Responses to reviewer 1

Reviewer 1'comments in blue

Responses from authors in black

Suggested new text blocks in italic

Reviewer 1

In this paper, the authors studied the influence of forest diversity, environmental soil factors, and microbial biomass on soil-litter BVOC emissions and GHG fluxes in Amazonian forests. Three different ecosystems were selected, and a broad range of drivers was analyzed. Two GHGs (CH_4 and CO_2) were measured, along with various BVOCs. This paper presents novel research in a field that requires further investigation. To date, limited research has focused on BVOCs from the soil compartment of forests. This is important, as the Amazon contains the largest tropical forest in the world, and global BVOC emissions are predominantly from natural sources. Despite the extensive number of variables measured and therefore, data obtained, the authors made a selection for their main text.

We would like to thank the reviewer for the time spent on the review, and for your positive words.

General remarks:

Overall, the authors should ensure consistency in the use of abbreviations; for example, "CH_4" is used in some instances, while "methane" is used in others. The same applies to the naming of forests, as well as to abbreviations like DMS and LMs.

Thank you for pointing this out, we checked the abbreviations carefully and have corrected this in the revised manuscript.

Regarding the use of Tedlar bags, do you expect any sorption/losses of gases when using these bags? Was this checked for the compounds of interest, or do you have any literature that supports this?

Yes, we are aware of the potential for adsorption and gas losses when using Tedlar bags. Although we have not previously tested these effects for the compounds of interest, we have followed literature recommendations. We briefly addressed the recommended time for storing samples in Tedlar bags in our manuscript (L204-205). We suggest to expand this information in the revised version, by adding the following lines:

"Beauchamp et al. (2008) demonstrated that compound losses can occur due to adsorption onto the inner walls of the bags and diffusion through the bag material, which can compromise the stability of stored samples. To minimize these effects, it is recommended to store samples at low temperatures and analyze them within 10 hours of collection. In our study, samples were stored for a maximum of eight hours before being analyzed using the PTR-QMS and Los Gatos instruments, which is within the period of time recommended by Beauchamp et al. (2008). Furthermore, to protect the integrity of the samples, the Tedlar bags were stored in opaque stainless-steel boxes placed inside containers with controlled air temperatures. These precautions ensured minimal adsorption and losses during the storage period."

N_2O is also a very important GHG emitted/consumed by soils; however, it is not mentioned, not even in the introduction. I wonder why the authors chose not to measure it.

We agree with the reviewer that it would have been very important to obtain N_2O flux data from the soil, as it is indeed an important greenhouse gas. Such measurements could have been an important contribution to this study. But, unfortunately, there was not N_2O instrument available for this study.

I am not sure if I overlooked this, but is there any explanation for the patterns of isoprene and monoterpenes along the transects?

This is indeed a complex question. Initially, we did not expect such distinct patterns between transects. However, the observed patterns of isoprene and monoterpene fluxes along the transects likely reflect the complex spatial and temporal variability in soil conditions, as well as external environmental drivers. These factors contributed to notable differences both within and between transects, as outlined in our results (Figures 8 and 9) and discussed in the sections 4.2 and 4.3.1.

Analyzing the results for each forest type, the ancient river terrace forest was the most homogeneous, but without clear patterns when comparing gas fluxes and tested environmental variables. In contrast, for the white sand forest, a distinct pattern emerged: BVOC uptake occurred in transect 1, while transect 2 showed emissions. We attribute this difference to the rainfall event before measurements in transect 2, which caused a 58% increase in soil moisture. This rise in soil moisture positively influenced BVOC emissions, as supported by our linear models identifying soil moisture as a key predictor of emissions in the white sand forest. Such an effect aligns with previous studies showing that soil moisture influences BVOC emissions by affecting microbial activity (Bourtsoukidis et al., 2018; Trowbridge et al., 2020).For the upland forest, we observed a somewhat similar pattern to the white sand forest; however, this response was more evident for acetaldehyde and DMS rather than all gases. Unlike in the white sand forest, soil moisture did not play a major role in the upland forest, as transect 2 was slightly drier than transect 1. Instead, microbial biomass emerged as a primary driver of BVOC fluxes in this forest type, corroborating findings that microbial community structure and activity can strongly influence soil BVOC dynamics (Insam & Seewald et al., 2010; Penuelas et al., 2014; Abis et al., 2020).

In summary, our linear models support three main conclusions: (1) soil moisture is a critical driver of BVOC emissions, especially in white sand forest; (2) microbial biomass exerts greater influence on BVOC fluxes in upland forest; and (3) the spatial heterogeneity of soil conditions and external factors such as rainfall, wind, and cloud cover, combined with measurements taken on different days, contribute to the high variability observed in fluxes. This complexity highlights the challenges in capturing soil gas fluxes accurately and underscores the need for long-term, multi-factorial studies to better understand BVOC flux dynamics in tropical forest soils.

Lastly, I am curious whether the authors were able to identify other BVOCs in their samples that could be relevant for future studies. Based on the sampling strategy and analytical techniques used, I suspect that more compounds were observed, and they could be tentatively identified using an MS library. This is, in my opinion, of great added value for future studies that decide to expand (in advance) their current list of BVOCs of interest, especially given the limited number of studies on the subject.

Yes, we indeed identified additional BVOCs in our measurements. Using cartridges, we detected compounds such as α-Cubebene, α-Copaene, Caryophyllene, D-Limonene, β-Pinene, β-Phellandrene, among others. We agree that these findings could provide valuable insights for future studies, and we encourage any interested reader to check all of them in the Supplementary Material (Figure S2). However, this data was primarily used for qualitative comparisons between forest types. Additionally, given the large amount of data presented in this study and the complexity of the study, we opted to focus on the main results to maintain conciseness. We also want to add that all datasets will become available upon publication, aiming to contribute to any future studies.

Specific remarks

Line 42: Wouldn't it be better to use the plural form soil-litter microorganisms?

Yes, we agree with the reviewer, and we added these key words in plural form in the revised manuscript.

Line 53: What about N_2O fluxes? N_2O is also produced and consumed by soils. Please add a few lines about this.

We agree with the reviewer, and will add this information to the Introduction, in the revised text:

"N_2O can be produced and consumed by soils through microbial nitrification and denitrification processes (Butterbach-Bahl et al., 2013; Snyder et al., 2009). These microbial processes, like those affecting other soil gases, are strongly influenced by environmental factors such as soil moisture, temperature, and nutrient availability (Saggar et al., 2013; Butterbach-Bahl et al., 2013). Together, these processes drive the net ecosystem exchange of BVOCs and GHGs between the soil-litter compartment and

the atmosphere, and the magnitude and direction of this exchange may vary across different ecosystem types."

Line 78: Indicate the most recent BVOC budget estimate for the Amazon basin.

We will modify this so that the text represents the most recent BVOC budget estimate for the Amazon basin:

*"Global emissions of BVOCs from terrestrial vegetation are estimated at approximately 760 Tg C yr^{-1}, with isoprene (C_5H_8) and monoterpenes ($C_{10}H_{16}$) accounting for around 70% and 11% of these emissions, respectively (*Tripathi et al., 2025*). Isoprene is a simple building block compound emitted in large quantities, particularly by tropical forests, whereas monoterpenes—such as α-pinene, β-pinene, and limonene—are structurally more complex (Guenther et al., 2012; Gomes Alves et al., 2016).*

Lines 78-84: This paragraph could be improved to better explain BVOC dynamics and their atmospheric effects. For example, BVOCs contribute to the formation of tropospheric ozone, an important GHG. Additionally, while SOAs indeed influence cloud properties, they also affect the Earth's radiation budget by scattering incoming solar radiation or absorbing outgoing longwave radiation. These aspects should be mentioned.

We agree with the reviewer, and we will add this information to the Introduction of the revised manuscript as following:

"Once released into the atmosphere, they actively participate in atmospheric chemistry and physics, influencing climate dynamics. BVOCs react with key atmospheric oxidants—including hydroxyl radicals (OH), ozone (O_3), and nitrate radicals (NO_3)—to form secondary organic aerosols (SOAs) (Artaxo et al., 2022; Yáñez-Serrano et al., 2020). SOAs, in turn, have a major influence on cloud properties, enhancing cloud condensation nuclei (CCN) concentrations, which impacts precipitation patterns and alters cloud lifecycles (Liu and Matsui, 2022). Depending on their chemical composition, SOAs can also influence the Earth's radiation budget by scattering incoming solar radiation (resulting in a cooling effect) or absorbing outgoing longwave radiation. Additionally, BVOCs contribute to the formation of tropospheric ozone—an important greenhouse gas and a major air pollutant (Vella et al., 2025) . Given these large-scale impacts, accurately quantifying BVOC fluxes in terrestrial ecosystems is essential for advancing our understanding of forest–atmosphere interactions and for improving Earth system models—thereby improving climate predictions."

Line 82: Support this statement with quantitative data. Is there already any estimate of BVOC emissions from vegetation vs BVOC emissions from the soil-litter compartment?

Yes, there is an estimate of BVOC emission contributions from vegetation. Tripathi et al. (2025) estimated that terrestrial vegetation emits approximately 760 Tg C yr^{-1} in BVOCs, with isoprene and monoterpenes contributing 70% and 11% of this total, respectively. The Amazon rainforest accounts for 40% of global BVOC emissions, making it a significant component of the global carbon cycle (Guenther et al., 2012). However, there is currently no information available on the specific contribution of the soil-litter compartment to the total BVOC budget of the Amazon Forest.

As previously pointed out, we suggest to add in the revised manuscript the following text:

"Global emissions of BVOCs from terrestrial vegetation are estimated at approximately 760 Tg C yr^{-1}, with isoprene (C_5H_8) and monoterpenes ($C_{10}H_{16}$) accounting for around 70% and 11% of these emissions, respectively (Tripathi et al., 2025). Isoprene is a simple building block compound emitted in large quantities, particularly by tropical forests, whereas monoterpenes—such as α-pinene, β-pinene, and limonene—are structurally more complex (Guenther et al., 2012; Gomes Alves et al., 2016). The Amazon rainforest alone contributes about 40% of global BVOC emissions, playing a critical role in the global carbon cycle (Guenther et al., 2012; Wang et al., 2024; Tripathi et al., 2025)."

Line 103: Remove "and?"

We will remove 'and'

Line 160: Why two Teflon inlets? What were they used for?

Thanks for pointing this out. We will add the below text in the Material and Methods section (2.3 and 2.4) for clarification:

"The two Teflon inlets were necessary to ensure the proper functioning of the semi-static chamber system. One inlet was connected to the pump for sampling, while the other one was connected to an open Teflon line to equilibrate the system and maintain internal pressure balance. It is important to mention that this semi-dynamic method does not assume a steady state condition. Additionally, a blank chamber was used to detect potential chamber effects or external influences."

Line 161: Sentence structure is not clear.

We agree, and we suggest rephrasing it as follows:

"Two Teflon inlets were connected to the top of the chamber, and an internal fan mixed the gases within the chamber headspace."

Lines 173-178: Rephrase for clarity. For example, in line 177, you refer to a continuous flow. Do you mean an external flow?

What we mean by continuous flow is the presence of a constant airflow within the semi-dynamic chamber system. In this setup, air accumulates inside the chamber while a pump continuously removes 500 sccm of air over 20 minutes through one of the inlets. At the same time, the second inlet remains open to allow external air to enter, thereby ensuring the system remains balanced and maintains equalized pressure.

For clarity, we rephrased:

"Gas sampling was conducted in December 2021, during the dry-to-wet season transition. Soil-litter gas fluxes (BVOCs, CO_2, methane) were collected using Tedlar bags (CEL Scientific, Cerritos, CA, USA). A semi-dynamic chamber system was employed, where constant airflow (500 sccm) was ensured by an air sampling pump (GilAir® Plus, Levitt Safety, Ottawa, ON, Canada) connected to one of the chamber outlets. While the pump continuously removed air from the chamber at 500 ml/min, a secondary inlet remained open to allow external air to enter the system, maintaining balanced pressure inside the chamber. After 20 minutes of air circulation under this continuous flow, a Tedlar sampling bag was connected to the outlet, and 5 L of air was collected over the course of 10 minutes. By the end of the 30-minute sampling process, a total of 15 L of air had flowed through the chamber."

Line 180: Specify what "los gatos analyzer" is. Someone not related to the field won't recognize the name. Refer to the section number rather than "see below".

We remove the "see below" and will put the section number (2.5) in the revised text. We give more details of Los Gatos instrument in the 2.5 section, with the following text:

"After PTR-QMS analysis, the bags were connected to a Los Gatos Ultraportable analyzer to measure the mixing ratios of CH_4 and CO_2 with high sensitivity and rapid response times. The Los Gatos analyzer is an instrument based on laser absorption spectroscopy specifically Off-axis Integrated Cavity Output Spectroscopy (OA-ICOS), enabling ultra-sensitive, precise, and real-time measurements of trace gases in gas samples (Pohlman et al., 2021; van Asperen et al., 2024). The air in the sample bag was measured for 3 minutes with an airflow of ~ 0.1 LPM, and an average was taken from the last 2 minutes of the measurement."

Line 184: Was the ventilation performed with ambient air?

Yes, it was. We will clarify this in the revised text as follows:

"Before placing the lid on the collar, the chamber was manually ventilated with ambient air to minimize collar-induced CO_2 accumulation. The chamber was then closed, the internal fan was turned on, and the lid was sealed with clamps. "

Line 220: Why was the air stream humidified, and what was the resulting relative humidity?

The air stream was humidified to better replicate the atmospheric conditions of the Amazon, where relative humidity is exceptionally high (90-100% during the wet season). Conducting the calibration with dry air would not accurately reflect the ambient air in the forest, potentially affecting the reliability and representativeness of the measurements. Relative humidity was estimated to be between 90-100%.

Line 237: Did you compare the DMS results from PTR measurements with those of GC-MS?

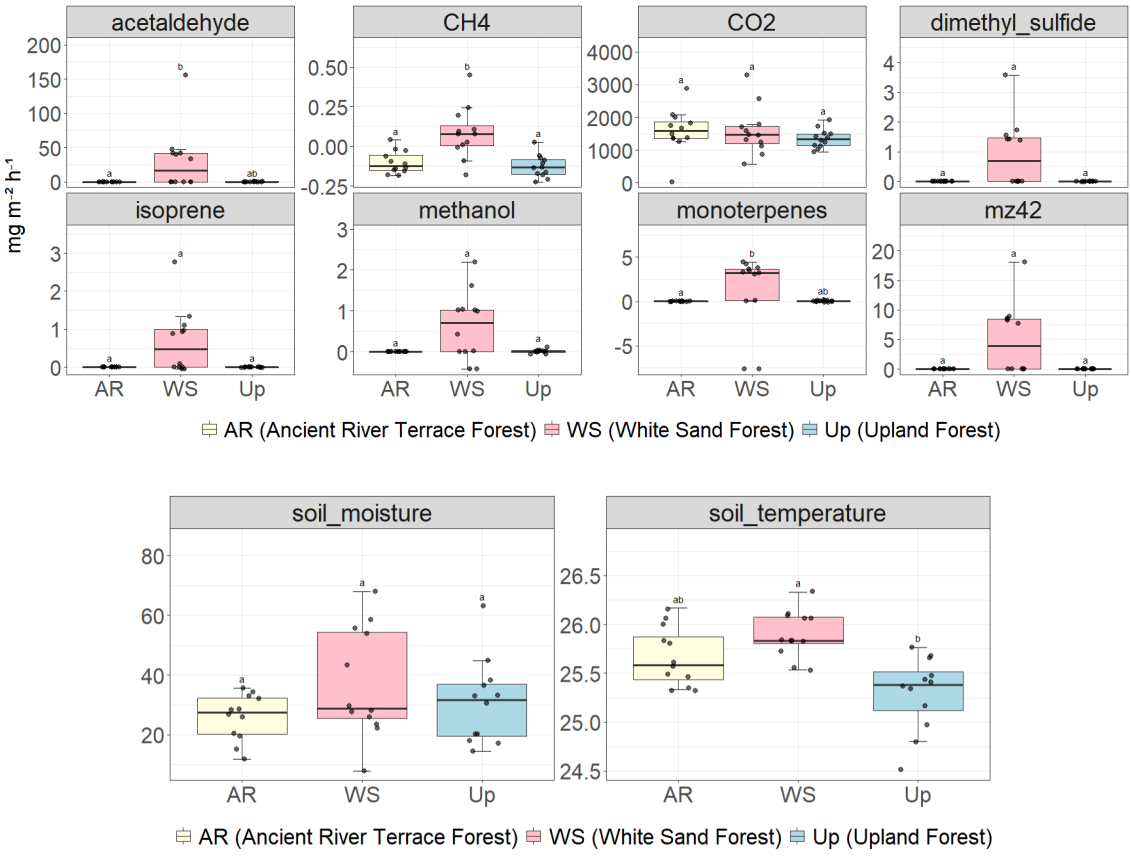
Unfortunately, we did not detect dimethyl sulfide (DMS) in the sorbent cartridges. It is very difficult to capture DMS with cartridges, due to high volatility, which makes it prone to breakthroughs rather than being captured.

Materials and methods: Refer to the supplementary material sections in the text using the specific section numbers.

We have modified this in the revised manuscript.

Figure 3: Consider splitting this figure into two panels: A (fluxes) and B (environmental soil variables). Also, clarify what is meant by “monoterpenes.” Which compounds are included in this group?

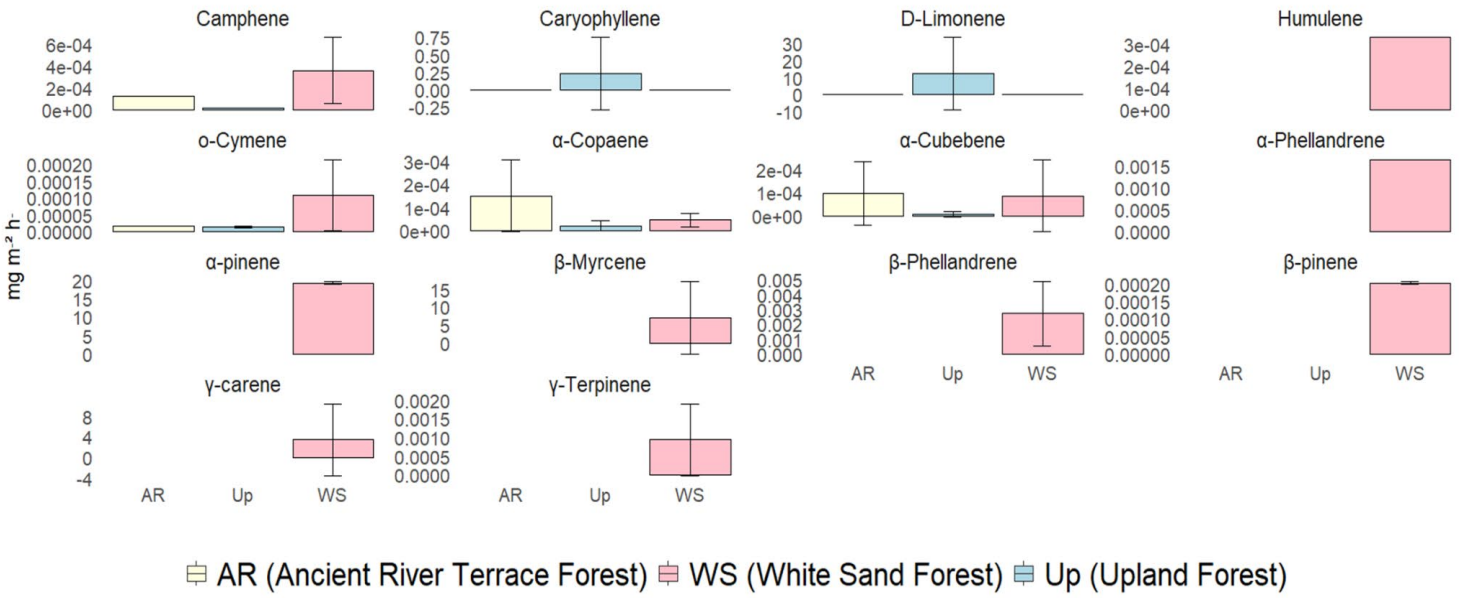
We agree with this idea and have split the plots. Our revised figure can be found below.



About the monoterpenes, as measurements were carried out with PTR-QMS, we can not specify which compounds were included in the sample. The PTR-QMS measures the total monoterpenes found in the sample. As for more general information on monoterpenes, we added the definition of this group in the introduction of the revised manuscript:

“Isoprene is a simple building block compound emitted in large quantities, particularly by tropical forests, whereas monoterpenes—such as α-pinene, β-pinene, and limonene—are structurally more complex (Guenther et al., 2012; Gomes Alves et al., 2016).”

Figure S2: Use the correct Greek symbols for compound names; i.e., “α” instead of “a”, “ɣ” instead of “g”, etc., and match the forest type colors with Figure 3.



Ok Thanks for pointing this out. We have corrected it in the revised manuscript.

Line 322: Is that consumption related to a specific compound? And a specific chamber?

The observed consumption refers to monoterpenes and DMS. This result is shown in Figure 3, which presents the boxplot for the two transects across each forest type. However, when analyzing Figure 9, we observed that monoterpene consumption occurred in two chambers of Transect 1. For DMS, consumption was observed in all chambers of Transect 1 (as shown in Figure S10, Supplementary Material).

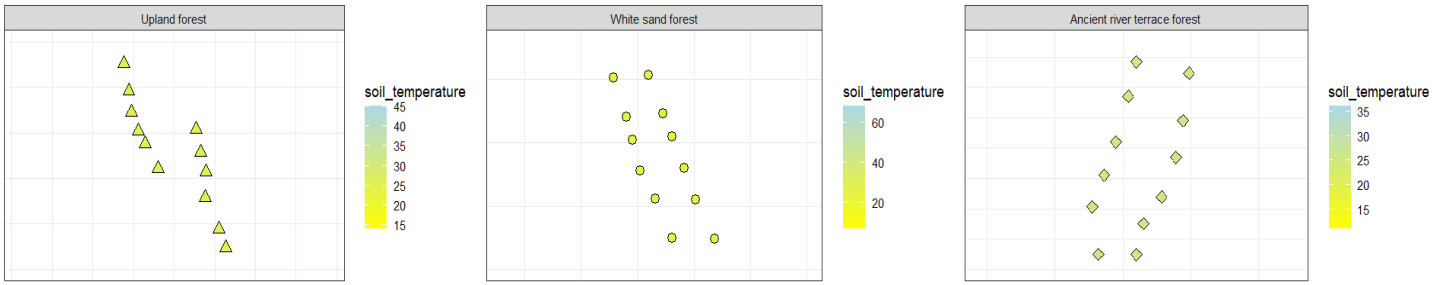
Table 4: Soil/litter characteristics and microbial biomass only explain a small proportion of the variance in CH_4. What could be the reason?

We believe the issue here is related to the forest type. Table 4 refers to the ancient river terrace forest, which contributed very little to the fluxes of most gases. As a result, linear models did not reveal clear patterns. We found an interesting relationship with a high R^2 for some gases (methanol, acetaldehyde, isoprene, and monoterpenes), where most of the selected drivers were characteristics of the soil and litter. However, for methane, the relationship was indeed poor, and the R^2 was low. We believe this may be due to spatial and temporal variability: methane fluxes are highly variable over small spatial scales and over time because of changing environmental conditions (e.g., moisture pulses,

temperature fluctuations), which can obscure direct links to biomass or soil and litter traits measured at a single time point. Additionally, spatial and temporal heterogeneity play a role: soil properties can vary greatly over small areas, and conditions change rapidly during events such as wetting or drying. Microbial communities respond quickly to these changes, and snapshot measurements of soil, litter, or biomass may not adequately capture these dynamics.

Figure S8: Keep the number of digits consistent among plots for soil temperature.

Thanks for pointing this out. We have corrected it in the revised manuscript.



Lines 459-462: Could you support these lines with numbers?

Yes, we cite values of BVOC from soil in section 4.2 (lines 560-586). Regarding this topic, our intention was to start the discussion by presenting the general aspects of our findings. However, specifically regarding your comment, we respond below:

Bourtsoukidis et al. (2018) reported maximum sesquiterpene emissions in upland forest soils following rain of approximately 10 µg m^{-2} h^{-1}, with model simulations reaching up to 20 µg m^{-2} h^{-1} (Fig. 6, A). Under drier conditions, sesquiterpene emissions decreased.

Llusià et al. (2022) showed that fertilization shifted soils from a sink to a source of BVOCs, with the highest terpene emissions recorded at upper elevations during the wet season after nitrogen addition (monoterpenes: 406 µg m^{-2} h^{-1}) and phosphorus addition (sesquiterpenes: 210 µg m^{-2} h^{-1}).

We suggest to modify the text to the following:

Previous studies investigated tropical soil BVOC fluxes using incubation (Bourtsoukidis et al., 2018) and fertilization experiments (Llusià et al., 2022), showing that fluxes are higher than previously anticipated. Bourtsoukidis et al. (2018) reported maximum sesquiterpene emissions of ~10 µg m^{-2} h^{-1} after rain (model simulations up to 20 µg m^{-2} h^{-1}), decreasing under drier conditions. Llusià et al. (2022) showed that fertilization converted soils from BVOC sinks to sources, with peak monoterpene emissions of 406 µg m^{-2} h^{-1} after nitrogen addition and sesquiterpenes of 210 µg m^{-2} h^{-1} after phosphorus addition.

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