively with ambient concentrations (Fig. 7), indicating there are different processes responsible for the soil fluxes of each MT.~~ The lack of a consistent pattern in the association between soil fluxes of individual MTs and ambient mixing ratios, PAR, temperature, and soil water content suggests that these exchanges are not governed by a single overarching mechanism. Instead, soil MT fluxes appear to result from compound-specific abiotic and biotic processes within the soil.

Line 408: SQT soil fluxes were mostly associated with temperature (Fig. 6), reflecting the observed seasonal trends. Microbial activity and/or abiotic release could be increased with temperature.

What is the VOC breakthrough volume in the adsorbent? Could higher ambient VOC levels cause cartridge breakthroughs and affect soil emission estimates?

Response: Cartridge breakthrough is indeed an important issue when sampling. We tested for breakthrough in our cartridges by sampling high volumes of diluted (up to 10 ppbv with up to 6 L collected volume) calibration gas (which included isoprene, MACR, MVK, selected MTs and β-caryophyllene; see manuscript text for exact composition) onto two cartridges in sequence. We could not detect any compound in the second cartridge (below the Limit of Detection). However, as we use two adsorbents in a row in our cartridges, there could be compounds breaking through the first

adsorbent Tenax and not being efficiently released by thermal desorption from the second adsorbent Carbograph.

We take this possibility into account with our calibration method in which we use the same handheld pumps to collect different concentrations of a compound from diluted calibration gas with the same volume of sample collected as in the environmental samples. Therefore, if we assume that in environmental samples the concentration of a compound is constant within the 30 minutes of collection, the same breakthrough in the two adsorbent or sampling losses should happen as in our calibration samples. Therefore, quantification should not be affected by a breakthrough through the first adsorbent bed.

For isoprene however we are not in the linear range of the GC-MS system anymore. As the method targeted the low-concentration MTs and SQTs, a higher split in the injection to avoid overload and saturation of the instrument was not possible. We adjusted the calibration curve for higher concentration by fitting two linear functions in the lower and higher range. However, high concentrations of isoprene could still be underestimated because of the saturated instrument. For the other two compounds MACR and MVK with lower volatility, the samples were still within the calibrated range.

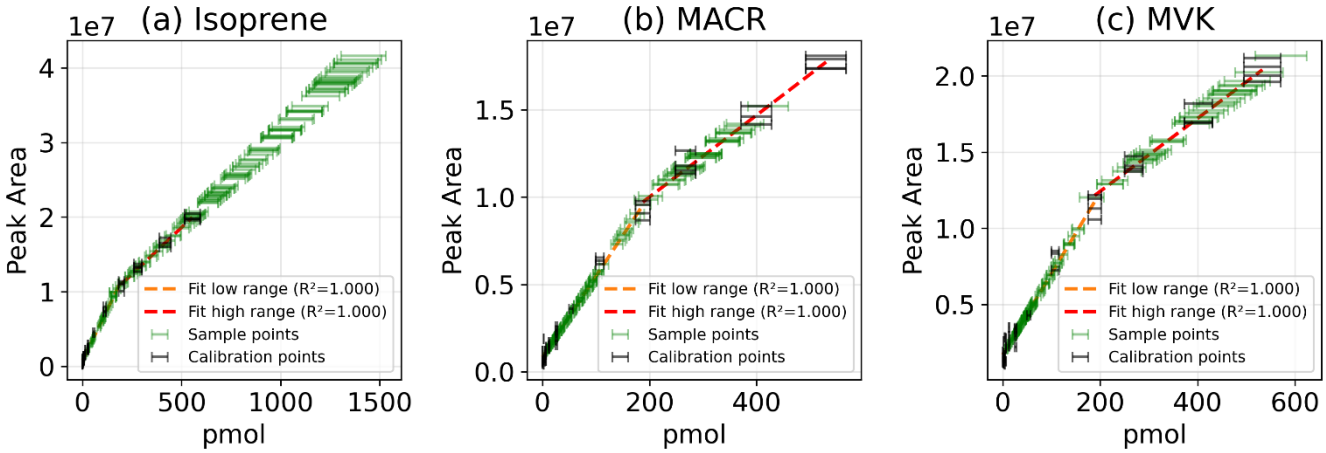

Figure A3 Calibration curve fit of integrated Peak Areas against substance on the cartridge in pmol for (a) isoprene, (b) MACR, and (c) MVK with calibration points with error bars plotted in black, linear fit in the lower range in orange and in the higher range in red, and sample points (from October 2024) in green with error bars.

Line 138: Breakthrough was tested using two sorbent cartridges in sequence, with calibration gas concentrations up to 10 ppbv, a 6 L sample volume, and a flow collection rate of 200 mL min^{-1}, resulting in no detectable targets in the second cartridge.

4.6 Limitations of this study and future directions

Line 534: Due to saturation of the instrument (outside linear range) for higher concentrations of isoprene vmr and therefore also fluxes could be underestimated in the dry seasons (Fig. A3).

The authors found that isoprene concentrations at soil level peaked at noon, while MT and SQT peaked later. Can you elaborate on this?

Response: Isoprene emission from plants is primarily controlled by temperature and light, as their synthesis is placed inside the chloroplasts (Guenther et al., 1991). As light intensity is highest at noon, we see the isoprene mixing ratios peak as expected. MT and especially SQT may partially be stored prior to emission, so SQT are usually less light-dependent, but have increased emission with temperature. Therefore, we see a peak a little later in the day when temperature is highest.

Line 376: As shown in Fig. 4, the isoprene mixing ratios follow diurnal cycles with peak levels around noon, which is consistent with light and temperature driven *de novo* emissions from the canopy above (Guenther et al., 1996; Crutzen et al., 2000; Yáñez-Serrano et al., 2015; Alves et al., 2016; Jardine et al., 2017; Gomes Alves et al., 2023). Isoprene is typically more light-dependent than MTs and SQTs and therefore the observed midday isoprene peak and later MT and SQT maxima are consistent with previous studies (Guenther et al., 1991; Kuhn et al., 2005; Yañez-Serrano et al., 2017).

The SQT soil fluxes reported here are 10 to 10,000 times lower than those in other studies. Is there a technical reason in the measurements or in the data analysis? Can the authors compare and discuss their measured mean air concentrations with published data?

Response: Certainly, the SQT fluxes from this study (4 ± 2 nmol m^{-2} $h^{-}1$) are lower than the few published values we can compare to, and warrant discussion. Very few studies have reported fluxes for SQTs in addition to MTs. When we compare SQT emission fluxes with other studies, it should be noted that previous studies were mostly done in very different ecosystems like a boreal forest where 3-171 nmol m^{-2} h^{-1} (Mäki et al., 2017, 2019) or tundra where 40.000 -180.000 nmol m^{-2} h^{-1} (Baggesen et al., 2021) were reported. These analyzed soil spots contained vegetation, while our chambers did not have plants inside them. In one study conducted in a tropical rainforest in French Guyana, the authors observed that uptake and emission of SQT differed between fertilized (122 nmol m^{-2} h^{-1}) and unfertilized soil **(**-323 nmol m^{-2} h^{-1}) (Llusià et al., 2022). Also uptake rates of -30 ± 16 nmol m^{-2} h^{-1} were reported for eucalyptus plantations (Mu et al., 2023)**.** The study of Bourtsoukidis et al. (2018) which was also performed with Amazon rainforest soil has reported fluxes for SQT of 489 nmol m^{-2} h^{-1} from an experiment in which they used zero-air (VOCs filtered out) to flush chamber cuvettes in the field as well as in a controlled laboratory experiment with soil samples. In part of their experiment, they used a diluted calibration gas to flush the chambers, but the calibration gas only contained α-pinene and no other MT or SQT. With this approach it is likely to generate strong emissions because of the artificial concentration gradient between the soil and the air above which will lead to much higher emission values (Ortega et al., 2008).

We discuss this in Line 485 onwards and clarified it more.

Line 485: The overall ~~low~~ mean emission flux for SQTs found here is 4±2 nmol m^{-2} h^{-1}. This is low i~~I~~n comparison to ~~another~~ a previous study conducted with soil from the same measurement site but mostly under laboratory conditions (Bourtsoukidis et al., 2018)~~,~~. T~~t~~he fluxes for SQTs identified here are lower by a factor of 10 to 10,000. However, ~~this~~ the laboratory study data was derived using ~~from a study where~~ BVOC-free air, which was cycled into the chambers and onto soil ~~at laboratory conditions~~, generating a maximum potential flux. The air was partially enriched with VOCs from a calibration mixture, ~~which~~ however this cannot reflect the greater chemical diversity of natural conditions with ~~their greater chemical diversity~~ as this calibration mixture only contained one MT (α-pinene) and no SQT. As ~~and~~ simultaneous soil uptake and emission occurs, the chosen method could be responsible for the observed difference in flux. Another study from a tropical forest in Guyana reported uptake and emission rates for SQT, depending on if they fertilized a soil spot (122 nmol m^{-2} h^{-1}) or left it unfertilized **(**-323 nmol m^{-2} h^{-1}) (Llusià et al., 2022). In an eucalyptus plantations also uptake rates of -30 ± 16 nmol m^{-2} h^{-1} were found (Mu et al., 2023), while in a boreal forest emission rates for SQT of 3-171 nmol m^{-2} h^{-1} (Mäki et al., 2017, 2019) and in a tundra 40.000 -180.000 nmol m^{-2} h^{-1} (Baggesen et al., 2021) were reported. However, we have to note that in the studies with higher emission rates vegetation was inside the used chambers as the soil was naturally covered by plants. So, the soil studies that report SQT fluxes we can compare this study with were mainly performed in vastly different ecosystems, used fertilization treatments, or derived under artificial laboratory conditions likely to maximize fluxes.

Line 502: ~~In other soil studies, SQT fluxes were also low, ranging between values for uptake in the range of -32 - -44 nmol m^{-2} h^{-1} (Llusià et al., 2022; Mu et al., 2023) and emission of 3-121 nmol m^{-2} h^{-1}~~ (Mäki et al., 2019; Llusià et al., 2022)~~.~~
~~The fluxes measured in this study are generally lower than those reported in the literature, which can be attributed to differences in environmental conditions.~~

There is extensive evidence that soil bacteria degrade isoprene, which may be useful to refer to (e.g., El Khawand et al., 2018; McGenity et al., 2018; Murrell et al., 2020).

Response: Thank you for drawing our attention to further literature evidence on soil bacteria degrading isoprene. We now include the citations of these more recent studies on isoprene consuming organisms.

Line 49: Microbes can consume isoprene using it as an energy source, and may emit it at low rates (Cleveland and Yavitt, 1997; Gray et al., 2015; El Khawand et al., 2016; McGenity et al., 2018; Murrell et al., 2020).

Line 387: Laboratory *in vitro* studies suggest that soil microorganisms like bacterial and fungal taxa consume isoprene (Cleveland and Yavitt, 1997; Gray et al., 2015; El Khawand et al., 2016; McGenity et al., 2018; Murrell et al., 2020) and use it as a carbon source.

Technical Corrections

I recommend adhering to SI (International System of Units) when presenting emission fluxes, ie, using seconds instead of hours.

Response: We agree that using SI units are the standard and thank you for suggesting to use seconds instead of hours. However, we would like to keep h^{-1} to facilitate comparisons with similar studies by Pugliese et al. (2023), Bourtsoukidis et al. (2018), Asensio et al., (2007) and others. Also, the data for this manuscript was already published in the unit [nmol m^{-2} h^{-1}] (Schüttler et al., 2025). We include a summary per second in the appendix.

Line 271: The net soil fluxes (see Table A1 in the appendix for an overview of all compound fluxes in [nmol m^{-2} h^{-1}] and an overview in SI units as [pmol m^{-2} s^{-1}]) of isoprene and two of its oxidation products methacrolein (MACR) and methyl vinyl ketone (MVK) (Fig. 3) were negative in all four measured seasons, indicating that these species were consistently taken up by the soil.

Table A1 Mean Retention times, mean measured flux in nmol m^{-2} h^{-1} with standard deviation and mean ambient ratio in pptv with standard deviation of all measured terpenoid substances in each season and below a summary of the fluxes of isoprene, MACR, MVK, total monoterpenes, and total sesquiterpenes in SI units pmol m^{-2} s^{-1}.

	mean flux [pmol m^{-2} s^{-1}]			
Substance	Jan 2023	Oct 2023	Apr-May 2024	Oct 2024
isoprene	-0.48 ± 1.33	-6.76 ± 6.19	-1.29 ± 2.03	-9.81 ± 7.31
MACR	-0.17 ± 0.36	-2.69 ± 2.19	-0.06 ± 0.25	-2.40 ± 1.96
MVK	-0.76 ± 1.27	-2.91 ± 2.50	-0.08 ± 0.33	-3.15 ± 2.86
Total monoterpenes	10.54 ± 14.00	5.44 ± 8.48	0.24 ± 10.26	-0.90 ± 8.44
Total sesquiterpenes	-0.26 ± 1.44	2.21 ± 2.57	1.18 ± 3.88	0.12 ± 1.43

L22: "Soil" may be removed or bracketed as the functions of those VOCs extend beyond soil ecology.

Response: Thanks. We agree that those VOCs could have an effect beyond the soil ecology and will therefore put soil in brackets

Line 23: Although soil BVOC fluxes contribute little to the overall atmospheric budget in rainforests dominated by the plant canopy, they may affect near-surface chemistry and play important roles in (soil) ecology.

L107: If measurements were taken across all seasons and the mean and SD are reported for each season, I assume the 23 refers to the total number of blank measurements rather than replicates of each season, year and field campaign conducted?

Response: Yes, 23 refers to the total number of blank measurements. We have now improved the sentence to make it clearer.

Line 107: A total of 23 replicates were measured from the blank chamber distributed across all seasons, and the mean blank fluxes and the standard deviation median are reported in Table A2 in Appendix A.

L130-135: Please clarify which standards were purchased as liquids and which were supplied by Apel-Riemer as a gas standard mixture.

Response: Thanks for asking about the liquid standards. We hope this might help anyone trying to do a similar experiment.

Line 127: Compounds were quantified using a gas standard calibration mixture and liquid standards injected at 1, 2, 4, 6, 8, and 10 µL in methanol-diluted compound mixtures with a syringe directly onto the sorbent cartridge. As liquid standards (−)-limonene (TCI), 3-carene (Merck), (−)-α-cedrene (Sigma-Aldrich), (+)-δ-cadinene (TCI), (+)-cyclosativene (Sigma-Aldrich), (+)-longifolene (PhytoLab), (-)-isolongifolene (Fluka), α-copaene (Biomol), trans-β-ocimene (LGC), (−)-α-phellandrene (Sigma-Aldrich), (−)-α-pinene (thermoscientific), (+)-α-pinene (Acros Organics), (+)-β-pinene (Fluka), sabinene (ChemCruz), β-caryophyllene (Sigma-Aldrich), a-terpinene (Sigma-Aldrich) and γ-terpinene (Sigma-Aldrich) were used. The gas standard mixture contained isoprene, MVK, MACR, tricyclene, (−) and (+)-α-pinene, (−)-β-pinene, (+) and (−)-camphene, sabinene, β-myrcene, (−)-α-phellandrene, (−)-3-carene, α-terpinene, (+)-limonene, γ-terpinene, terpinolene, m- p- and o-cymene, (+) and (−)-linalool, and β-caryophyllene (Apel-Riemer International, USA). When a calibration was performed with calibration gas and liquid standard, the calibration with gas standard was used, as it is more similar to the conditions when filling environmental samples than injecting methanol-diluted compound mixture.

L141-142: This section is unclear. Are you referring to technical variability? Please clarify and also report this variability.

Response: Yes, we mean technical variability in instrument and sampling set up as the variability in peak area of the same calibration step. As the calibration set-up was the same as when an environmental sample is collected this variability represents the total uncertainty in a sample.

Line 138: Peak areas were used to quantify concentrations ~~and~~. The sum of instrumental and sampling procedure ~~measurement~~ variability was assessed by ~~the deviation in measurements from at least~~ a minimum of five calibration samples with the same amount of standard calibration gas ~~or liquid standard injection~~. The standard deviation of the integrated area between these measurements of the

same concentration was used as variability in measurement. The values as mean values across all measurement campaigns are reported in percent (as standard deviations divided by the mean values) and were 4% for isoprene, 13% for MACR, 10% for MVK, 7% for (−)-α-pinene, 12% for (+)-α-pinene, 6% for β-myrcene, 9% for tricyclene, 9% for (+)-camphene, 6% for (−)-camphene, 16% for sabinene, 6% for α-terpinene, 10% for (+)-limonene, 6% for γ-terpinene, and 12% for β-caryophyllene. For substances with liquid calibration the variability of (−)-α-pinene was used.

The following reported values were the minimum and maximum values of different campaigns/calibration curves. Now we report mean values instead.

Line 146: The flux error was calculated by the variability of the BVOC concentration derived from repeated measurements of a known concentration of analyte and ranged between 7% for α-pinene and up to 16% for sabinene as this peak was not separated well in the chromatogram.

Table A1: include the retention index.

Response: While retention indexes can be very useful tools in GC, we unfortunately did not use n-alkane standards in our instrument runs and can therefore not calculate retention indexes. This is something we will consider in future studies.

Enantiomer analysis of VOC is not standard practice in the VOC research community. It would be informative to include a chromatogram in the supplement.

Response: Indeed, it can be informative for some readers to see the chiral identification for chiral compounds with our method in a chromatogram. We now provide this in Figure A2.

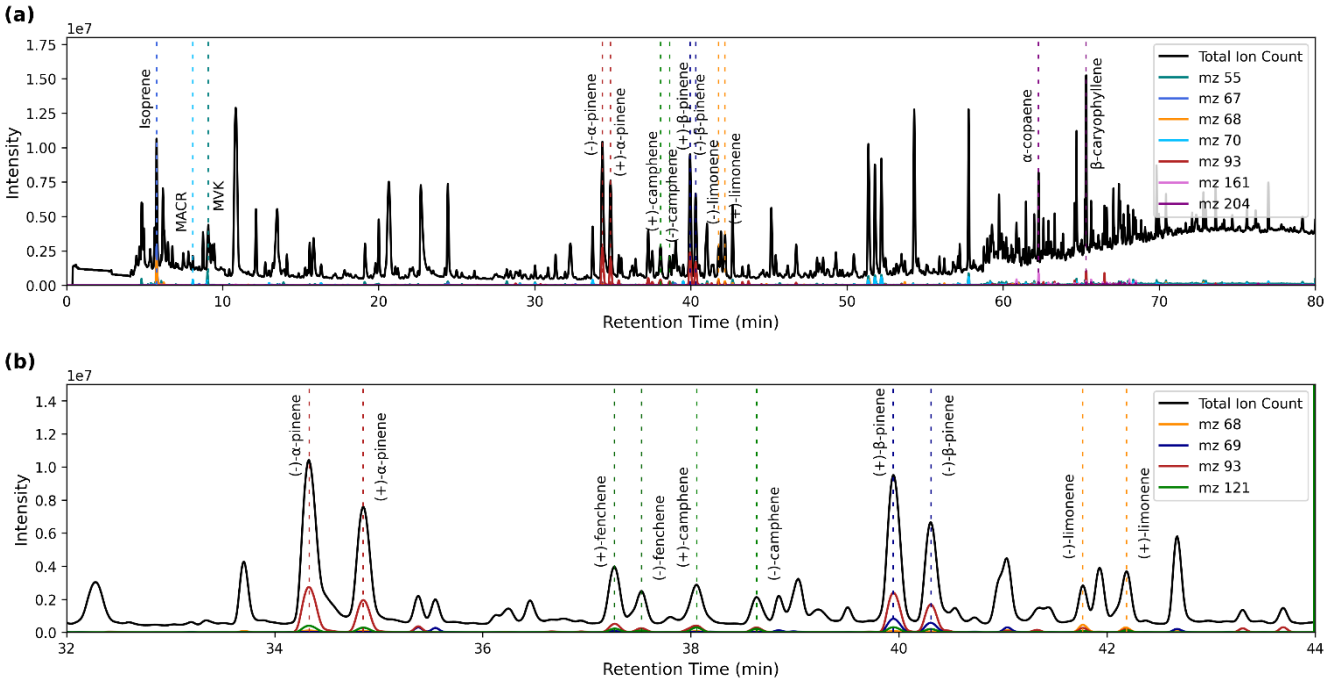

Figure A2 (a) Example Chromatogram of a soil chamber sample from October 2023 with annotation of isoprene, MACR, and MVK peaks, the chiral monoterpenes, and the two most prominent sesquiterpenes (b) Zoomed into the chiral monoterpene resolution.

Reply to comments from Reviewer 3:

General comments

The topic of this manuscript is of importance, as soil emissions have been severely neglected in the BVOC field and little is known about the processes affecting the magnitudes and types of emissions. While canopy emission especially in the tropic have been studied extensively, we still know next to nothing about how emissions and uptake from soil will change in the changing climate or due to extreme weather conditions, such as the El Niño. I also find the inclusion of stereoisomers into the larger discussion of terpenes interesting, especially if they can be used to track or estimate changes in biological processes due to environmental stressors.

Response: We thank the reviewer for taking the time to review our work, for providing comments, and for recognizing the importance of soil BVOC fluxes.

While the manuscript is in general well written and has merit, I have some critical comments – especially regarding the methodology and research questions. My main concerns are:

1. As I understand, authors measured BVOC fluxes from 3 separate locations close by to each other, but all differing; 1 without litter, 1 with litter, 1 near a termite nest. This means that only one true biological replicate per location was measured, which is – in my opinion – not sufficient for an ecological study. Authors have done pseudoreplication within one chamber for seasonal changes, but as the location of two of the chambers were changed between seasons, temporal comparison even within one chamber is difficult. Same applies for blanks, where only one spot was sampled, resulting in pseudo/technical replicates, not representative of the true natural variation. While the authors express that their aim was to "screen" differing extremes by placing the chambers in distinct locations, why not have multiple chambers in those distinct locations instead of one? Why blanks were only measured in one location? As the authors themselves express, the litter density varied significantly even within a few meters – which may also be the case for soil microbiome, roots etc. – all possibly affecting the BVOC fluxes observed. It is also evident from the results (Fig.4) that spots 2 and 3 differ from spots 4 and 5. As such, the lack of replication in this study is my main concern, and authors must be careful when expressing what can be concluded based on their results.

Response: Thank you for raising this important methodological concern. We agree that our study design can be viewed as pseudo-replication due to the stated objective of the study. Faced with high heterogeneity in the soils at the site, we opted to explore which chemical species are emitted and uptaken by soils that differ markedly in respiration rates, organic content and litter. This allowed us to look for soil emission markers and to determine which of the species we have been measuring in ambient air from the ATTO tower may be affected by soil (particularly the chiral species). As soil surveys become available, the sampling strategy will change to what the reviewer has in mind, characterizing the most widespread soil types with a high number of replicates. In order to take this point on-board, and following a comment from Reviewer 1, we therefore changed the statistical model to linear mixed-effect models to account for the pseudo-replication when looking at statistical significance.

We agree that changing the chamber locations between January 2023 and October 2023 makes it difficult to compare the first measurement season and the three following seasons. However, in October 2023, April-May 2024, and October 2024, the location was not changed, and the chambers remained in the exact same locations throughout the three measurement campaigns. This was done intentionally to compare the same chamber spot locations across seasons and look into possible long-term trends.

To transparently account for the lack of biological replication we make our discussion and conclusion statements more carefully as the reviewer has suggested.

4.6 Limitations of this study and future directions

Line 527: The study was conducted at three locations on a Terra Firme rainforest plateau, and the samples exhibited significant variability between soil spots. Having only one biological replicate for each of the three soil spots limits possible conclusions to the overall ecosystem soil BVOC flux.

5. Conclusion

Line 536: The soil-atmosphere exchange of terpenoids and their enantiomers in the Amazon rainforest at the site of the soil chambers from this study is strongly connected to season and environmental conditions like temperature and soil moisture. For the uptake of isoprene, MACR, and MVK, ambient concentrations and temperature seem to be the primary drivers. MT and SQT emissions and uptake were found to be governed by the litter layer and season, as well as showing very local differences from spot to spot in the composition of the total flux.

2. Why was ambient air used as carrier gas in the BVOC sampling? As authors state in the introduction, soil fluxes are typically orders of magnitude smaller than canopy/vegetation emissions, so would it not make more sense to use zero air (VOC free air) when investigating soil emissions, rather than ambient air with potentially high levels of BVOCs? I assume authors have aimed to record the background with the other tube measured in parallel to the soil chamber, but this does not address this issue. Furthermore, in Fig.1, it looks like the ambient tube was sampled separately "near" the actual chamber. Why not have a T-piece at the inlet of the chamber with part of the ambient air going into the chamber and part into the ambient tube? This would ensure that the ambient tube captures all possible analytes and contaminants going into the chamber.

Response: Using zero air as a flow through a chamber would cause an artificial burst of VOC emissions due to the generation of an unnatural concentration gradient. It would also preclude the characterization of VOC uptake. Furthermore, it does not reflect the real environmental conditions (Ortega et al., 2008; Veres et al., 2014; Zeng et al., 2022). This could be one of the reasons Bourtsoukidis et al. (2018) saw such high emission values for sesquiterpenes (SQTs). A more recent study by Edtbauer et al. (2021) investigating emissions and uptake from mosses and lichen has shown both processes to be important. While mosses were found to emit sesquiterpenes, they also take up oxygenated products of photooxidation, thereby influencing ambient levels of both species.

The tube for ambient measurements was placed directly next to the chambers. We were concerned that using a T-piece could have resulted in partially taking air from inside the chamber instead of from only the incoming flux, as the outlet and inlet pumps were set to the same flux and would therefore compete. However, as the ambient measurement was a maximum of 10 cm distant from the chamber inlet (Fig. 1 (c)), we assume the same air entering the chamber was collected as the background ambient sample.

Related to this, how were emission rates (Fig.3) for ambient samples calculated (what were Ct and C0)? What are the mean and standard error for (N=?); ambient air taken from the different locations, and the blank from another location? It is evident from emissions/uptakes that variation can be high between locations (e.g., monoterpenes), and for me, it's impossible to say if they actually differ from the blank. Because no true replication for the blanks were conducted, authors cannot show what the natural variation was. Emission of monoterpenes in Jan 2023 and uptake of isoprene in Oct 2023 and 2024 are more evident, but otherwise, they may well be within blank levels.

Response: For ambient samples, there was no calculation of emission rates. In Figure 3 (a), we report volume mixing ratios (ppbv) measured in the ambient samples and (b) soil fluxes measured with chambers and calculated as describes in the method section. To use as blanks for ambient air, cartridges

were transported to Brazil along with the normal air samples. They were opened and closed at the site, but no air volume was sampled on them (Zero-volume cartridge blanks). These blank cartridge samples (minimum two per measurement campaign) did not contain any target substances (below LOD) when analyzed in Mainz. We will update Table A1 in the Appendix with the LODs; see the answer to reviewer 2.

Line 103: In each campaign a minimum of two cartridges were not sampled with a volume of air, but opened and closed at the site and transported along the sampled cartridges. These transport blank cartridges did not contain any of the target compounds (below LOD).

For the soil flux calculation C_0 was the ambient air sample and C_t the sample from within a chamber, both as volume mixing ratios in ppbv. We always took two ambient samples at the same time, directly next to two of the chambers (8-12 cm above ground; 2 to a maximum of 10 cm distance to the chamber inlet). We report the number of measurements for ambient samples and flux data in Table A1 in the Appendix.

As chamber flux blanks (different from the zero-volume cartridge blanks), we took samples from a soil chamber that was closed at the bottom with Teflon foil isolating the chamber system from soil contact. The blank chamber was placed between the locations of the sample soil chambers and exposed to the rainforest air as for the other chambers. The blank flux was calculated as the difference between an ambient air sample and a sample from this closed-bottom blank chamber. These fluxes are reported in Table A2 and serve to assess possible background VOC fluxes from the chamber materials.
However, we don't think the location within the 15m radius of the sample chambers has a big impact on the ambient air concentrations, and therefore, the blank chamber location is not as relevant as long as it is exposed to the same ambient air and environmental variables (i.e., meteorological conditions).

Emissions of monoterpenes (MTs) could be within the blank levels when looking at the total monoterpene flux. In Table A2 it is shown that the blank flux differs for each monoterpene. So, the blank mean of the flux for Total Monoterpenes is the combination of positive and negative blank values by the individual MTs.
Same is valid for the SQT. The here found most relevant SQT β-caryophyllene had very low flux values in the blank chambers (see Table A2 with 0±0 to 0.21 ± 1.04 nmol m^{-2} h^{-1} as mean blank values and Table A1 with mean seasonal emission values of up to 1.90 ± 4.82 nmol m^{-2} h^{-1}).

3. Manuscript lacks hypothesis and research questions. Why measure enantiomers of terpenes or isoprene oxidation products? As the authors point out, soil BVOC fluxes are poorly understood, so the introduction would benefit from more detail for the reader's benefit. At the moment, the introduction is vague and many important points are only mentioned but not elaborated on.

Response: We are happy to elaborate further on our research questions. We have developed a means of determining the stress state of an ecosystem by the enantiomeric ratio of (+) and (–)- α-pinene in ambient air. This is based on measurements made in an enclosed rainforest (BIOSPHERE 2) and at the ATTO site (Byron et al., 2022, 2025). Additionally we saw a height gradient for the chiral ratio α-pinene, possibly indicating distinct sources below the canopy (Zannoni et al., 2020). The enantiomeric signature could potentially be affected by the soil if it were selective to one or the other enantiomer. Specifically, we wanted to know if soil emissions or uptake of the enantiomers are enhanced across seasons. This has not, to our knowledge, been examined before. We now clarify this better in the introduction as requested.

Line 60: In this study, we investigated chirally resolved soil BVOC fluxes in the Amazon rainforest to assess the relevance of soils to the total terpenoid BVOC budget in this ecosystem and to determine whether soils influence enantiomeric ratios. In particular, we are interested in the soil effect on the enantiomers of α-pinene, as these have been shown to be indicators of ecosystem drought stress (Byron

et al., 2022, 2025), and a height gradient was observed at the ATTO tower site (Zannoni et al., 2020). We measured across four seasons, including the El Niño drought period in the dry season 2023 to account for and look into seasonal differences.

Line 66: The effect of temperature, soil moisture, soil properties, litter content and terpenoid ambient concentrations on soil terpenoid fluxes in terms of diurnal cycle, magnitude, flux direction (emission and/or uptake), and chemical composition, including chiral speciation, was investigated.

The section about atmospheric implications should, in my opinion, be omitted. Authors did not measure radical reactions, nor do they know what the in situ OH concentrations are. Furthermore, because of the issues with replication, the emission rates reported in this study should be considered tentative, and consequently, any estimates on atmospheric impact are rough at best and do not provide any usable information e.g., for modeling purposes.

Response: There appears to be a misunderstanding here regarding the atmospheric reactivity data given in the paper. In our view, it is important to show which of the soil emissions has the greatest impact on the local atmospheric oxidants. For this assessment, no knowledge of the radical concentrations is required, merely the rate coefficients of the species with the respective oxidant. As these are all available in the literature from previous laboratory measurements, we can present to atmospheric scientists the relative impacts of the emissions on OH and O3. To be clear, even though a species is at low concentration, it's atmospheric impact can be high if the rate coefficients are fast. This information is valuable to atmospheric modelers who may include only one representative SQT in the model, and our assessment allows them to select appropriate rate coefficients to reflect the real emission profile. In summary we would prefer to keep this atmospherically valuable information in the paper as we think it provides the context to understand and assess the effect of soil emissions on the local atmospheric chemistry.

Specific comments

Introduction:

Response: The changes in the manuscript for the introduction are summarized in one paragraph below.

38-41: As chirality is highlighted in this manuscript, I would like to know more about possible impacts of specific enantiomers being emitted. Authors should elaborate on what is known about (biogenic) processes and BVOC chirality and why differentiating between emissions of enantiomers is important. How can this information be used when assessing soil processes or atmospheric impacts?

Response: We now elaborate more on the possible impacts of specific enantiomers being emitted (see below).

45-47: Authors should elaborate how vegetation, soil properties etc. affect fluxes from soil. It would be beneficial for the reader if authors first describe some of the processes controlling BVOC fluxes from soil in general and then move on to describe what we know about tropical forests.

Response: As suggested we now first describe some of the processes controlling soil BVOC fluxes in general and then refer to what we know about tropical forests (see below).

50-52: Authors should elaborate how these factors (water content, nutrient composition, temperature ect.) can affect soil uptake or emissions.

Response: We now elaborate more on these processes (see below).

57-69: Again, authors should give more details about how weather conditions can affect (soil) BVOC fluxes in general and then describe what we know about their effects in rainforests. El Niño (and other

extreme weather events) causes drought, which has been shown in previous studies to increase BVOC emissions, which again, can exacerbate extreme weather conditions. This cycle is worth elaborating on in the introduction, with relevant references.

Response: We now elaborate more on the effect of El Niño on BVOCs in general (see below).

61-64: What were the hypothesis and research questions? Why did you measure isoprene's oxidation products – not otherwise mentioned in the introduction – and how do they link to the larger context or the study?

Response: This study focused on BVOC compounds like MTs, SQTs, isoprene and two of isoprene's oxidation products. Most of them were designated targets for which we have calibration standards, with the exception of a few additionally found tentatively identified SQTs calibrated to another SQT with the most similar mass spectra and molecular structure of which we had a calibration standard. For species for which we have calibration standards and that we measure in the ambient forest air, we can use this information to assess the role of soil in their concentrations.

We measured isoprene's oxidation products because it was part of the calibration gas and this permitted quantification of the signals. It is interesting in this context because it has been shown that MACR and MVK can be directly emitted by plants (Tani et al., 2010; Jardine et al., 2012; Fares et al., 2015), so it is also potentially emitted by soil microbes and/or roots. We have also seen uptake by cryptogamic species (Edtbauer et al., 2021). Also, they are the most dominant oxidation products of isoprene and while it is known that isoprene can be consumed by soil microbes, we were also interested if the oxidation products would be consumed as well.

We now include the aforementioned reasons in the text for greater clarity as to the motivation for the measurements and how they fit into the overall context of research at the site.

Line 38: Many plants and other organisms, like insects, emit one ~~These~~ enantiomers~~s~~ in excess, reflecting the dominant biosynthetic pathway in the species, the tissue or chemotype and arises from a stereoselective terpene synthase enzyme (Yassaa and Williams, 2007; Song et al., 2014; Staudt et al., 2019; Zannoni et al., 2020). The atmospheric reactivity of enantiomers towards ozone and OH radicals is identical. However, the further reaction and dimer formation might have stereochemical preferences for (+)-α-pinene and (+)-limonene enantiomers (Bellcross et al., 2021; Gao et al., 2025). Although chirality does not play a role in total atmospheric reactivity, organisms use specific enantiomers in order to communicate via the atmosphere to predators or conspecifics. The (−)-α-pinene enantiomer was found to play a role in plant-insect interactions, attracting beetles to already weaker trees (Norin, 1996). The (+)-α-pinene/(−)-α-pinene ratio can be elevated in response to mechanical stress (Eerdekens et al., 2009), and in spruce plants, a response to drought stress was found to result in higher emission rates of the (−)-enantiomers of limonene, β-phellandrene, α- and β-pinene (Daber et al., 2025). In a rainforest biome *de novo* synthesized (−)-α-pinene responded differently to increasing drought than (+)-α-pinene which is derived mainly from storage pools (Byron et al., 2022). Recently, it was shown that the enantiomeric ratio can be used to determine how the ecosystem responds to drought (Byron et al., 2025). ~~can have distinct biological impacts and their emissions may be linked to different biological processes making enantiomer-resolved studies increasingly important (Williams et al., 2007; Yassaa and Williams, 2007; Song et al., 2014; Staudt et al., 2019; Zannoni et al., 2020; Byron et al., 2022, 2025; Daber et al., 2025). For instance, abiotic stress by drought periods has been shown to alter the chiral ratio of the plant emitted monoterpene α pinene (Byron et al., 2022, 2025)., y~~ Yet enantiomer-resolved soil BVOC fluxes have not been reported.

Line 43: Soils are recognized as both a source and sink of BVOCs, however, compared to canopy BVOC, understanding of soil BVOC fluxes seasonal and diurnal dynamic, enantiomeric resolution, and

the environmental thresholds controlling flux direction and speciation remain poorly constrained (Rinnan and Albers, 2020). Flux magnitudes and speciation are difficult to assess as they are the result of biotic, soil microbiome emissions and uptake as well as root exudates, and abiotic processes, like evaporation, diffusion, and sorption processes (Cleveland and Yavitt, 1997; Horváth et al., 2012; Rinnan and Albers, 2020). These processes in turn are sensitive to temperature, soil moisture, and soil porosity and interconnected with available ambient BVOCs and litter material, and therefore organic matter and nutrients, which can boost the microbial communities (Peñuelas et al., 2014; Weikl et al., 2016; Mäki et al., 2017; Kivimäenpää et al., 2018). ~~vary with vegetation, litter, soil properties, and available ambient VOCs (Gray et al., 2014; Kivimäenpää et al., 2018; Mäki et al., 2019; Tang et al., 2019; Rinnan and Albers, 2020; Ghirardo et al., 2020; Llusià et al., 2022; Mu et al., 2023)~~. Across ecosystems, soil BVOC fluxes are typically one to two orders of magnitude lower than canopy emissions, partly due to concurrent microbial uptake (Cleveland and Yavitt, 1997; Owen et al., 2007; Peñuelas et al., 2014; Drewer et al., 2021). Microbes can consume isoprene as an energy source and may emit it at low rates (Cleveland and Yavitt, 1997; Gray et al., 2015). The isoprene oxidation product methyl vinyl ketone (MVK) was found as volatile metabolite from a bacteria and active against fungal spore germination (Herrington et al., 1987). Methacrolein (MACR) and MVK can both be directly emitted or uptaken by plants (Tani et al., 2010; Jardine et al., 2012; Fares et al., 2015). MT and SQT emissions in contrast were associated with plant roots (Mäki et al., 2017; Tsuruta et al., 2018), SQT especially also with soil fungi (Horváth et al., 2012), as well as with microbes (Asensio et al., 2008; Weikl et al., 2016). In tropical forests, terpenoids ~~MTs~~ are uptaken or emitted depending on the environmental conditions such as the soil water content, nutrient composition in soil, temperature, season, and vegetation (Bourtsoukidis et al., 2018; Drewer et al., 2021; Llusià et al., 2022). Amazonian soils are reported to act as a strong source of SQTs under certain conditions (Bourtsoukidis et al., 2018). ~~SQT emissions are associated with plant roots (Mäki et al., 2017; Tsuruta et al., 2018), soil fungi, and microbes (Asensio et al., 2008; Horváth et al., 2012; Weikl et al., 2016)~~. Soil BVOC fluxes in an artificial tropical forest have been reported to alter strongly under drought conditions (Pugliese et al., 2023).

Line 56: El Niño climate events, which occur semi-periodically (every 2-7 years), impact the Amazon rainforest by decreasing rainfall and elevating temperatures. It was shown in a modeling study that the isoprene emission flux increases as a response of the vegetation to a strong El Niño event (Vella et al., 2023). MT have also been shown to generally increase with temperature and due to drought-stressed vegetation in tropical forest ecosystems (Byron et al., 2022; Gomes Alves et al., 2022; Werner et al., 2022). The 2023-24 El Niño event caused a record drought in the Amazon rainforest (Espinoza et al., 2024). Climate projections indicate that the frequency and intensity of El Niño events are likely to increase under continued greenhouse gas emissions, with potentially profound effects on the Amazon and its BVOC dynamics (Geng et al., 2024).

Line 60: In this study, we investigated chirally resolved ~~measured~~ soil BVOC fluxes in the Amazon rainforest to assess the relevance of the soils to the total terpenoid BVOC budget in this ecosystem and see if soils have an influence on enantiomeric ratios. We measured across four seasons, including the El Niño drought period in the dry season 2023, to account for seasonal differences. Soil fluxes of isoprene, two of isoprene's oxidation products methyl vinyl ketone (MVK) and methacrolein (MACR), and enantiomer-resolved MTs and SQTs were quantified using thermal desorption-chiral gas chromatography-time of flight mass spectrometry (TD-GC-ToF-MS). The measurements were conducted at the Amazon Tall Tower Observatory (ATTO) research station (Andreae et al., 2015) located 150 km north-east of Manaus (Brazil). The effect of temperature, soil moisture, soil properties, litter content and terpenoid ambient concentrations on soil terpenoid fluxes in terms of local time of day, magnitude, flux direction (emission and/or uptake), and chemical composition, including chiral speciation was investigated.

Methods:

84: Define “close proximity”.

Response: The chambers were installed within a radius of 15 m to each other.

Line 84 The three PVC collars were installed at three different locations within a radius of 15 m ~~in close proximity~~ to each other

86: How much before sampling were the collars installed?

Response: The collars were installed at least 24 hours prior to measurements.

Line 84-85 The three PVC collars were installed at three different locations within a radius of 15 m ~~in close proximity~~ to each other near the 325 m tall tower and at least 24 hours prior to measurements.

Fig.1. This figure would benefit from a schematic showing the different sampling spots (1-5) and which were with/without litter, effected by the El Niño etc.

Response: Thanks for this feedback. We included an index for the effect of El Niño in Table 1 instead.

Table 2 Overview of measurement campaigns with attributed season, Oceanic Niño Index representing 3-month average temperature anomaly in the oceanic surface waters around the respective measurement period (NOAA’s Climate Prediction Center, 2026), start date, end date, measured chambers and the number of flux data points

Name in plots	Season	Oceanic Niño Index	Start Date	End Date	Number of flux data points	Chambers Measured
Jan 2023	Dry-to-wet	-0.5 (La Niña/Neutral)	2023-01-22	2023-01-26	20	Spot 1, Spot 2, Spot 3 without litter
Oct 2023	Dry	1.8 (around El Niño peak)	2023-10-01	2023-10-14	39	Spot 1, Spot 4, Spot 5
Apr-May 2024	Wet	0.8 (El Niño influenced)	2024-04-24	2024-05-02	35	Spot 1, Spot 4, Spot 5
Oct 2024	Dry	-0.2 (Neutral)	2024-10-11	2024-10-20	37	Spot 1, Spot 4, Spot 5
			2024-10-18	2024-10-20	6	Spot 5 without litter

103-104: Storage for up to 2 months seems excessive, especially because highly volatile compounds, like isoprene, were targeted in this study. How did the authors check that the long storage did not result in loss of analytes? Was an internal standard used?

Response: Indeed, shorter storage times are always preferred for adsorbent cartridges. However, due to instrument usage and availability, a shorter storage time was not possible for all campaigns. While we did not test storage times ourselves, Helin et al. (2020) tested MTs and SQTs and found the recovery of 97±4% and 94±5%, respectively, for 2 months of storage at 4°C. There could have been an issue with highly volatile compounds like isoprene, however our found values for ambient concentration are within the expected range from previous measurements at the site with PTR-MS (Andreae et al., 2015; Yáñez-Serrano et al., 2015; Nölscher et al., 2016; Yáñez-Serrano et al., 2018; Gomes Alves et al., 2023).

Still, we would like to mention this constraint:

4.6 Limitations of this study and future directions
Line 534 The storage time of up to two months of the adsorbent cartridges could have resulted in some loss of the higher volatile compounds like isoprene, MACR and MVK. For MTs and SQTs these storage times have been tested previously (Helin et al., 2020).

129-130: Liquid standards were injected into the sorbent tubes under a nitrogen/helium flow I assume, not directly? Authors list the composition of the gas mixture, but what about the liquid standards?

Response: Yes, we used a nitrogen flow after injecting the liquid mixtures to remove the used solvent methanol prior to the GC-MS analysis. We now also list the used compounds in the liquid standard mixture with suppliers.

Line 127: Compounds were quantified using a gas standard calibration mixture and liquid standards injected at 1, 2, 4, 6, 8, and 10 µL in methanol-diluted compound mixtures with a syringe directly onto the sorbent cartridge. Afterwards the cartridge was purged with nitrogen for 10 min to remove the methanol. As liquid standards (−)-limonene (TCI), 3-carene (Merck), (−)-α-cedrene (Sigma-Aldrich), (+)-δ-cadinene (TCI), (+)-cyclosativene (Sigma-Aldrich), (+)-longifolene (PhytoLab), (-)-isolongifolene (Fluka), α-copaene (Biomol), trans-β-ocimene (LGC), (−)-α-phellandrene (Sigma-Aldrich), (−)-α-pinene (thermoscientific), (+)-α-pinene (Acros Organics), (+)-β-pinene (Fluka), sabinene (ChemCruz), β-caryophyllene (Sigma-Aldrich), a-terpinene (Sigma-Aldrich) and γ-terpinene (Sigma-Aldrich) were used in the concentration range between 0.49 to 84.52 nmol L^{-1}. The gas standard mixture contained isoprene, MVK, MACR, tricyclene, (−) and (+)-α-pinene, (−)-β-pinene, (+) and (−)-camphene, sabinene, β-myrcene, (−)-α-phellandrene, (−)-3-carene, α-terpinene, (+)-limonene, γ-terpinene, terpinolene, m- p- and o-cymene, (+) and (−)-linalool, and β-caryophyllene (Apel-Riemer International, USA). When a calibration was performed with calibration gas and liquid standard, the calibration with gas standard was used, as it is more similar to the conditions when filling environmental samples than injecting methanol-diluted compound mixture.

140: Identifying enantiomers without authentic standards purely based on spectral library comparison is tentative at best. I would like to see how well compounds were separated in the chromatograms? While PARADISe is able to resolve convoluted peaks, I would be very careful with identification and quantification of compounds without an authentic standard when doing targeted analysis.

Response: We agree that identifying enantiomers without authentic standards is impossible, as mass spectra are practically identical. Even for different MTs, it is challenging due to similar mass spectra. However, we did not base our identification only on spectral library comparisons but used authentic standards. For the enantiomers of α-pinene, limonene, camphene and β-pinene we used enantiomerically pure standards to know the elution order with our column and method. PARADISe

was used in the data analysis to improve peak integration of almost coeluting MTs like (−)-α-phellandrene and 3-carene and to integrate unknown SQTs. Unknown SQTs were identified as being a SQT by comparison with mass spectral library, but because of the before mentioned challenges they were not assigned to a specific individual SQT due to lack of an authentic standard for every single SQT. These tentatively as a SQT identified SQTs are still included when Total SQT are reported. The most dominant SQT α-copaene and β-caryophyllene, as well as (−)-α-cedrene, (+)-δ-cadinene, (+)-cyclosativene, (+)-longifolene, and (-)-isolongifolene were identified with authentic standards.

Please see an example chromatogram below, which we now include in the appendix.

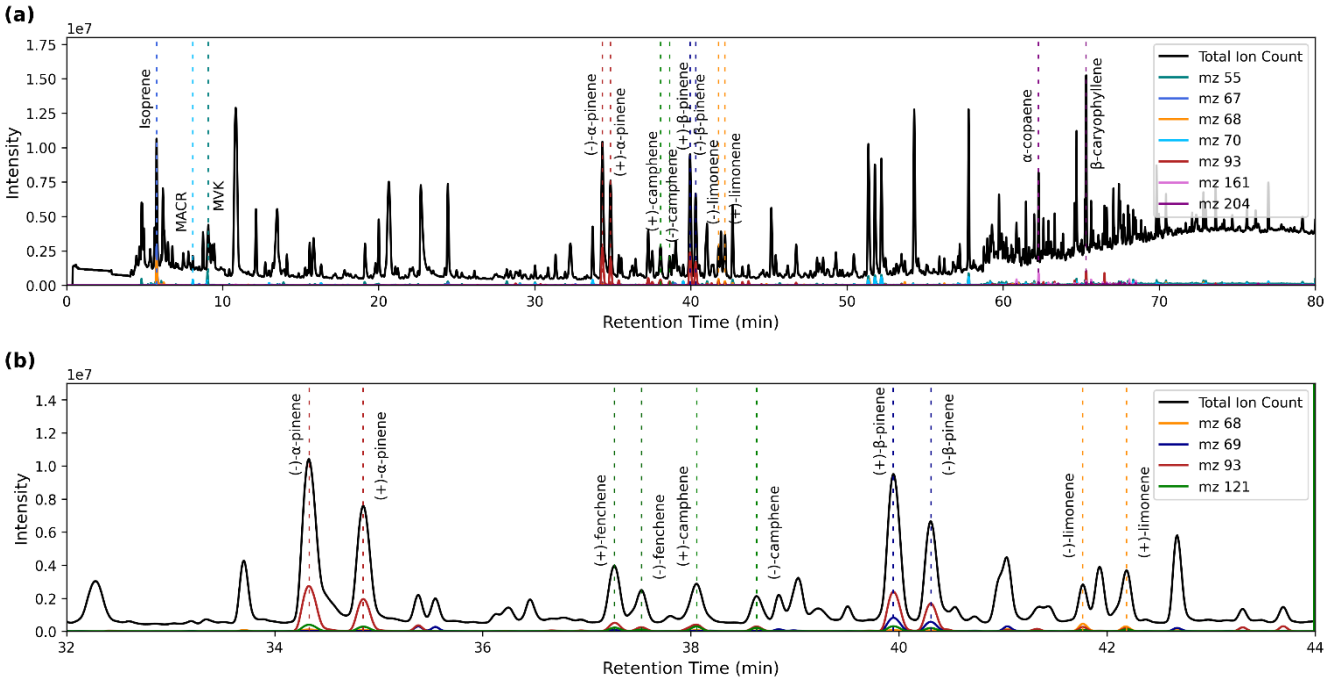

Figure A1 (a) Example Chromatogram of a soil chamber sample from October 2023 with annotation of isoprene, MACR, and MVK peaks, the chiral monoterpenes, and the two most prominent sesquiterpenes (b) Zoomed into the chiral monoterpene resolution.

Line 136: Compounds in the sample chromatogram were identified by matching retention times with those of the standards.~~, and c~~Enantiomers~~s~~ elution order of α-pinene, limonene, camphene and β-pinene were ~~confirmed~~ identified by spiking with enantiomerically pure standards (see Fig. A1 for enantiomer resolution in a chromatogram).

155: Define “near”.

Line 152: Soil samples were collected in June 2023 approximately 0.5-1.5 m near spots 1, 2, and 3, where VOCs had been measured in January 2023.

164-168: As authors have sampled VOCs from chambers with and without litter, the litter composition should not be ignored. Did authors conduct any additional analysis of the litter or only the dry weight?

Response: Unfortunately, we did not do that. From visual inspection, the litter composition was mixed from various plants and in different stages of decomposition. We agree that is would be beneficial for future studies to better assess the litter and plant composition at the measurement site.

188: Why was soil moisture/temperature measured so far away from the BVOC sampling site? Was any replication conducted?

Response: We had soil sensors for moisture and temperature closer to the sampling site, however the instrumentation broke during measurement campaigns. For this reason, we chose to use the consistent

moisture and temperature measurements from the site that was further away. This ensured consistent values when looking at the impact of these environmental parameters on the flux.

199: Statistical analysis needs to be explained more thoroughly, especially because pseudoreplication was used and the sampling sites changed in between samplings. Was the flux data normalized? Did you use repeated measurements ANOVA for dry-wet seasons and before-after litter removal? Authors need to specify which test was used for which parts of the data.

Response: Thank you for raising this concern about the statistical analysis. Following the feedback of Reviewer 1, we changed our statistical model and now use linear mixed-effect models instead of ANOVA tests, because of different sample size and pseudo-replication. We describe the now used statistical model in the methods, changed the corresponding results for soil spot, seasonal, and chiral ratio differences and adjusted Figure 6 and 8. While some p-values changed slightly under the new statistical model, the overall trends and conclusions were not affected when comparing fluxes and chiral ratios per seasons and soil spots.

2.6 Statistical analysis

Statistical analyses were performed using Python (version 3.12.4) with the following packages: numpy (v.2.0.0), pandas (v.2.2.2), matplotlib (v.3.9.1), seaborn (v.0.13.2), statsmodel (v.0.14.5~~ 2~~), and scipy (v 1.16.0). Data visualization was conducted using matplotlib and seaborn.

Statistical differences were assessed using linear mixed-effect models, because the dataset contains repeated measurements over time from the same soil chambers and ambient sampling points, which violates assumptions of independence of simpler tests. Local time (hour-of-day as a categorical factor), was included as a fixed effect in all models, because we expected a diurnal pattern for the measured VOC fluxes and mixing ratios. ~~between soil fluxes measured in different seasons and from different soil plots~~ were ~~determined using the Tukey HSD (Honestly significant difference) test following a significant result from ANOVA~~ To assess seasonal differences in fluxes, a linear mixed-effects model was implemented with season, chamber spot location and local time as fixed effects and the sampling date spot as random effects. Differences between soil spots within a single season were assessed using chamber spot location and local time as fixed effects, and sampling date as a random effect. Using the Holm–Bonferroni method, p-values were adjusted for multiple comparisons afterwards in both cases.

For comparisons of enantiomeric ratios between atmospheric and soil chambers and between seasons, a linear mixed-effect model with fixed effect for local time and a random effect for the sampling date and chamber spot or ambient air sampling location was used. Ratios in both cases were log-transformed prior to analysis to stabilize variance and improve residual normality.

We fitted linear mixed-effects models with fixed effects for environmental predictors and local time, and random intercepts for measurement date and chamber spot location, to quantify the association of predictors with fluxes. Regression slopes (β) represent the change in flux per unit increase in the predictor. ~~, the non-parametric Mann-Whitney U test was used due to non-normality of the data~~. ~~For correlations between the fluxes and environmental parameter Pearson coefficients were calculated.~~

Statistical significance was accepted for $p < 0.05$.

Results:

Fig.2. Mean and standard deviation of what (N=?)?

Line 245: **Figure 2 Meteorological data during the measured seasons with (a) temperature (red) and (c) relative humidity (blue) measured at 26 m at the Instant tower, (b) soil temperature (orange) and (d) soil water-content (green) measured at 10 cm depth and (e) photosynthetically active radiation (PAR) incoming at 81m at the Instant tower across the four measurement periods in the different seasons. The line represents the mean and shaded area is the standard deviation from the dates of the measurement campaigns specified in Table 1 (number of dates N=5 for Jan 2023; N= 14 for Oct 2023; N= 9 for Apr-May 2024, N= 9 for Oct 2024).**

264: Authors should list which signals have been summed as total MT and SQT.

Line 260: Figure 3 summarizes the mean measured mixing ratios for ~~terpenoids (~~isoprene~~, total monoterpenes, and total sesquiterpenes)~~, methacrolein (MACR) and methyl vinyl ketone (MVK), total monoterpenes (sabinene, β-myrcene, tricyclene, both enantiomers of α-pinene, 3-carene, both enantiomers of α-fenchene, both enantiomers of camphene, both enantiomers of β-pinene, β-ocimene, both enantiomers of limonene, γ-terpinene, α-terpinene, terpinolene), and total sesquiterpenes (β-caryophyllene, α-copaene, (+)-cyclosativene, (+)-longifolene, (-)-isolongifolene, (-)-α-cedrene and a per campaign differing number of unknown SQTs (good confidence with NIST that they are a SQTs, but with no authentic standard to confirm which exact SQT) at the soil level (outside of the chambers) over the four seasons sampled.

280-282: How was emission highly seasonal, time-of-day dependent, and specific to soil conditions? Did you test this and their interaction with ANOVA? SQTs were significantly different in dry seasons 2023, did you test this and what was the p value?

Response: As we changed the statistical model used, we now perform linear mixed-effect models. The p-values are summarized in two tables in the appendix now (see below). We agree that we should phrase this sentence more carefully.

Line 277: The emission or uptake was ~~is highly~~ seasonal, time-of-day dependent and mostly specific to the individual soil spot ~~conditions~~ (see Table A6 and A7 for statistical tests). Interestingly, the SQT emission was significantly higher in the dry season 2023 compared to the dry-to-wet season 2023 ($p < 0.001$) and the dry season 2024 ($p < 0.01$) ~~other measured seasons~~.

Fig.3. See my general comment 2.

Response: See above.

Fig.4. If these are mean fluxes, why not show standard deviation? Could you indicate in the figure which differences were statistically different.

Response: We decided against showing the standard deviation in this figure to keep readability. Instead, we now report standard deviations for each season in Table A1. We agree that in this way, we do not report standard deviation of hourly values per season. We can add another long table in the appendix, but we are not sure if this is beneficial to the reader. The total dataset can be accessed online.

Fig.5. Same comments as for Fig.4. Also, I would again be careful how the different spots are compared. Spot 2 and 3 are different, so authors cannot include them in their statistical analysis before and after litter removal the same way they would the same spot 5. As statistical methods were only briefly described by the authors, it's also difficult to say what tests were used and how (e.g., repeated measures ANOVA or something else).

Response: We changed our statistical methods to mixed-effect models which now accounts for these constraints. Please see the changes to the statistical method section as reported above and for clarification this part of the answer to reviewer 1:

"We acknowledge that our data is not independent due to repeated measurement over time from the same soil spots when we compare seasons or soil spots. To address this, we have re-analyzed the data using linear mixed-effects models, which accounts for the structure of our time-series data by including random intercepts for each measurement date. We describe the now used statistical model in the methods, changed the corresponding results for soil spot, seasonal, and chiral ratio differences and adjusted Figures 6 and 8. While some p-values changed slightly under the new statistical model, the

overall trends and conclusions were not affected when comparing fluxes and chiral ratios per seasons and soil spots."

Discussion:

I would combine the discussion about the emission rates with section 4.1 and consider very carefully what can be concluded from the results done with pseuduoreplicates. While the discussion about the different drivers behind the observed levels and blends of BVOCs is valid, the discussion about the measured emission rates (which do not reflect the natural variation in the environment) could be significantly reduced. Authors can discuss overall trends, but comparing hard numbers for emissions rates measured with pseudoreplicates is not valid and could be partly behind differences found between this study and others. As such, I would also omit the comparison with canopy emissions and the atmospheric impacts, and instead, expand on the discussion e.g., about the chirality which is a novel topic.

Response: On reflection, a discussion of the fluxes and a comparison with values found by other studies should not be omitted. As described in the answers above, the potential of the soil to influence chiral ratios is of interest. However, the limitations of our studies should be emphasized as we did in section 4.6. and also expanded as mentioned above.

407-12: Could authors elaborate on how and how quickly soil microbiome can shift during extreme weather events? As no microbial analysis was done for this manuscript, it would be beneficial if authors demonstrate with relevant references if the time-scale of shifting microbiome is enough to explain the observed variations.

Response: Thank you for making us think more about the velocity with which soil microbiomes can shift. By taking into account the literature about the microbiome in tropical forest soils (Kivlin and Hawkes, 2016; Buscardo et al., 2018, 2022), we think it is reasonable to expect the microbiome to change to some degree between seasons.

Line 235: So, the roots, as well as the microbiome, could have contributed to the different MT species fluxes. In a study from similar Amazon rainforest terra firme soil, bacterial communities were observed to shift between dry and wet seasons due to seasonality-related changes in soil nutrient and moisture regimes (Buscardo et al., 2018). In tropical forest soils in Costa Rica bacterial biomass, richness, and enzyme activity peaked at wetter conditions (Kivlin and Hawkes, 2016). Fungal groups in Amazonian soil were observed to shift within 2 months following a nitrogen pulse and returned to their original community microbiome within 5 months (Buscardo et al., 2022).

Soil microorganisms, particularly fungi, are known to be significant sources of SQTs (Horváth et al., 2012; Ditengou et al., 2015; Gfeller et al., 2019). A study by Bourtsoukidis et al. (2018a) has shown that Amazonian soils can emit SQTs at rates comparable to the plant canopy during dry season conditions. In contrast, our study did not observe consistent SQT emissions during the two dry seasons of 2023 and 2024. Only in the El Niño-influenced dry season 2023 was an emission pattern of SQT evident. In the subsequent dry season 2024, SQTs were even partly uptaken by the same soil spots.

Appendix:

Could authors provide the results from their statistical tests (p and F values, degrees of freedom) e.g., as table.

Response: Following suggestions from Reviewer 1, we changed our statistical methods because of pseudo replications from ANOVA to linear mixed-effect models. Here we provide a table of the effect size as β-coefficients, the 95% confidence interval, and Holm-Bonferroni adjusted p-values.

The linear mixed-effects model was generated using the smf.mixedlm function which is based on the python package statsmodel (v.0.14.5) which is based on the lmer function from the R package lme471. We used season, local time, and soil spot ID as fixed factors and date as random effect.

The use of linear mixed-effects models analysis is necessary to account for repeated measures, since failure to do so would violate the assumption of independent observations.

2.6 Statistical analysis

Statistical analyses were performed using Python (version 3.12.4) with the following packages: numpy (v.2.0.0), pandas (v.2.2.2), matplotlib (v.3.9.1), seaborn (v.0.13.2), statsmodel (v.0.14.5 ~~2~~), and scipy (v 1.16.0). Data visualization was conducted using matplotlib and seaborn.

Statistical differences were assessed using linear mixed-effect models, because the dataset contains repeated measurements over time from the same soil chambers and ambient sampling points, which violates assumptions of independence of simpler tests. Local time was included as a fixed effect in all models, because we expected a diurnal pattern for the measured VOC fluxes and mixing ratios. ~~between soil fluxes measured in different seasons and from different soil plots~~ were ~~determined using the Tukey HSD (Honestly significant difference) test following a significant result from ANOVA~~ To assess seasonal differences in fluxes, a linear mixed-effects model was implemented with season, chamber spot location and local time as fixed effects and the sampling date spot as random effects. Differences between soil spots within a single season were assessed with chamber spot location and local time as fixed effect and the sampling date as random effect. Using the Holm–Bonferroni method, p-values were adjusted for multiple comparisons afterwards in both cases.

For comparisons of enantiomeric ratios between atmospheric and soil chambers and between seasons, a linear mixed-effect model with fixed effect for local time and a random effect for the sampling date and chamber spot or ambient air sampling location was used. Ratios in both cases were log-transformed prior to analysis to stabilize variance and improve residual normality. To assess the effect size (β coefficient) of environmental parameters on fluxes, mixed-effects models were fitted with fixed effect of local time and adjusting for random effects of measurement date and chamber spot location. ~~, the non-parametric Mann-Whitney U test was used due to non-normality of the data~~.

We fitted linear mixed-effects models with fixed effects for environmental predictors and local time, and random intercepts for measurement date and chamber spot location, to quantify the association of predictors with fluxes. Regression slopes (β) represent the change in flux per unit increase in the predictor.~~For correlations between the fluxes and environmental parameter Pearson coefficients were calculated.~~ Statistical significance was accepted for $p < 0.05$.

Table A6 Overview of seasonal differences of the fluxes of isoprene, MACR, MVK, total monoterpenes, and total sesquiterpenes by linear mixed-effect models with the formula "Flux ~ C(Season_renamed) + C(Hour) + C(Chamber_spots)" and Date as the random effect; β-coefficients are the estimated change between the baseline season to the compared season (Compared-Baseline) in nmol m^{-2} h^{-1}; 95% CI is the confidence interval; p-value (adj) is the adjusted p-value after Holm-Bonferroni correction for multiple comparisons. Significance: * p < 0.05, ** p < 0.01, *** p < 0.001.

Substance	Baseline Season Compared Season	Jan 2023 Oct 2023	Jan 2023 Apr–May 2024	Jan 2023 Oct 2024	Oct 2023 Apr–May 2024	Oct 2023 Oct 2024	Apr–May 2024 Oct 2024
isoprene	β-coefficient	-29.029	-4.994	-47.96	20.385	-14.165	-31.634
	95% CI	-44.6 – -13.458	-10.862 – 0.873	-67.105 – -28.814	11.632 – 29.139	-28.297 – -0.032	-44.452 – -18.816
	p-value (adj)	7.75e-04 ***	0.099	5.47e-06 ***	2.00e-05 ***	0.099	6.59e-06 ***
MACR	β-coefficient	-14.602	0.19	-14.325	10.098	-0.212	-9.586
	95% CI	-21.059 – -8.145	-0.752 – 1.132	-21.246 – -7.403	7.113 – 13.083	-4.651 – 4.227	-13.348 – -5.824

	p-value (adj)	3.72e-05 ***	1.000	1.49e-04 ***	2.01e-10 ***	1.000	2.96e-06 ***
MVK	**β-coefficient**	-8.565	4.524	-10.144	10.802	-2.163	-12.113
	95% CI	-16.229 – -0.9	2.039 – 7.01	-21.799 – 1.511	8.353 – 13.251	-7.893 – 3.567	-17.341 – -6.886
	p-value (adj)	0.086	0.001 **	0.176	3.25e-17 ***	0.459	2.79e-05 ***
Total MTs	**β-coefficient**	-72.842	-77.208	-93.614	-12.864	-19.672	-4.739
	95% CI	-96.934 – -48.751	-107.677 – -46.738	-125.306 – -61.923	-32.544 – 6.817	-36.833 – -2.512	-29.031 – 19.553
	p-value (adj)	1.86e-08 ***	2.73e-06 ***	3.53e-08 ***	0.400	0.074	0.702
Total SQTs	**β-coefficient**	11.828	15.698	-1.261	-2.072	-7.71	-4.039
	95% CI	5.678 – 17.978	5.988 – 25.408	-5.912 – 3.389	-6.445 – 2.301	-10.996 – -4.424	-8.98 – 0.902
	p-value (adj)	8.17e-04 ***	0.006 **	0.706	0.706	2.55e-05 ***	0.327

3.3 Diurnal and seasonal dynamics of soil terpenoid exchanges

Line 272: The fluxes of isoprene showed strong seasonal variation, with higher uptake fluxes in the dry seasons compared to the dry-to-wet and wet seasons (~~Tukey test~~ Holm–Bonferroni adjusted $p < 0.001$; see Table A6).

Line 272: Interestingly, the SQT emission was significantly higher in the dry season 2023 compared to the ~~other measured~~ dry seasons ~~2024~~.

Table A7 Overview of differences per spot within each season of the fluxes of isoprene, MACR, MVK, total monoterpenes, and total sesquiterpenes by linear mixed-effect models with the formula "Flux ~ C(Chamber_spots) + C(Hour)" and Date as random effect; β-coefficients are the estimated change between the baseline spot to the compared spot (Compared-Baseline) in nmol m^{-2} h^{-1}; 95% CI is the confidence interval; p-value (adj) is the adjusted p-value after Holm-Bonferroni correction for multiple comparisons. Significance: * p < 0.05, ** p < 0.01, *** p < 0.001.

Substance	Season	Baseline Spot	Compared Spot	β-coefficient	95% CI	P-value (Adj)
isoprene	Jan 2023	Spot 1 (N=6)	Spot 2 (N=14)	0.963	-2.633 – 4.558	1.000
		Spot 1 (N=6)	Spot 3 without litter (N=7)	1.782	-1.558 – 5.122	1.000
		Spot 2 (N=14)	Spot 3 without litter (N=7)	0.819	-3.307 – 4.946	1.000
	Oct 2023	Spot 1 (N=52)	Spot 4 (N=24)	0.102	-9.952 – 10.157	1.000
		Spot 1 (N=52)	Spot 5 (N=25)	18.133	8.017 – 28.249	0.012 *
		Spot 4 (N=24)	Spot 5 (N=25)	18.031	4.106 – 31.957	0.223
	Apr–May 2024	Spot 1 (N=31)	Spot 4 (N=27)	2.472	-0.207 – 5.151	1.000
		Spot 1 (N=31)	Spot 5 (N=31)	3.336	0.807 – 5.865	0.214
		Spot 4 (N=27)	Spot 5 (N=31)	0.864	-1.714 – 3.441	1.000
	Oct 2024	Spot 1 (N=17)	Spot 4 (N=24)	-0.26	-10.637 – 10.116	1.000
		Spot 1 (N=17)	Spot 5 (N=25)	33.689	21.938 – 45.44	0.000 ***
		Spot 4 (N=24)	Spot 5 (N=25)	33.949	20.988 – 46.91	0.000 ***

		Spot 5 (N=25)	Spot 5 without litter (N=12)	-25.058	-41.86 – -8.256	0.083
MACR	Jan 2023	Spot 1 (N=6)	Spot 2 (N=14)	-1.746	-3.235 – -0.256	0.475
		Spot 1 (N=6)	Spot 3 without litter (N=7)	0.46	-0.944 – 1.863	1.000
		Spot 2 (N=14)	Spot 3 without litter (N=7)	2.205	0.831 – 3.58	0.043 *
	Oct 2023	Spot 1 (N=52)	Spot 4 (N=24)	2.746	-0.247 – 5.739	1.000
		Spot 1 (N=52)	Spot 5 (N=25)	5.209	2.207 – 8.211	0.019 *
		Spot 4 (N=24)	Spot 5 (N=25)	2.463	-1.603 – 6.529	1.000
	Apr–May 2024	Spot 1 (N=31)	Spot 4 (N=27)	0.127	-0.334 – 0.589	1.000
		Spot 1 (N=31)	Spot 5 (N=31)	-0.293	-0.735 – 0.149	1.000
		Spot 4 (N=27)	Spot 5 (N=31)	-0.42	-0.894 – 0.053	1.000
	Oct 2024	Spot 1 (N=17)	Spot 4 (N=24)	2.034	-0.465 – 4.532	1.000
		Spot 1 (N=17)	Spot 5 (N=25)	5.12	2.465 – 7.774	0.005 **
		Spot 4 (N=24)	Spot 5 (N=25)	3.086	0.501 – 5.67	0.463
		Spot 5 (N=25)	Spot 5 without litter (N=12)	-2.074	-5.698 – 1.55	1.000
MVK	Jan 2023	Spot 1 (N=6)	Spot 2 (N=14)	-1.005	-4.237 – 2.226	1.000
		Spot 1 (N=6)	Spot 3 without litter (N=7)	0.777	-1.827 – 3.381	1.000
		Spot 2 (N=14)	Spot 3 without litter (N=7)	1.782	-0.872 – 4.436	1.000
	Oct 2023	Spot 1 (N=52)	Spot 4 (N=24)	3.458	-0.054 – 6.971	1.000
		Spot 1 (N=52)	Spot 5 (N=25)	5.544	2.017 – 9.07	0.058
		Spot 4 (N=24)	Spot 5 (N=25)	2.085	-2.549 – 6.72	1.000
	Apr–May 2024	Spot 1 (N=31)	Spot 4 (N=27)	0.148	-0.407 – 0.703	1.000
		Spot 1 (N=31)	Spot 5 (N=31)	0.364	-0.289 – 1.017	1.000
		Spot 4 (N=27)	Spot 5 (N=31)	0.215	-0.417 – 0.848	1.000
	Oct 2024	Spot 1 (N=17)	Spot 4 (N=24)	1.826	-0.851 – 4.502	1.000
		Spot 1 (N=17)	Spot 5 (N=25)	5.003	1.703 – 8.304	0.077
		Spot 4 (N=24)	Spot 5 (N=25)	3.178	-0.075 – 6.431	1.000
		Spot 5 (N=25)	Spot 5 without litter (N=12)	-7.969	-12.97 – -2.969	0.054
Total MTs	Jan 2023	Spot 1 (N=6)	Spot 2 (N=14)	-104.079	-125.254 – -82.904	0.000 ***
		Spot 1 (N=6)	Spot 3 without litter (N=7)	-76.622	-98.679 – -54.565	0.000 ***
		Spot 2 (N=14)	Spot 3 without litter (N=7)	27.457	0.767 – 54.147	0.963
	Oct 2023	Spot 1 (N=52)	Spot 4 (N=24)	-6.642	-22.658 – 9.374	1.000

		Spot 1 (N=52)	Spot 5 (N=25)	-12.25	-27.813 – 3.313	1.000
		Spot 4 (N=24)	Spot 5 (N=25)	-5.608	-27.575 – 16.36	1.000
	Apr–May 2024	Spot 1 (N=31)	Spot 4 (N=27)	-21.177	-40.488 – -1.866	0.758
		Spot 1 (N=31)	Spot 5 (N=31)	-26.435	-45.807 – -7.063	0.195
		Spot 4 (N=27)	Spot 5 (N=31)	-5.258	-24.104 – 13.588	1.000
	Oct 2024	Spot 1 (N=17)	Spot 4 (N=24)	-13.325	-29.115 – 2.465	1.000
		Spot 1 (N=17)	Spot 5 (N=25)	-2.222	-22.226 – 17.781	1.000
		Spot 4 (N=24)	Spot 5 (N=25)	11.102	-8.798 – 31.003	1.000
		Spot 5 (N=25)	Spot 5 without litter (N=12)	-6.272	-37.851 – 25.308	1.000
Total SQTs	Jan 2023	Spot 1 (N=6)	Spot 2 (N=14)	-0.849	-6.357 – 4.659	1.000
		Spot 1 (N=6)	Spot 3 without litter (N=7)	1.137	-3.524 – 5.798	1.000
		Spot 2 (N=14)	Spot 3 without litter (N=7)	1.986	-3.301 – 7.274	1.000
	Oct 2023	Spot 1 (N=52)	Spot 4 (N=24)	-2.627	-6.901 – 1.647	1.000
		Spot 1 (N=52)	Spot 5 (N=25)	-5.737	-10.125 – -1.349	0.229
		Spot 4 (N=24)	Spot 5 (N=25)	-3.11	-8.475 – 2.255	1.000
	Apr–May 2024	Spot 1 (N=31)	Spot 4 (N=27)	-12.758	-20.51 – -5.007	0.033 *
		Spot 1 (N=31)	Spot 5 (N=31)	-12.775	-20.404 – -5.146	0.029 *
		Spot 4 (N=27)	Spot 5 (N=31)	-0.016	-7.481 – 7.448	1.000
	Oct 2024	Spot 1 (N=17)	Spot 4 (N=24)	4.034	1.024 – 7.043	0.207
		Spot 1 (N=17)	Spot 5 (N=25)	5.974	2.789 – 9.16	0.007 **
		Spot 4 (N=24)	Spot 5 (N=25)	1.941	-1.362 – 5.244	1.000
		Spot 5 (N=25)	Spot 5 without litter (N=12)	-3.442	-7.927 – 1.043	1.000

Line 289: For isoprene, in the dry-to-wet season 2023 and wet season 2024, no significant differences in fluxes were found ($p > 0.05$) (Fig. 4a) between the measured soil chambers. However, in the ~~other three~~ two dry seasons there was a significantly higher isoprene uptake by spot 1 than spot 5 (Holm–Bonferroni adjusted $p < 0.05$~~1~~ for ~~dry season 2023 and $p < 0.001$ for dry season 2024~~ ~~all~~) and in the dry season 2024 also in spot 5 than spot 4 (Holm–Bonferroni adjusted $p < 0.001$).
Comparing fluxes of MTs from different soil spots, we note clear monoterpene speciation differences (Fig. 4b). The highest emission rates were observed for soil spot 1 in the dry-to-wet transition season 2023. Here, the flux was significantly higher compared to the other two spots (Holm–Bonferroni adjusted $p < 0.0001$).

3.4.1 Effect of litter removal

Line 289: When litter was removed from the soil plot, in the two seasons dry-to-wet season 2023 and dry season 2024, no significant difference was found in the fluxes for isoprene and total MTs ($p > 0.05$).

Could authors provide some example chromatograms to show the separation of enantiomers and corresponding identification for chiral compounds.

Response: Indeed, good idea, this can also be informative for some readers to see the chiral identification for chiral compounds. Please see above Figure A1 that we will include in the Appendix.

Technical corrections

In chemical formulas, numbers should be subscripts

Response: Thanks. We noticed that we missed this in a chemical formula in Table 2 and appreciate the chance to improve that.

Line 235: ~~CaCl2~~ $CaCl_2$

In discussion, the verb tense should be consistent throughout, e.g. past tense.

Response: Thanks, we will revise the verb tense throughout the discussion when we resubmit the reviewed manuscript.

Figure texts overall are too small.

Response: Thank you for pointing that out. We will increase all figure text font size to the same as the manuscript text.

43-45: Sentence is really long and hard to understand.

Response: We improved readability:

~~Soils are recognized as both a source and sink of BVOCs, however, compared to canopy BVOC, understanding on soil BVOC fluxes seasonal and diurnal dynamic, enantiomeric resolution, and the environmental thresholds controlling flux direction and speciation remain poorly constrained.~~

While soils are increasingly recognized as sources and sinks of BVOCs, they remain poorly constrained compared to canopy BVOCs. Understanding seasonal and diurnal patterns, as well as their enantiomeric resolution and environmental thresholds that control those fluxes is important for better assessing the impact of soil on ecology and atmospheric chemistry.

71: You already define the abbreviation (ATTO) in the introduction.

Response: Thank you for pointing that out. We will refer to ATTO instead here.

98-104: You could combine the information about the sorbent tubes and their preconditioning/storage to it's on paragraph – separate from the description of sampling.

Response: We will move this paragraph to section 2.2 BVOC analysis

140: Define NIST and the version used.

Line 140: Compounds lacking standards were identified by comparing their mass spectra with those in the NIST library (NIST 14 Mass Spectral Library)

199: Define ANOVA.

Response: As we changed the statistical model, we do not use ANOVA anymore. We define the now used linear mixed-effect model as above.

215: I think the sentence is missing something.

Response: Thanks for noticing this.

Line 215: Soil respiration was more than three times higher in the chambers with litter content than in the chamber without litter in the dry-to-wet season 2023.

395: play only a minor role

Response:

Line 394: MACR and MVK are the dominant first-generation oxidation products of isoprene (Pierotti et al., 1990), but they can also be directly emitted by plants (Jardine et al., 2012). MACR and MVK have been reported to have a bidirectional flux in and from trees (Fares et al., 2015) and can be absorbed by tree saplings (Tani et al., 2010). However, while MACR and MVK play only a minor role in plant emissions, they ~~MACR and MVK~~ have been reported to have a bidirectional flux in and from trees (Fares et al., 2015) and can be absorbed by tree saplings (Tani et al., 2010).

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