Reply to comments from Reviewer 2:

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

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*The reviewer comments are included here 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).*

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 positive 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 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 spot 2 and 3 were moved to spot 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). Spot 4 and 5 were located within 15 m of spot 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 to 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 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 before mentioned 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. The blank values varied in each season, and the number of blanks taken in each campaign is different (Table A2). 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.

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 effects of different soil properties as 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 a discussion of the relationship between SWC and the soil fluxes should be extended in the text. As the SWC and temperatures co-variate, so interpretation has to be done careful.

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 of 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 the enantiomers. While the effect size differs between enantiomers, the direction (negative, positive or close to zero) of the β coefficients 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 $p < 0.05$.

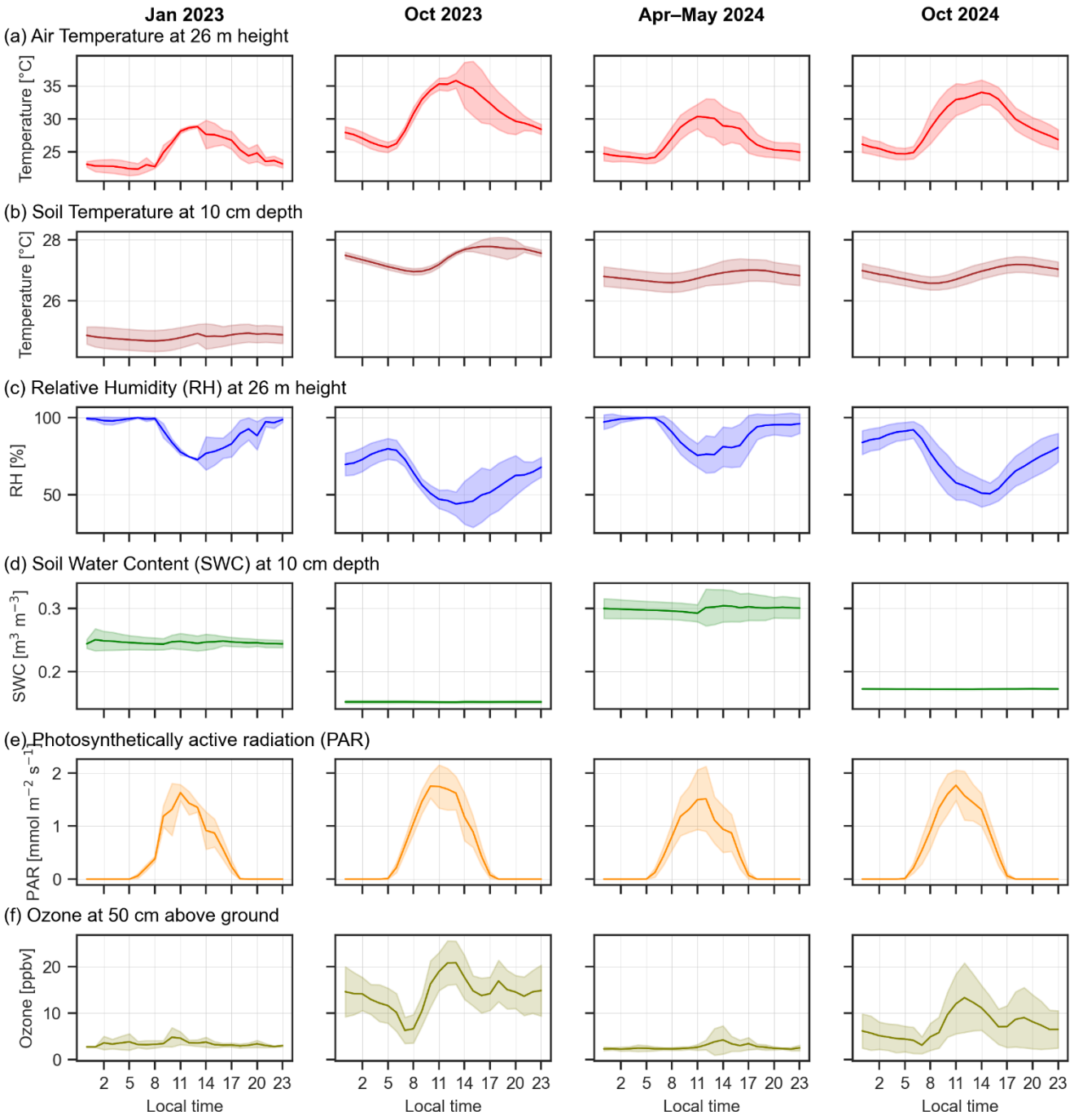

Figure 2 Meteorological data during the measured seasons with (a) temperature (red) and (c) relative humidity (blue) measured at 26 m at the Instant tower, (b) soil temperature (orange) and (d) soil water-content (green) measured at 10 cm depth and (e) photosynthetically active radiation (PAR) incoming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

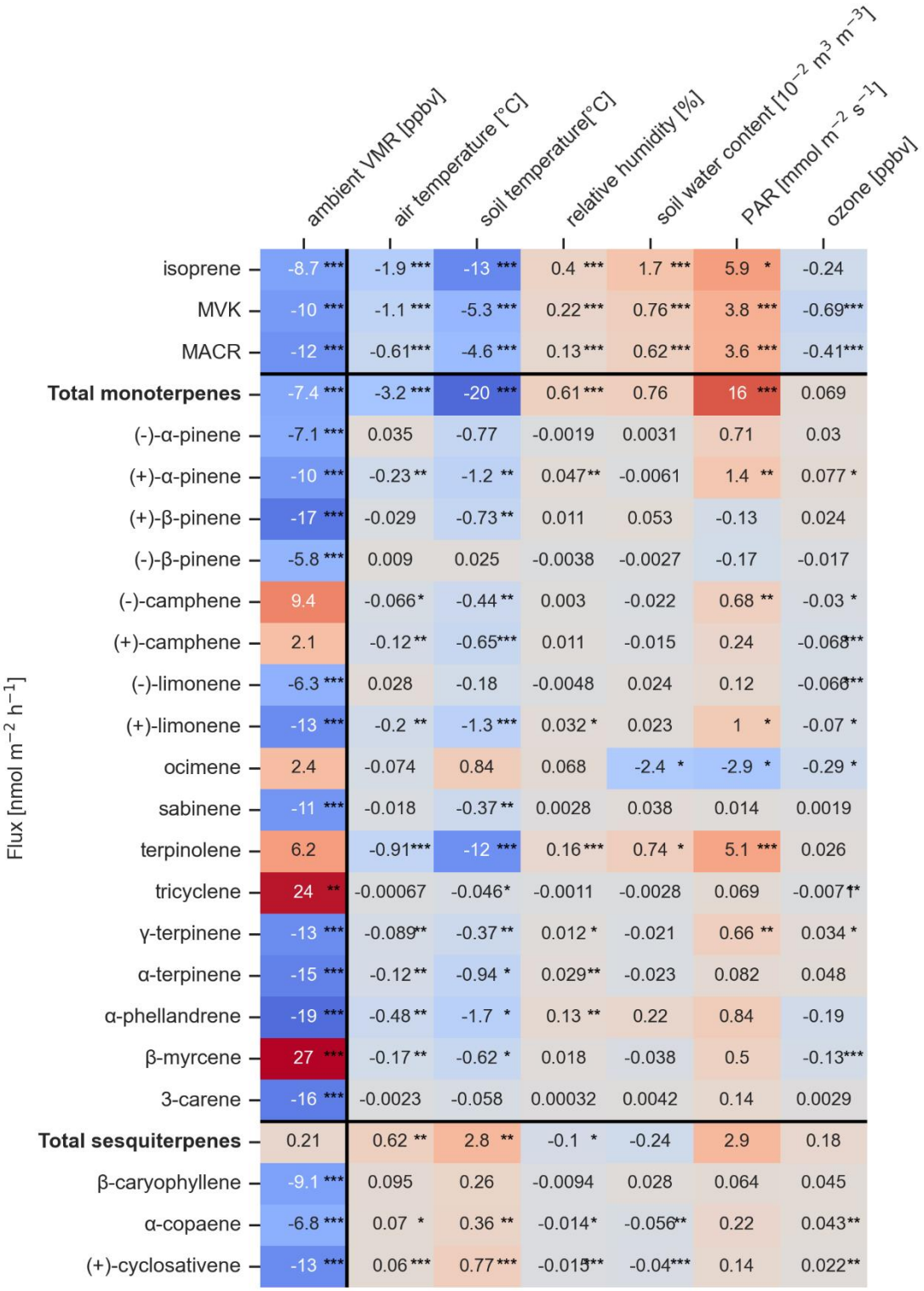

Figure 6 Heatmap of ~~correlations between the~~ β coefficients from linear mixed-effects models quantifying the change of flux in nmol m^{-2} h^{-1} of measured compounds ~~and~~ per unit change in environmental variables, after adjusting for the fixed effect of chamber spot location and random effect for measurement date. ~~various e~~ Environmental variables are: 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

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 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 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: 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 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 concentrated MTs and SQTs, a higher split in the injection to avoid overload and saturation of the instrument was not possible to use. 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.

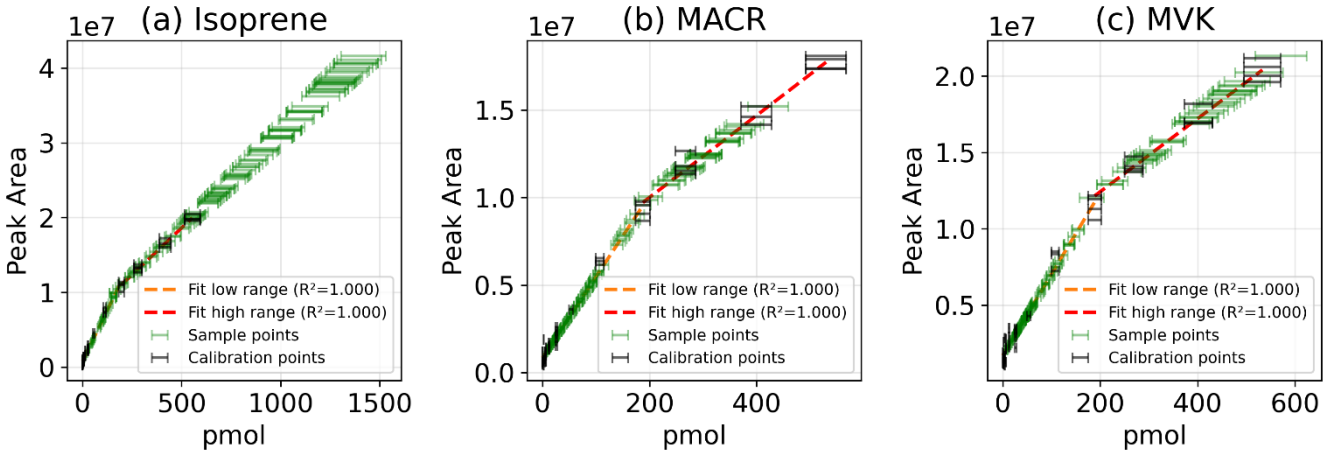

Figure A3 Calibration curve fit of integrated Peak Areas against substance on the cartridge in pmol for (a) isoprene, (b) MACR, and (c) MVK with calibration points with error bars plotted in black, linear fit in the lower range in orange and in the higher range in red, and sample points (from October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

Line 138: Breakthrough was tested by two sorbent cartridges in sequence and concentrations of the calibration gas of up to 10 ppbv with a 6 L sample volume and flow collection rate of 200 mL min^{-1}, which resulted 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 SQTs 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

which 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 496: ~~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 in 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

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.

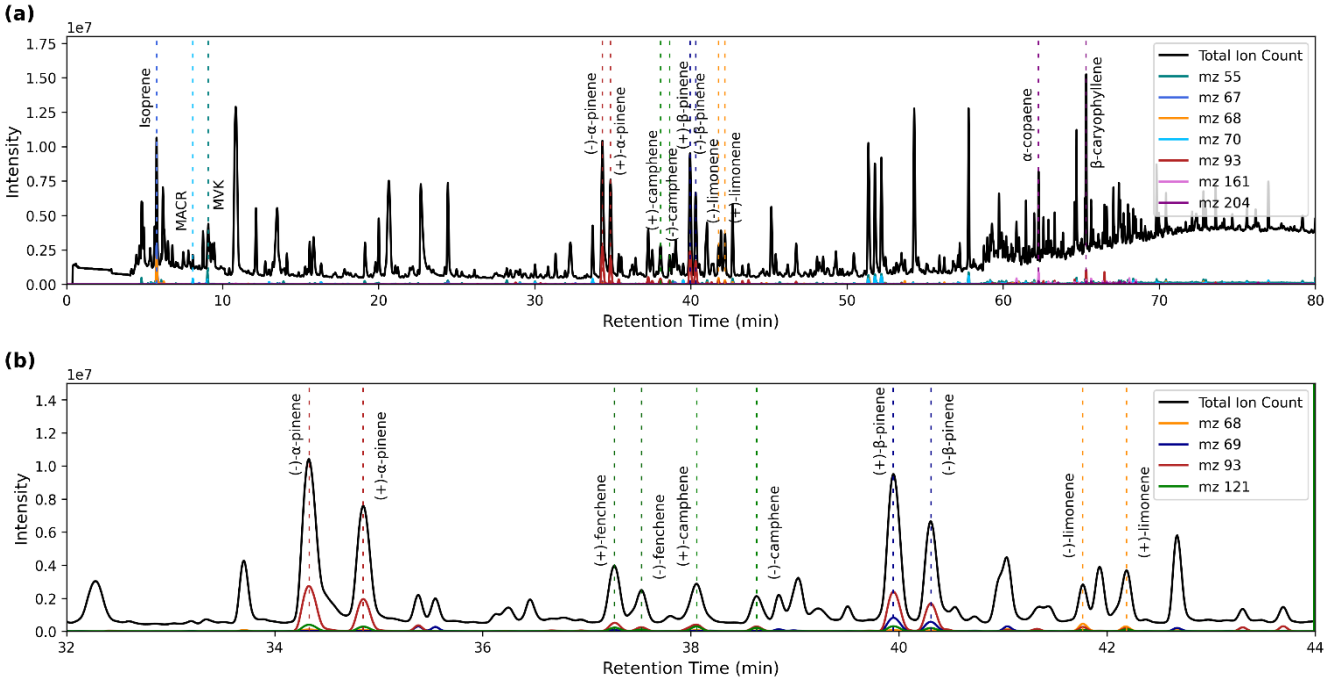

Figure A2 (a) Example Chromatogram of a soil chamber sample from October 2023 with annotation of isoprene, MACR, and MVK peaks, the chiral monoterpenes, and the two most prominent sesquiterpenes (b) Zoomed into the chiral monoterpene resolution.

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