LINE numbers refer to the revised manuscript with Track changes turned off.

REVIEWER 3 Dr. Amélie Saunier

The article entitled "Temperate marine macrophytes are highly variable sources of BVOC - a comparative study from the Bastic Sea and NE Atlantic" evaluated BVOC emissions from 3 macrophytes (Zostera marina, Fucus vesiculosus and Ulva intestinalis) from two different locations (Ireland and Finland) by on line measurements through the use of Vocus PTR-TOF. The results presented in the paper are really interesting, highly qualitative and filled the gap of knowledge on marine BVOC. My main issue is about the statistical analyses (see below).

We would like to thank Dr. Saunier for their valuable feedback that has helped us improve the manuscript considerably. We agree with Dr. Saunier's concerns about the statistics and have reanalysed the data and rewritten the Data analysis and Results-sections. In depth answers are provided below to each of the raised concerns.

- line 219 => it is not clear to me if Zostera marina were collected with their roots or not?

The shoots were all attached to rhizomes, we rewrote the sentence to clarify this LINE 219:

Instead of individual plants, Zostera replicates consisted of bundles of shoots (each shoot attached to an individual rhizome), averaging 22 shoots in Ireland and 15 in Finland.

- line 227-228 => not sure that only a leaf rinsing is enough to remove all the epibiota from individuals. I would be more careful with this statement (providing maybe pictures of before/after the rinsing)

We agree, and have changed the wording of the sentence LINE 228**:**

Before measuring their BVOC-emissions, the macrophytes were carefully cleaned and rinsed with filtered seawater to remove sediment and ~~potential~~ most visible epibiota.

We also added a new sentence to clarify the presence of some epiphytes LINE 229:

After cleaning, small amounts of filamentous algae remained attached to Zostera and Fucus replicates, but over 95% of the biomass consisted of the target species.

- line 268 => LPM is a bit weird to me. I would rather write L.min-1.

Changed throughout the manuscript.

- line 320 => I would say deposition or uptakes rather than consumption, just to be more careful.

We have changed "consumption" to "uptake" throughout the manuscript

- For the PCA graphs, you could mix the A and B together with a selection of the main variable (by adding a threshold in your code) affecting the individual repartiton. Since there are a lot of information in this PCA, maybe in this new combined graph, no need to put the compounds where no elemental formula were found (but keep it for the statistical analyse of course). Also, I would rather put the real formula and not the protonated one. Finally, PCA is dedicated to parametric data which is often not the case for BVOC emissions (due to high variability), so most of the time, it is better to run a PLS-DA, more suitable for these types of data unless you have checked the normality and homoscedasticity of all compounds detected for all treatments.

Thank you for the suggestions, we agree that a PCA is likely not the optimal method to deal with our data and have therefore redone the analysis with a NMDS that can handle non-parametric data. We chose to use Euclidean distance for the NMDS, because it can handle negative values (BVOC uptake). This contrasts with many commonly used ecological dissimilarity measures (e.g. Bray–Curtis) that require non-negative data and would therefore necessitate transformation of the data that could obscure the direction of the BVOC flux.

We have rewritten the Method section concerning the ordination-analysis to reflect the reanalysis. The ordination plot or the trends that can be drawn from the ordination plot, did not significantly change with the changed methodology.

We also tried combining panels A and B together but feel that this change made the figure overcrowded and very hard to interpret. Panels A and B are also on different scales which also speaks against combining panels. We realise that the differing scales were not properly highlighted before and have fixed this issue in the figure text. Therefore, we have decided to keep the panels separate. However, to increase readability we have reduced the number of compounds shown in panel B, to only include compounds that significantly correlated with the NMDS ordination shown in panel A (approach described in the Methods). The two panels have been moved from Fig 2. to their own Figure (now Fig 3). Additionally, we chose not to include ellipses in the NMDS like we did in the PCA, because NMDS does not assume multivariate normality and ellipses can therefore be misleading.

- lines 380-393 => I don't really understand how you ran the PERMANOVA. Even though, it is non-parametric analyses, it works like MANOVA that handles several parametric variables. So according to your experimental design, you should run a two-ways PERMANOVA with species and regions as factors. Then you would obtain p-values for the effect of species, for the effect of region and the interaction between both factors. Then to highlight where are the differences (if you got significant effect of at least on factor), you should run a pairwise test. However, in the description of the results, it seems that PERMANOVA was used as a pairwise test which is not correct but maybe I misunderstood something. And you could add the results of the PERMANOVA in this PCA graph.

We agree that our methods were poorly described and that the suggested approach is better suited to our data. Previously we performed the PERMANOVA only to directly examine differences between the six groups (i.e. Macrophyte*Region), after which we performed the pairwise test. We have now reanalysed the data and included Macrophyte and Region as fixed factors, in combination with their interaction that was previously tested. Previously we used Bray-Curtis distance for the PERMANOVA, but in the reanalysis we used Euclidean distance to

match the NMDS. Euclidean distance can handle negative values (BVOC uptake). The method section concerning the PERMANOVA has consequently been changed to match the reanalysis, and we have also added additional information of our approach to improve clarity of the analysis.

The reanalysis revealed that both fixed factors (Macrophyte and Region) significantly affected BVOC composition. The interaction (Macrophyte * Region) also had a significant effect. We have rewritten the results to convey these new results. The reanalysis also slightly changed the results of the pairwise comparison. While previously two pairs were not significantly different from each other, according to the reanalysis all pairs showed significantly different BVOC composition. This is in line with the NMDS, where each experimental group formed a distinct cluster.

The PERMANOVA results have been added the NMDS figure (Fig. 3).

- Also to illustrate your results in a very simple way, you could build Venn diagram that quickly highlights shared and unique compounds among treatments

Thank you for this nice suggestion. Instead of a Venn diagram we built an UpSet plot, that serves the same purpose but is better equipped to show differences between many groups (six groups here). The UpSet plot has been added to Fig 2.

Due to this new Figure, we realized that we had somehow miscalculated (at LINE 386) the number of compounds shared by all groups (previously reported as 32, in reality 31) and species (previously 72, in reality 70). Also, the numbers presented at Lines 403 & 516 were slightly incorrect. All these numbers have now been corrected in the manuscript. Luckily, the miscalculations were minor (likely had to do with improper accounting of unidentified compounds) and do not affect any of our conclusions.

- for the ANOVA on total BVOC emissions, I have the same issue than for the PERMANOVA. According to your experimental design, you should run a two-ways ANOVA with species and regions as factors followed by a posthoc test.

Again, we agree that the original analyses were flawed. Our data exhibited strong right-skewness (evident in Fig.4b & c) and unequal variance across groups, and we were not able to transform data to satisfy assumptions for a standard two-way ANOVA. Therefore, the data were analysed using a generalized linear model (GLM) with a Gamma distribution and log link, which appropriately accounts for right-skewed data and non-constant variance. Macrophyte species, Region, and their interaction were included as fixed effects. Model significance was assessed using analysis of deviance (χ^2 tests), and Tukey-adjusted pairwise comparisons were performed using estimated marginal means. The methods have been updated to reflect this new statistical approach.

The reanalysis offered increased detail in how macrophyte species, regions and their interaction affected emission rates (total, DMS and sesquiterpenes). The results of the pairwise analysis changed slightly for DMS and sesquiterpenes. We have rewritten the results-section based on the new statistical approach and newly found results. We have also updated the boxplots in Fig. 4 (previously Fig.3).

- usually, emissions unity is written with . instead of / like ng.g-1.h-1. That needs to be changed throughout the manuscript

Changed to ng g^{-1} h^{-1} throughout the manuscript.

- throughout the manuscript, I would rather write the real molecular formula than the protonated one.

Due to our untargeted approach, we cannot reliably designate the real molecular formula to majority of compounds. For instance, we detected dozens of compounds that likely are fragments of larger unidentified compounds. Certain well-studied compounds could with relatively good probability be assigned the real formula (e.g., DMS), but these are in minority. The PTR measures ions, and we believe our results are most accurate, transparent, and easy to understand when all detected compounds are presented as the ions by which they were detected. Therefore, we have decided not to change the chemical formulas.

- no physiological data was measured during this experiment that could better explain the differences obtain according to region?

No, unfortunately not. This manuscript presents data from our first exploratory experiment, which main purpose was to develop and test our ability to measure macrophyte BVOCs, and to acquire a first glimpse into macrophyte BVOC-data. Therefore, data beyond the VOC-emissions was not gathered.

- lines 486 - 495 => here, I don't think that the use of GC-MS instead of PTR-MS is the only reason that you detect more compounds in your study. We could add at least two other explanation: 1) the method used were not the same. You used a dynamic sampling method by adding an airflow into the system while it was not the case at least in Coquin et al. 2024 and Bravos Linares et al. 2010 (headspace + SPME) ; 2) you might have detected the emissions from epibiota (I don't think that only rinsing remove all the organisms from the leaves since some of them can be really well attached to it, see my comment above) while in Coquin et al. 2024 it was removed with a scalpel (not enough though to remove everything I think). This part need to be developed.

Thank you for these helpful suggestions. We have added a new point to the highlighted sentence (LINE 522):

3) the use of a dynamic sampling approach with continuous airflow may have enabled detection of a wider range of emitted compounds, compared with static methods (e.g., headspace SPME; Coquin et al., 2024)

We have also added a sentence about potential influence of epiphytes later in the discussion (LINE 644):

Finally, although the replicates primarily consisted of the target species in terms of biomass, we cannot exclude the possibility that some of the detected compounds were emitted by epiphytes (filamentous algae) growing on the macrophytes.