## **Reviewer #2**

Title: Litter decomposition enhances volatile organic compound emission from a freshwater wetland: insights from year-round in situ field experiments

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MS No.: egusphere-2023-2998

The authors report VOC fluxes from the decaying leaf litter of a freshwater wetland plant, Phragmites australis. The reported fluxes include an impressive list of 62 VOCs: 5 aromatics, 15 OVOCs, 30 NMHCs, 9 HVOCs, 3 VOSCs. Many of these compounds are difficult to quantify well, thus requiring proof of careful methodology. The proof, unfortunately, is not provided, leading to doubts about data quality. The problem is compounded by the use of incomplete references. The methods section cites two papers (Wang et al, 2023 and Liu et al. 2021) to broadly explain 'analysis steps'. The cited studies employ slightly different methods and themselves cite a range of earlier papers for broad methodological statements. It becomes a nearly impossible task to follow the actual methods of VOC quantification. Specific papers should be cited for

specific methods, and only if they truly align. For example, Wang et al., 2023, uses a different instrument altogether (GC-MSD-FID from Agilent vs. a GC-MSD from Shimadzu), so it should be made evident why this paper is cited. Below, I compile a checklist for methodology that should be included for field measurements of VOC fluxes. While several items are addressed in this study, other critical aspects are missing.

**Reply:** Thanks for the comments and suggestions. We are very sorry that Reviewer #2 was concerned about the methodology used in this study, which in fact has been evaluated and also

used in previous studies published in *Biogeosciences*. Below we tried to address your concerns and provided more detailed explanation.

Furthermore, litter BVOCs are diverse and complex, and we only reported 62 VOCs in this work. Well over 62 different VOCs have been reported in the previous studies. For example, Svendsen et al. (2018) reported 84 different VOCs emitted from the *Salix* litter and the similar semi-quantification method was used in their study.

As for the literatures cited, the main analysis steps of them were the same as ours. First, VOCs are concentrated by a pre-concentrator, and then are analyzed by GC-MSD. Although the instrument type was different between this work and Wang et al. (2023), the running procedures of the pre-concentrator and GC-MSD, such as temperature setting in each step, were the same. We did refer the studies to conduct the lab analysis and thus cited them. In addition, the lab analysis described in the manuscript was also clear enough for readers to understand.

Anyway, based on your comments, we have deleted the cited references in original manuscript and modified the description on laboratory analysis.

### References

Svendsen, S.H., Priemé, A., Voriskova, J., Kramshøj, M., Schostag, M., Jacobsen, C.S., Rinnan, R., 2018. Emissions of biogenic volatile organic compounds from arctic shrub litter are coupled with changes in the bacterial community composition. Soil Biology and Biochemistry. 120, 80-90. https://doi.org/10.1016/j.soilbio.2018.02.001.

### Methodology

### Sample storage:

## **Canister stability**

Stability of compounds was not tested for the compounds of interest, even though they have different degrees of stability in canisters. Compounds with poor stability or even production within canisters from other compounds (e.g., VOSCs) need to be checked.

Reply: Thanks for the comments. Canister sampling for VOCs was widely used in field observations and recommended by US EPA. The method used here for collecting VOCs referred to the previous chamber-based flux studies (Hellén et al., 2006; Whelan and Rhew, 2016) and the method of PAMS and TO-15, which reported that under conditions of normal usage for sampling ambient air, most VOCs can be recovered from canisters near their original concentrations after storage times of thirty days up to (https://www.epa.gov/sites/default/files/2019-11/documents/to-15r.pdf, Section *1.3*). Haapanala et al. (2006) reported that 24 different NMHCs and 7 halocarbons can preserve in the canisters for at least one week. In fact, US EPA has sponsored the investigation of the SilcoSteel<sup>TM</sup> process of coating the canister interior with a film of fused silica to reduce surface activity. Of course, it should be admitted that humidity could influence the storage time of VOCs in canister. For example, some VOCs are not stable for more than a week under 50% of relative humidity. In addition, Guo et al. (2010) also reported that DMS collected in canisters was stable for a week. Yu et al. (2024) reported that VSCs (DMS, COS and CS<sub>2</sub>) were stable for sixteen-days storage in canisters at room temperature. However, our samples collected by silonite-treated stainless steel canisters were immediately transported to the laboratory after

sampling and were analyzed within two days.

### References

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  Measurements of hydrocarbon emissions from a boreal fen using the REA technique. *Biogeosciences.* 3, 103-112. https://doi.org/10.5194/bg-3-103-2006.
- Hellén, H., Hakola, H., Pystynen, K.H., Rinne, J., Haapanala, S., 2006. C<sub>2</sub>-C<sub>10</sub> hydrocarbon emissions from a boreal wetland and forest floor. *Biogeosciences.* 3, 167–174. https://doi.org/10.5194/bg-3-167-2006.
- Whelan, M.E., Rhew, R.C., 2016. Reduced sulfur trace gas exchange between a seasonally dry grassland and the atmosphere. *Biogeochemistry*. 128, 267–280. https://doi.org/10.1007/s10533-016-0207-7.

# **Field sampling:**

# Flux chamber blank (sampling equipment only)

Chamber blank/control experiments using empty chambers were not conducted, which is concerning because of the use of foam board to make the chamber float. Separate issue: chambers are described as 1.1 m x 1.1 m x 1.1 m (Line124), but it appears that another smaller chamber (35 x 35 x 15cm) is then placed inside the larger box (Line 140), is that correct?

**Reply:** Thanks for the comments.

1) In fact, we also considered this issue when conducting the experiments. Therefore, the foam board was completely wrapped with Teflon film and installed around the chamber. In addition, previous studies have already reported that most of the VOCs released from urethane foam were siloxane-type compounds and the VOCs reported in our study were not detected. (Lattuati-Derieux et al., 2011; Hornyák-Mester et al., 2023). Moreover, the chamber blank experiment was actually conducted before formal sampling. Based on your comments, we have added the relevant information in *Supplement* as below:

"A blank test was carried out by enclosing the chamber with the Teflon film. First, high-purity  $N_2$  (99.999%) was used to purge the chamber and remove the ambient air in the chamber. After that, cleaned canisters were used to collect the first air sample from the chamber and then the second sample and third sample were collected after 30 and 45 minutes, respectively. During sampling, high-purity  $N_2$  was gently added into the chamber to equalize the gas pressure. The target VOCs reported in this study were not detected." (Text S1)

2) Yes. Nine  $1.1m \times 1.1m \times 1.1m$  stainless steel cuboidal boxes without the cover were installed in the freshwater wetland. Three treatments (A: no litter; B: 1.4 kg litters; C: 2.8 kg litters) were set and each of the treatment consisted of three parallel groups, as shown in the Fig.1 in the manuscript. The static chamber (35cm  $\times$  35 cm  $\times$  15cm) used for flux measurement were put inside the cuboidal boxes.

# References

- Hornyák-Mester, E., Mentes, D., Farkas, L., Hatvani-Nagy, A.F., Varga, M., Viskolcz, B.,
  Muránszky, G., Fiser, B., 2023. Volatile emissions of flexible polyurethane foams as a function of time. *Polym. Degrad. Stab.* 216, 110507.
  https://doi.org/10.1016/j.polymdegradstab.2023.110507.
- Lattuati-Derieux, A., Thao-Heu, S., Lavédrine, B., 2011. Assessment of the degradation of polyurethane foams after artificial and natural ageing by using pyrolysis-gas chromatography/mass spectrometry and headspace-solid phase microextraction-gas chromatography/mass spectrometry. *J. Chromatogr. A.* 1218, 4498-4508. https://doi.org/10.1016/j.chroma.2011.05.013.

#### Leakage / sample replacement

A correction is needed to account for the removal of 3.2 L samples taken from an 18 L chamber. This is not a large correction, given that the first sample is taken at the beginning when outside air = inside air, but it should be considered (if using 3 points instead of 2). Leakage/sample replacement may be responsible for the non-linearity of concentration change that was observed. **Reply:** Thanks. In this work, two-point flux calculation was used, which was also suggested and used in the previous studies (Hellén et al., 2006; Maier et al., 2022). The collection of a 3.2 L sample accounted for < 20% of the chamber and we therefore did not correct for the effects of sample replacement. It is acknowledged that this manual sampling may introduce uncertainty in the actual flux estimate. However, this sampling behavior remained consistent across the three treatments and 11 samplings in this work, allowing the results to be compared.

# References

- Hellén, H., Hakola, H., Pystynen, K.H., Rinne, J., Haapanala, S., 2006. C<sub>2</sub>-C<sub>10</sub> hydrocarbon emissions from a boreal wetland and forest floor. *Biogeosciences.* 3, 167–174. https://doi.org/10.5194/bg-3-167-2006.
- Maier, M., Weber, T.K.D., Fiedler, J., Fuß, R., Glatzel, S., Huth, V., Jordan, S., Jurasinski, G., Kutzbach, L., Schäfer, K., Weymann, D., Hagemann, U., 2022. Introduction of a guideline for measurements of greenhouse gas fluxes from soils using non-steady-state chambers. *J. Plant Nutr. Soil Sci.* 185, 447–461. https://doi.org/10.1002/jpln.202200199.

## Instrument:

Hardware and chromatography details. Yes. Entech pre-concentrator with Shimadzu GC-MSD. Temperature programming. Note that running in SCAN mode is less precise than SIM (Lines 156-170).

**Reply:** Thanks for the comments. For GC/MSD, SIM mode does offer better sensitivity and selectivity than SCAN mode. SIM mode is very convenient when only a select number of analytes are important for the study and all other compounds present in the sample can be ignored. However, if we have no preconceived notion about what might be present in the environmental samples, as shown in this work and previous studies on BVOCs (Leff and Noah, 2008; Ryde et al., 2022), a SCAN mode is therefore appropriate (Geer Wallace et al., 2017).

## References

- Leff, J. W., and Fierer, N., 2008. Volatile organic compound (VOC) emissions from soil and litter samples. *Soil Biol. Biochem.* 40, 1629–1636, https://doi.org/10.1016/j.soilbio.2008.01.018.
- Ryde, I., Davie-Martin, C.L., Li, T., Naursgaard, M.P., Rinnan, R., 2022. Volatile organic compound emissions from subarctic mosses and lichens. *Atmos. Environ.* 290, 119357. https://doi.org/10.1016/j.atmosenv.2022.119357.
- Geer Wallace, M., Pleil, Joachim D., Mentese, S., Oliver, K.D., Whitaker, D.A., Fent, K.W., 2017. Calibration and performance of synchronous SIM/scan mode for simultaneous targeted and discovery (non-targeted) analysis of exhaled breath samples from firefighters.

J. Chromatogr. A. 1516, 114-124. https://doi.org/10.1016/j.chroma.2017.07.082.

Instrument precision. Not reported. Precisions are needed for each compound.

**Reply:** We added this in the updated version. (Text S1)

# "Text S1 Quality assurance and quality control

Before sampling, the canisters were repeatedly filled and evacuated pure nitrogen at least three times to remove the potential contaminants and then the evacuated canisters were placed in laboratory for 24 hours. After that, 10% evacuated canisters were selected randomly to be refilled with pure nitrogen and were analyzed in the same way as the samples. Only when no targeted VOCs were detected, the canisters were considered as clean ones and can be used for formal sampling.

A blank test was carried out by enclosing the chamber with the Teflon film. First, high-purity  $N_2$  (99.999%) was used to purge the chamber and remove the ambient air in the chamber. After that, cleaned canisters were used to collect the first air sample from the chamber and then the

second sample and third sample were collected after 30 and 45 minutes, respectively. During sampling, high-purity  $N_2$  was gently added into the chamber to equalize the gas pressure. The target VOCs reported in this study were not detected.

Field blank canisters refilled with pure nitrogen were brought to sampling site and returned to laboratory (lab) for analysis in the same way as samples. The targeted VOCs were not detected or presented the level below the method detection limits (MDLs). During the period of lab analysis, lab blank (the canister filled with pure nitrogen) was analyzed firstly to check if any contaminants remained in the system of GC/MSD coupled with pre-concentrator. All VOCs detected in this work were identified based on their retention times (RTs) in GC and m/zobtained from MSD and were quantified by calibration curves. The VOCs without standards were identified by their match to the NIST library and were semi-quantified based on the species whose RTs were close to them or based on the species which have a similar chemical structure to them. The gas standards were prepared by dynamically diluting the Photochemical Assessment Monitoring Stations (PAMS) standard mixture and TO-15 standard mixture (Linde Spectra Environment Gases, USA) to 0.5, 1, 5, 15 and 30 ppb, respectively. The pure liquid standards of dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) were purchased from Sigma-Aldrich and were dynamically diluted to 0.1, 0.5, 1, 2 and 4 ppb, respectively. The calibration curves were obtained by running the five diluted standards, plus the humidified zero air, in the same manner as the collected samples. Each day before the sample analysis, the system was checked by high pure nitrogen (99.999%) and calibrated with a one-point calibration (1ppb). If the relative percent difference from the initial calibration curve were > 15%, the recalibration is made. For calibrated compounds, the analytical precision determined as a standard deviation of the calibration standards (n=7) was < 12%. The MDLs of these calibrated VOCs ranged from 0.001 ppb to 0.040 ppb. Further, the minimum detectable flux (MDF) of the chamber for VOCs was estimated based on their MDLs (Pihlatie et al., 2013). By assuming a minimum increase of VOC concentration within the chamber headspace equal to its MDL during the 10 mins of chamber enclosure. The resulting MDFs for chamber ranged from 0.002  $\mu$ g m<sup>-2</sup> h<sup>-1</sup> to 0.074  $\mu$ g m<sup>-2</sup> h<sup>-1</sup>." (Text S1)

Duplicate runs. Not reported.

**Reply:** We added this in the updated version. (Text S1)

Detection limit. Not reported.

Reply: We added this in the updated version. (Text S1)

Use of Gas standards. Diluted PAMS and TO-15 standard mixtures are cited. DMS and DMDS standards were used but not described. For VOCs without standards, they were "semi-quantified based on the species whose RTs were close to them or based on the species which have a similar chemical structure to them." This is not an appropriate method, as there is no reason to believe the instrument sensitivity or ion fragmentation will be the same. These compounds must be removed from any quantitative analysis.

**Reply:** Thanks for the comments. We agreed with you that an appropriate quantification of VOCs ideally requires the use of specific standards for the VOCs presented in samples. However, this manuscript aims to explore what kind of VOCs involved in the exchange process

between wetland and atmosphere, the standards for each compound measured here are thus not known a priori. Other method that allows semi-quantification of a compound using the nearest or similar components within the chromatogram can be used. Previous studies on BVOCs also met similar problems and the semi-quantification method was commonly used by them (Frazier et al., 2022; Ruiz-Hernández et al., 2018; Ryde et al., 2022), and some of these studies were also published in *Biogeosciences* (Brachmann et al., 2023; Jaakkola et al., 2023; Kleist et al., 2012; van Meeningen et al., 2016, 2017).

## References

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- Brachmann, C.G., Vowles, T., Rinnan, R., Björkman, M. P., Ekberg, A., Björk, R.G., 2023. Herbivore–shrub interactions influence ecosystem respiration and biogenic volatile organic compound composition in the subarctic. *Biogeosciences*. 20, 4069-4086. https://doi.org/10.5194/bg-20-4069-2023.

- Jaakkola, E., Gärtner, A., Jönsson, A.M., Ljung, K., Olsson, P.O., Holst, T., 2023. Spruce bark beetles (Ips typographus) cause up to 700 times higher bark BVOC emission rates compared to healthy Norway spruce (Picea abies). *Biogeosciences.* 20, 803-826. https://doi.org/10.5194/bg-20-803-2023.
- Kleist, E., Mentel, T. F., Andres, S., Bohne, A., Folkers, A., Kiendler-Scharr, A., Rudich, Y., Springer, M., Tillmann, R., Wildt, J., 2012. Irreversible impacts of heat on the emissions of monoterpenes, sesquiterpenes, phenolic BVOC and green leaf volatiles from several tree species. *Biogeosciences.* 9, 5111-5123. https://doi.org/10.5194/bg-9-5111-2012.
- van Meeningen, Y., Schurgers, G., Rinnan, R., Holst, T., 2017. Isoprenoid emission response to changing light conditions of English oak, European beech and Norway spruce.
   *Biogeosciences.* 14, 4045-4060. https://doi.org/10.5194/bg-14-4045-2017.
- van Meeningen, Y., Schurgers, G., Rinnan, R., Holst, T., 2016. BVOC emissions from English oak (Quercus robur) and European beech (Fagus sylvatica) along a latitudinal gradient.
   *Biogeosciences.* 13, 6067-6080. https://doi.org/10.5194/bg-13-6067-2016.

Gas standard traceability. Missing. Need to add source of standard and standard traceability (if available).

**Reply:** We added this in the updated version.

"Photochemical Assessment Monitoring Stations (PAMS) standard mixture and TO-15 standard mixture (Linde Spectra Environment Gases, USA)" (Text S1)

"The pure liquid standards of dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) were purchased from Sigma-Aldrich and were dynamically diluted to 0.1, 0.5, 1, 2 and 4 ppb" (Text Cal Curve over range of concentrations. May not be valid, as VOC concentrations / ranges were not reported. Calibration curves might not be appropriate for the range of concentrations observed. If concentrations < 0.5 ppb (or <100 ppt for VOSCs), then the calibration curve is a one point calibration.

**Reply:** Thanks. There might be some misunderstanding in description of establishing calibration curves. In fact, our calibration curves included the origin which used the humidified zero air. The following figure shows the calibration curves of typical VOCs measured in this work. Thus, the VOC concentrations above the method detected limits can be quantified by the calibration curves ( $\mathbb{R}^2 > 0.99$ ). We modified the description on establishing the calibration curves, which was provided as below:

"The calibration curves were obtained by running the five diluted standards, plus the humidified zero air, in the same manner as the collected samples." (Text S1)



# **Flux calculation**

Curve fitting procedures."VOC concentrations in chamber headspace had already leveled off at 30 min. Thus, here the first two points, which could capture the initial fluxes, were used to calculate VOC fluxes (Zhang et al., 2021)." What is meant by 'leveled off', and do all chambers show this trend? Zhang et al. only used 2 point flux calculations for one compound and only in the <10% of cases when r2<0.75. Here, the 2-point method appears to be used for all VOCs. Is the system leaky or is there a dilution effect from withdrawing large samples (see above)? **Reply:** Thanks for the comments. In fact, the VOC concentrations leveling off in the chamber headspace was not a phenomenon unique to this work, but a common problem with static chamber approach. The VOC concentrations in the chamber headspace increase asymptotically during the sampling period and it leads to very high VOC accumulation in the chamber headspace, which in turn inhibits the continued increase in headspace VOC concentration as wetland to atmosphere gradient is altered. Gao and Yates (1998) have already reported this

phenomenon (see Figure 2 in their article).

Due to VOC accumulation within the chamber headspace, the flux measured by a static chamber during its placement underestimates the actual flux. The longer the chamber placement time, the more severe the underestimate is (Gao and Yates, 1998). Therefore, the VOC concentrations in the chamber headspace might exhibit a nonlinear increase with time, particularly over a long sampling period. This is the reason why many studies used the initial stage of chamber placement to calculate gas flux, in which the target gas concentrations are more likely to increase linearly with time (Heinemeyer and McNamara, 2011). This to some degree prevents flux underestimation due to a build up of headspace VOC concentrations. The flattening of headspace VOC concentration was not observed in all chambers in this work. We used the first two points to calculate flux to keep VOC flux calculations consistent in three treatments. In this way, we can compare the VOC fluxes in different treatments and in different seasons. Similarly, this method to calculate BVOC fluxes was also used by Hellén et al. (2006). We acknowledged that this could induce uncertainty in calculating VOC fluxes. However, if the third point (30 min) was included, VOC fluxes could be considerably underestimated as suggested by Gao and Yates (1998). In addition, although Zhang et al. (2021) employed two-point flux calculations only in the < 10% of cases, this still reflects that the method could capture the initial gas fluxes and the calculated results were acceptable. If not, they could have excluded the flux data directly instead of continuing to report the results in their study.

Anyway, we greatly appreciate your comments and suggestions. In the revised manuscript, we added some sentences as follows:

"In fact, VOC concentration in chamber headspace has already leveled off at 30 minutes in some cases, which could be because the accumulation of VOCs in the chamber significantly decreases the natural VOC concentration gradient between wetland and atmosphere (Heinemeyer and McNamara, 2011). It was a common problem for static chamber approach. Previous studies suggested shortening the sampling time or using the initial stage of chamber placement to calculate the gas fluxes (Gao and Yates, 1998; Hellén et al., 2006; Heinemeyer and McNamara, 2011; Silva et al., 2015; Zhang et al., 2021). In addition, to compare the VOC fluxes measured in different treatments and in different seasons, the first two points, which could capture the initial fluxes, were thus used to calculate VOC fluxes." (Line 156-165)

"Furthermore, online measurements can obtain more VOC concentration data in the initial

stage of chamber placement, reducing the uncertainty in flux calculation, as shown in the present study." (Line 456-458)

# References

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- Zhang, W., Jiao, Y., Zhu, R., Rhew, R.C., Sun, B., Dai, H., 2021. Chloroform (CHCl<sub>3</sub>) emissions from coastal Antarctic tundra. *Geophys. Res. Lett.*, 48, e2021GL093811. https://doi.org/10.1029/2021GL093811.

Flux detection limits. Given the precision of each compound, what are their flux detection limits? When is a flux determined to be not significantly different from zero? How are these observations treated?

**Reply:** Thanks for the comments. Based on your comments, we have added the flux detection limits in the updated version (Text S1). In addition, the VOC fluxes were considered here when they were greater than the minimum detectable flux (MDF), which was used for the zero flux

test.

"Further, the minimum detectable flux (MDF) of the chamber for VOCs was estimated based on their MDL (Pihlatie et al., 2013). Assuming a minimum increase of VOC concentration within the chamber headspace equal to its MDL during the 10 minutes of chamber enclosure. The resulting MDFs for chamber ranged from 0.002 to 0.074  $\mu$ g m<sup>-2</sup> h<sup>-1</sup>." (Text S1)

#### References

Pihlatie, M.K., Christiansen, J.R., Aaltonen, H., Korhonen, J.F.J., Nordbo, A., Rasilo, T., Benanti, G., Giebels, M., Helmy, M., Sheehy, J., Jones, S., Juszczak, R., Klefoth, R., Lobo-do-Vale, R., Rosa, A.P., Schreiber, P., Serça, D., Vicca, S., Wolf, B., Pumpanen, J., 2013.
Comparison of static chambers to measure CH<sub>4</sub> emissions from soils. *Agric. For. Meteorol.* 171-172, 124-136. https://doi.org/10.1016/j.agrformet.2012.11.008.

In summary, numerous revisions are needed to address the methodological omissions and to improve the quality of flux data. Measuring 62 VOC fluxes is impressive, but how many of these fluxes are real? I suspect several of the measurements have larger uncertainties than presented and/or are below detection levels. Also, aggregating fluxes by VOC type can summarize a large set of results, but this process obscures what is happening with specific compounds. By aggregating by mass, heavier compounds can dominate the overall picture. Please provide more information about the measurements that are defensible, add caveats where needed, and exclude results that are unreliable. Only then can the reader be fully able to assess the scientific contributions of the work.

Reply: Thanks for the comments and suggestions. We acknowledged that, as in many static

chamber studies, there are some uncertainties in this work that could lead to the fluctuation in the VOC fluxes calculated here. However, the variation trend of wetland-atmosphere exchange of VOCs, such as the uptake or release of VOCs and the relationship between VOC flux and temperature, as well as the impacts of litter decomposition on VOCs in freshwater wetlands, would not change. Based on your comments and suggestions, we have added descriptions and explanations of these uncertainties to provide our readers with a clearer understanding and to enable subsequent researchers to conduct their studies accordingly.