Reply to comments from Reviewer 3:

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

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 reviewing our work, providing comments, and crediting 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 measured 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 pump 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 ~~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. 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 local time of day, 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: Plants and other organisms, like insects, often emit one ~~These~~ enantiomers~~s~~ in excess, reflecting the dominant biosynthetic pathway in a species, a given tissue or a chemotype by using 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). Although an influence from the soil was not yet investigated. ~~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 using it 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 1 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

Name in plots	Season	Oceanic Niño Index	Start Date	End Date	Number of flux data points	Chambers Measured
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.

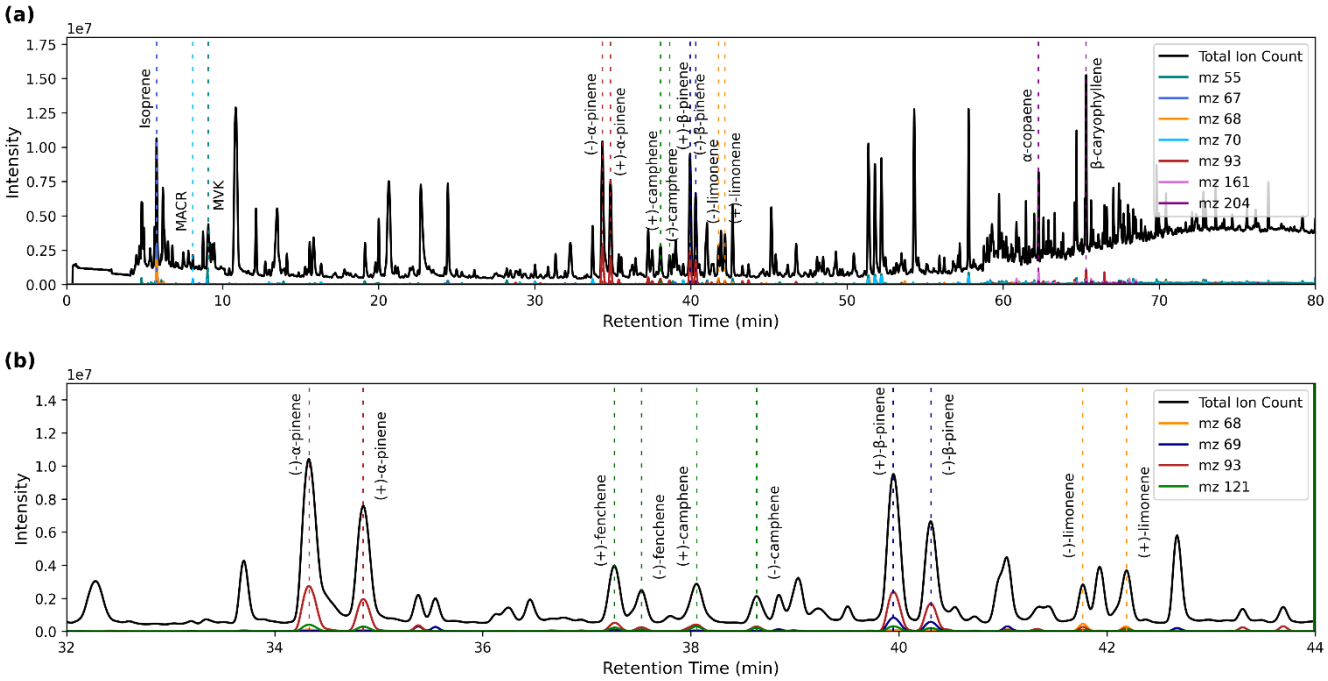

Figure A1 (a) Example Chromatogram of a soil chamber sample from October 2023 with annotation of isoprene, MACR, and MVK peaks, the chiral monoterpenes, and the two most prominent sesquiterpenes (b) Zoomed into the chiral monoterpene resolution.

Line 136: Compounds in the sample chromatogram were identified by matching retention times with those of the standards., ~~and c~~Enantiomers ~~confirmed~~ elution order of α-pinene, limonene, camphene and β-pinene were 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 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.

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 come back 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, compared to canopy BVOC they remain poorly constrained. 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: CO_2 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, MACR and MVK play only a minor role in plant' emissions.

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