

## Reply to comments from Reviewer 1:

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

5 *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).*

10 **Response:** During a re-examination of the linear mixed-effects models used to evaluate the effects of environmental parameters on soil VOC fluxes, we identified a coding error. We corrected the error, re-ran the analyses, and updated Figure 6 and the corresponding discussion. The corrected results are now more consistent with the Pearson correlations analysis reported in the preprint. All other results from the revised linear mixed-effects models remain as stated in our previous response to Reviewer 1:

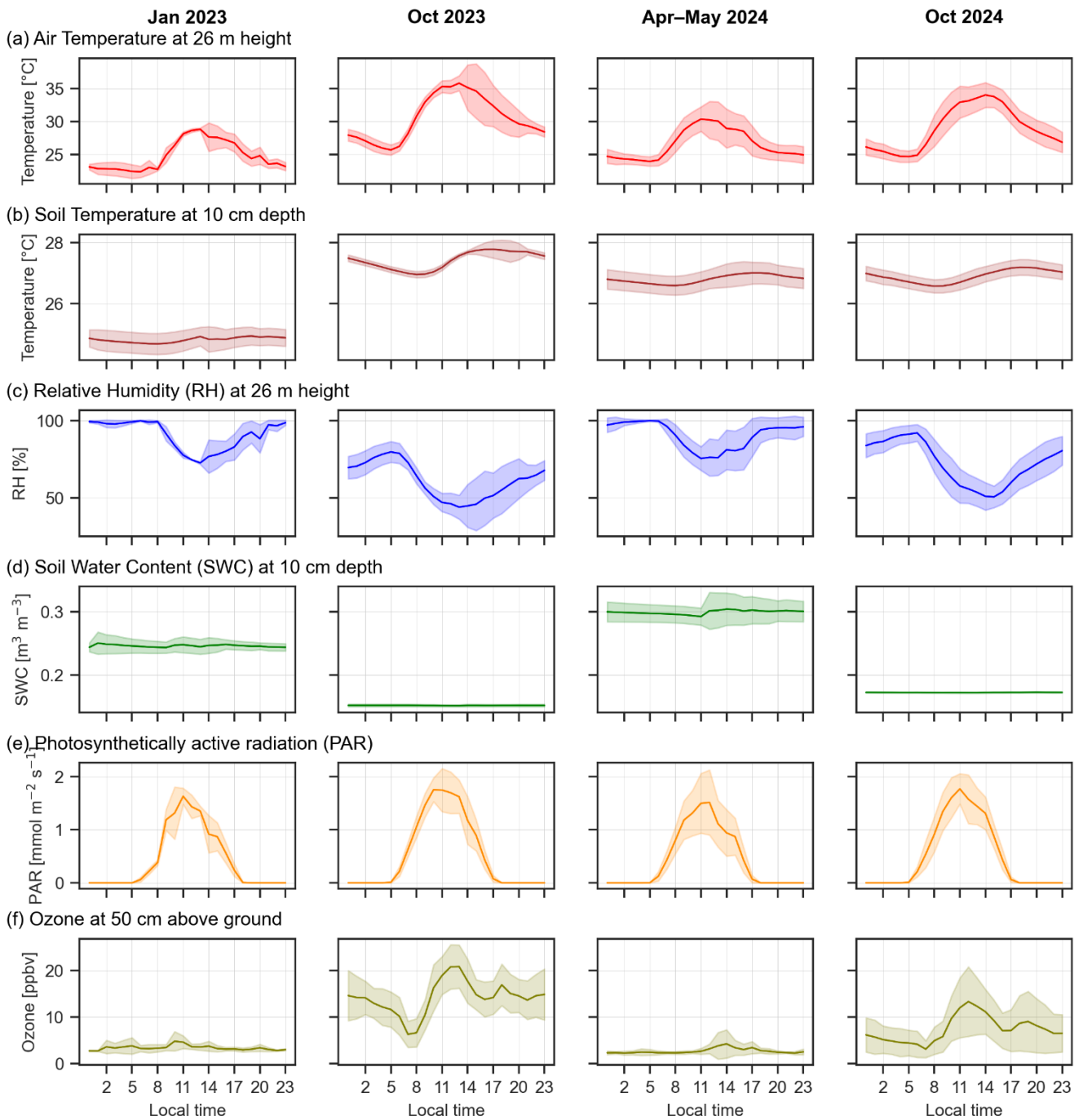
15 We also adjusted the order of ambient parameters in Figure 6 to the same order as was used in Figure 2, added ozone to Figure 2 (f), and adjusted the unit for PAR from  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to  $\text{mmol m}^{-2} \text{s}^{-1}$ .

### 2.6 Statistical analysis

20 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 ( $\beta$ ) 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  $p < 0.05$ .

### 3.2 Meteorological conditions

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**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.

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### 3.4.2 Soil fluxes VS environmental conditions

	ambient VMR [ppbv]	air temperature [°C]	soil temperature [°C]	relative humidity [%]	soil water content [ $10^{-2} \text{ m}^3 \text{ m}^{-3}$ ]	PAR [ $\text{mmol m}^{-2} \text{ s}^{-1}$ ]	ozone [ppbv]
isoprene	-8.7 ***	-1.9 ***	-13 ***	0.4 ***	1.7 ***	5.9 *	-0.24
MVK	-10 ***	-1.1 ***	-5.3 ***	0.22 ***	0.76 ***	3.8 ***	-0.69 ***
MACR	-12 ***	-0.61 ***	-4.6 ***	0.13 ***	0.62 ***	3.6 ***	-0.41 ***
<b>Total monoterpenes</b>	<b>-7.4 ***</b>	<b>-3.2 ***</b>	<b>-20 ***</b>	<b>0.61 ***</b>	<b>0.76</b>	<b>16 ***</b>	<b>0.069</b>
(-)- $\alpha$ -pinene	-7.1 ***	0.035	-0.77	-0.0019	0.0031	0.71	0.03
(+)- $\alpha$ -pinene	-10 ***	-0.23 **	-1.2 **	0.047 **	-0.0061	1.4 **	0.077 *
(+)- $\beta$ -pinene	-17 ***	-0.029	-0.73 **	0.011	0.053	-0.13	0.024
(-)- $\beta$ -pinene	-5.8 ***	0.009	0.025	-0.0038	-0.0027	-0.17	-0.017
(-)-camphene	9.4	-0.066 *	-0.44 **	0.003	-0.022	0.68 **	-0.03 *
(+)-camphene	2.1	-0.12 **	-0.65 ***	0.011	-0.015	0.24	-0.068 ***
(-)-limonene	-6.3 ***	0.028	-0.18	-0.0048	0.024	0.12	-0.068 ***
(+)-limonene	-13 ***	-0.2 **	-1.3 ***	0.032 *	0.023	1 *	-0.07 *
ocimene	2.4	-0.074	0.84	0.068	-2.4 *	-2.9 *	-0.29 *
sabinene	-11 ***	-0.018	-0.37 **	0.0028	0.038	0.014	0.0019
terpinolene	6.2	-0.91 ***	-12 ***	0.16 ***	0.74 *	5.1 ***	0.026
tricyclene	24 **	-0.00067	-0.046 *	-0.0011	-0.0028	0.069	-0.007 **
$\gamma$ -terpinene	-13 ***	-0.089 **	-0.37 **	0.012 *	-0.021	0.66 **	0.034 *
$\alpha$ -terpinene	-15 ***	-0.12 **	-0.94 *	0.029 **	-0.023	0.082	0.048
$\alpha$ -phellandrene	-19 ***	-0.48 **	-1.7 *	0.13 **	0.22	0.84	-0.19
$\beta$ -myrcene	27 ***	-0.17 **	-0.62 *	0.018	-0.038	0.5	-0.13 ***
3-carene	-16 ***	-0.0023	-0.058	0.00032	0.0042	0.14	0.0029
<b>Total sesquiterpenes</b>	<b>0.21</b>	<b>0.62 **</b>	<b>2.8 **</b>	<b>-0.1 *</b>	<b>-0.24</b>	<b>2.9</b>	<b>0.18</b>
$\beta$ -caryophyllene	-9.1 ***	0.095	0.26	-0.0094	0.028	0.064	0.045
$\alpha$ -copaene	-6.8 ***	0.07 *	0.36 **	-0.014 *	-0.056 **	0.22	0.043 **
(+)-cyclosativene	-13 ***	0.06 ***	0.77 ***	-0.013 **	-0.04 ***	0.14	0.022 **

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Figure 6 Heatmap of correlations between the  $\beta$  coefficients from linear mixed-effects models quantifying the change of flux in  $\text{nmol m}^{-2} \text{ h}^{-1}$  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 environmental variables are: ambient mixing ratio (VMR) of each compound in ppbv, air temperature at 26 m in  $^{\circ}\text{C}$ , incoming photosynthetically active radiation (PAR) at 81 m in  $\text{mmol m}^{-2} \text{ s}^{-1}$ , soil temperature in  $^{\circ}\text{C}$  and soil water content (scaled by  $10^{-2}$ ) at 10 cm depth in  $10^{-2} \text{ m}^3 \text{ m}^{-3}$ . Statistical significance of the Pearson correlation  $\beta$  coefficients is indicated by asterisks: (\*) for  $p < 0.05$ , (\*\*) for  $p < 0.01$  and (\*\*\*) for  $p < 0.001$ .

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### 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 ( $\beta = -8.7, -10, \text{ and } -12 \text{ nmol m}^{-2} \text{ h}^{-1}$  per 1 ppbv increase respectively;  $p < 0.001$ ; Fig. 6), after accounting for repeated chamber spot location

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50 measurements and dates in the linear mixed-effect model (Fig. 6). This indicates 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  $\beta$ -myrcene and tricyclene, which exhibited a positive association with ambient mixing ratios ( $\beta = +27$  and  $+24 \text{ nmol m}^{-2} \text{ h}^{-1}$  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.

55 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 ( $\beta = 16 \text{ nmol m}^{-2} \text{ h}^{-1}$  per  $\text{mmol m}^{-2} \text{ s}^{-1}$ ;  $p < 0.001$ ).

60 Different MT species like  $\alpha$ -phellandrene,  $\beta$ -carene,  $\gamma$ -terpinene, limonene, and  $\beta$ -ocimene, also show negative correlation with their mixing ratios ( $-0.21$  to  $-0.82$ ;  $p < 0.001$ ), while  $\beta$ -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  $\alpha$ -copaene ( $0.26$  and  $0.24$ ;  $p < 0.001$ ).

#### 4.1.1 Isoprene and the oxidation products MACR and MVK

65 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 ( $\beta = -8.7$ ,  $-10$ , and  $-12 \text{ nmol m}^{-2} \text{ h}^{-1}$  per 1 ppbv respectively;  $p < 0.001$ ) and key environmental parameters as soil temperature ( $\beta = -13$ ,  $-5.3$ , and  $-4.6 \text{ nmol m}^{-2} \text{ h}^{-1}$  per  $1^\circ\text{C}$ ;  $p < 0.001$ ), while decreased with increasing soil water content ( $\beta = 1.7$ ,  $0.76$ , and  $0.62 \text{ nmol m}^{-2} \text{ h}^{-1}$  per  $0.01 \text{ m}^3 \text{ m}^{-3}$ ;  $p < 0.001$ ) (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

75 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

80 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.

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

## References:

90 Alves, E. G., Jardine, K., Tota, J., Jardine, A., Yáñez-Serrano, A. M., Karl, T., Tavares, J., Nelson, B., Gu, D., Stavrou, T., Martin, S., Artaxo, P., Manzi, A., and Guenther, A.: Seasonality of isoprenoid emissions from a primary rainforest in central Amazonia, Atmospheric Chemistry and Physics, 16, 3903–3925, <https://doi.org/10.5194/acp-16-3903-2016>, 2016.

Bourtsoukidis, E., Pozzer, A., Williams, J., Makowski, D., Peñuelas, J., Matthaios, V. N., Lazoglou, G., Yañez-Serrano, A. M., Lelieveld, J., Ciais, P., Vrekoussis, M., Daskalakis, N., and Sciare, J.: High temperature sensitivity of monoterpene emissions from global vegetation, *Commun Earth Environ*, 5, 95 23, <https://doi.org/10.1038/s43247-023-01175-9>, 2024.