

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

REVIEWER 2

Main comment: This study presents the BVOC composition and emission rates of three marine macrophytes (*Fucus vesiculosus*, *Ulva intestinalis*, and *Zostera marina*) across two regions (Ireland and Finland) and in two seasons (spring and summer). This manuscript is overall well written and the discussion is solid. I suggest providing greater methodological detail (see specific comments below). Otherwise, I believe this manuscript provides valuable information on BVOC composition and emission rates from macrophytes, whose cover has declined over the past century. There is therefore a growing need to accumulate data from similar studies for inclusion in marine BVOC budgets.

We would like to thank the reviewer for their valuable feedback that has helped us improve the manuscript. We have responded to all the questions & concerns below.

Methods:

Line 192: I disagree with the term “treatment”. This is a species-region combination whereas a treatment implies active application or manipulation of the samples. Instead, I suggest using the term group/category/sample type throughout the manuscript.

We have replaced the term with *experimental group* throughout the manuscript.

Line 214: “kept submerged in mesh bags”. In situ water? At in situ seawater temperature?

We kept the macrophytes in the nearby sea, this is now clarified at **LINE 214:**

Macrophytes were collected in the morning of emission measurements and kept in mesh bags submerged in the sea prior to incubations.

Line 219: “4 m depth” for consistency with previous sentence.

Fixed.

I suggest changing the PTR-TOF acronym to PTR-TOF-MS throughout the manuscript. This is a Time-of-flight mass spectrometer, not just a “Time of flight”.

The suggested change has been applied throughout.

What was the Incubation flask made of?

The flask was made of borosilicate glass; this is now clarified in the text **LINE 268:**

Incubations were performed in a 5-liter borosilicate glass filtration flask (Schott Duran).

Line 266: Vocus Zero-air Generator?

Fixed.

In Figure 1, I also suggest changing the terminology to “VOC-free air generator” or “zero-air generator” as “clean air generator” doesn’t mean much.

Done.

Line 277: “the flask was filled with 4.5L of pre-bubbled filtered seawater, leaving 500 mL of headspace for BVOC measurements by PTR-TOF-MS.”

Thank you for pointing this out. The headspace was in fact 1L, because the flask has a neck that is not included into the volume for the normal use of the flask (filtration). We have clarified this:

LINE 280 *For the background measurements, the flask was filled with 4.5L of pre-bubbled filtered seawater, leaving 1L of headspace for BVOC measurements by PTR-TOF-MS (yielding a total of 5.5 L, as the neck volume is excluded from the nominal flask capacity).*

Line 282: how different was the Incubation temperature (20°C) from in situ temperature at collection site?

Clarified at **LINE 286:**

The seawater temperature in all incubations was ~20° C, similar to ambient seawater temperatures during both campaigns (see 2.1 Study regions).

Lines 282-283: does it mean that BVOC emissions were only measured once for 30min for each macrophyte replicate? Were replicates measured sequentially? Was it in replicate incubation flasks or in the same flask? If the latter, was the water changed in between incubations? – All these details need to be provided

Thank you for pointing out the lacking explanations. We have clarified our methodology:

LINE 215 *During both campaigns, we collected and measured all replicates (n=5) per species during the same day and all three species during the same week.*

LINE 288 *BVOC emissions from both background and macrophyte replicates were purged and measured continuously for 30 minutes. All replicates were measured in the same glass flask, which was rinsed twice with Milli-Q water between replicates and subsequently filled with fresh pre-bubbled filtered seawater.*

Lines 332-341: Since emission rates are expressed in ng BVOC/g DW/hr, it seems like a factor of 10^9 is missing in this formula to go from g to ng. This is probably implied, but it could be said explicitly.

Indeed, thank you for pointing this out. We have added a clarifying sentence to the calculation (LINE 348):

To express emission rates in $\text{ng g}^{-1} \text{h}^{-1}$, the calculated emission rates are multiplied by 10^9 to convert from grams to nanograms.

Also, the Nppb of 2.5×10^{10} molecules/cm³ assumes conditions of 25°C. This needs to be adjusted for the incubation temperature of 20°C (line 282).

According to our calculations 2.5×10^{10} molecules/cm³ is a fair value for 20°C and therefore we have not changed the emission rate calculations. Our value is based on the ideal gas law $pV = NkT$ which can be converted to $N/V = p/(kT)$, which gives that air, assuming it is an ideal gas at 20°C and 1013 mbar, has 2.504×10^{25} molec/m³ or 2.504×10^{19} molec/cm³. Lastly, 1 ppb means dividing by 1×10^9 , which results in the value 2.504×10^{10} molec/cm³.

Results:

See previous comment about the “treatment” terminology.

The term has been changed to *experimental group*.

I suggest referring to the figure in order. Therefore, Fig 2c should be swapped with Fig2a (and therefore Fig2a & b become b & c).

We have changed the composition of Figure 2 and added a new Figure 3 (ordination plots), but the panels are now in order of referring.

Line 372: “Only 32 compounds were measured in every treatment...” is confusing. Do you mean “32 compounds were consistently detected across all treatments”?

Yes, this was changed (LINE 386):

*Only 31 compounds were consistently detected across all groups (Macrophyte*Region), but 72 of the total compounds were emitted by each macrophyte species in at least one region.*

Line 377: It is an assumption that these compounds were only consumed. It could also be that consumption was greater than production in this particular context (i.e. one-off 1h measurements under light exposure). Also, is it clear that the presence of macrophytes led to a loss of these BVOCs or were these particular compounds also degrading in the background? If so, their decay could be a consequence of photochemical or chemical processes more than “consumption”. In the same way as you are talking about “emission”

(and not “production”), I would suggest talking about “uptake” or “loss/decay/degradation” rather than “consumption”.

We agree that we should be more conservative with our statement and have changed “consumption” to “uptake” throughout the manuscript. However, it is clear that the addition of macrophytes triggered the uptake. All instances we have labelled as uptake, followed the pattern shown in Fig 1c., where a stable background emission clearly reduced in size after macrophyte addition. With that being said, we cannot prove that macrophytes or associated organisms (microbes) actually took up the compounds or if some other process led to the reduced emissions.

Lines 412-413. Please refer to Fig. 3b and 3c when mentioning DMS and sesquiterpenes. I also suggest adding the molecules’ names (DMS and sesquiterpenes) on the y-axes of these 2 figures.

Done.

Lines 420-424: I suggest removing parenthesis after “were” and “by”.

Done.

Lines 424-426: Same here, I suggest replacing the parentheses after “compound” and “halocarbon” by a coma.

We removed the double parentheses, sentence now reads (LINE 448):

A nitrogen-containing compound (C₇H₁₂NO⁺, potentially 1-Azabicyclo[2.2.2]octan-3-one) and a volatile halocarbon-fragment (CHBr₂⁺) was also present in the highest quartile of emissions (Fig. 5).

Discussion:

General comment on VOC profile (interpreting the first paragraph of the Results section): there is a small, robust common core of VOCs (32), but each species has a much wider potential emissions profile (72+) that varies with regional context, suggesting strong environmental or biogeographic modulation of VOC emissions.

We added a sentence highlighting this observation to the first paragraph of the Discussion. The addition also functions as introduction for our later discussion, where these issues are discussed in more detail.

(LINE 476): *This suggests that macrophyte BVOC emissions are strongly influenced by environmental or biogeographic factors.*

Lines 463-465: It is important to precise that DMS is only one of the degradation products of DMSP as it is produced through the breakdown of DMSP by enzymatic cleavage (the breakdown of DMSP via the demethylation pathway leads to MeSH, which is in fact the dominant degradation pathway of DMSP). Isn't it possible that in this particular context (heat wave or geo-temporal context), the metabolic switch in DMSP degradation led to more DMS than MeSH (see Wang et al 2022 - Oxidative Stress Regulates a Pivotal Metabolic Switch in Dimethylsulfoniopropionate Degradation by the Marine Bacterium *Ruegeria pomeroyi*).

Thank you for this suggestion. We agree that our previous wording did not clearly convey that DMS is only one of several products formed during DMSP degradation. We have therefore revised the text to explicitly mention the demethylation pathway and the production of MeSH. Although the substantially higher DMS emissions relative to MeSH may suggest a greater contribution of DMS-producing pathways, our study was not designed to resolve the mechanisms regulating DMSP degradation. We therefore refrain from speculating about a potential metabolic switch or invoking mechanisms described for marine bacterial systems, as it is unknown how well these findings and processes translate to marine macrophytes.

The revised section now reads (LINE 488):

DMS is one of several products of dimethylsulfoniopropionate (DMSP) degradation. Specifically, DMS is formed through enzymatic cleavage of DMSP, whereas the demethylation pathway produces methanethiol (MeSH) (e.g., Curson et al., 2011). In our study, DMS emissions substantially exceeded MeSH emissions, with MeSH even exhibiting net uptake rather than emissions in three of the six experimental groups (Ulva FIN, Ulva IRL, and Zostera IRL; Table S2). DMSP and its degradation products play important roles in the physiology and stress responses of marine primary producers, including antioxidation and osmoregulation (Zhang et al., 2019 and references therein). Indeed, the role of DMSP as an osmolyte offers a potential explanation to why DMS emissions were lower in the brackish Baltic Sea compared to the fully saline Atlantic.

We have added Curson et al. 2011 to the Reference list.

Lines 541-542: Do the authors mean that they should have tried to mimic in situ temperature at collection sites instead of maintaining temperature the same in all incubations? It seems like maximum temperature was 22°C on the Finnish coast and 20°C at Mace Head, which doesn't seem like a massive difference for incubations conducted at 20°C. What was the in-situ temperature during collection though? If not that different, this comment could be removed and instead discussion could focus on temperature conditions prior to collection?

The purpose of the sentence is to highlight that the measured differences are likely not an artefact from the incubation setup. We believe the sentence in question makes a simple but important point and have rewritten the section to better convey our message (LINE 571):

Although light and temperature were kept comparable throughout all incubations and in situ seawater temperatures were broadly similar during the campaigns, differing seasons and the

markedly different annual temperature regimes in the two regions likely contributed to the observed differences in emissions.

Lines 546-548: the comment on whether BVOCs from macrophytes' flowers could attract pollinators sounds like a stretch when you are already trying to understand whether the intraspecific variation of macrophyte BVOC emissions is not related to seasonality or this specific heat wave. I understand mentioning growth processes, which are linked with seasonality, but I suggest removing this comment.

We rewrote the sentence and removed the two references from the manuscript. Sentence now reads (LINE 574):

For instance, in terrestrial plants, factors like flowering, leaf senescence, and dormancy affect BVOC emissions (Kesselmeier & Staudt, 1999), but how these processes affect BVOC emissions from macrophytes remains unknown.

4.3.1. Methodological insights and issues: I think it is important here to remind the reader that this study report one-off measurements that were only conducted over an hour period and under artificial light conditions. These measurements thus don't capture circadian variations in BVOC emissions from these organisms.

We have expanded on these issues in the discussion. The diel variability is mentioned at LINE 642:

Moreover, it is important to note that macrophyte BVOC emissions exhibit pronounced diel- and seasonal variability (e.g., Saunier et al., 2025b; Broadgate et al., 2004, Coquin et al., 2026). ...

The above-mentioned factors are not captured in our dataset and should be kept in mind when comparing results across studies.

Artificial light conditions now mentioned at LINE 653:

In this study, we removed the macrophytes from their natural habitat, which likely altered the BVOC-emissions of the organisms to some degree, e.g., due to increased stress, artificial light conditions or wounding.

Line 585: I suggest replacing “online measurements” by “real-time continuous measurements”.

Done.

Lines 594-596: here you could replace “online” and “offline” with “continuous” and “Time integrated” measurements, respectively.

Done.

Lines 601-602: The Vocus ToF MS allows for compound identification with high mass resolution. This is obviously not perfect, and for some isomeric compounds, you would benefit from coupling PTR-ToF-MS and GC measurements, but I see this argument as a positive point rather than a drawback. I suggest rewording as follows: “Although the PTR-TOF-MS allows for (list advantages)...this study could benefit from combining instrumental approaches...(develop).”

We have reworded the section and added information about the high mass resolution (LINE 629):

The PTR-TOF-MS also enables compound identification with very high mass resolution, allowing it to clearly distinguish between closely spaced peaks that may overlap when using other analytical methods. However, despite its many advantages, the methodology used here also has some drawbacks.

Line 606: “it is impossible to capture...”

Fixed.

This comment applies to PTR-MS measurements in general. The principle of Proton Transfer reaction remains the same for PTR-TOF-MS and PTR-quadrupole-MS.

Noted, sentence now reads (LINE 636):

For instance, with any PTR-MS, volatile halocarbons cannot be measured because their proton affinities are lower than that of water, which makes the ionization reaction thermodynamically unfavourable.

Lines 680-682: “...showcasing a chemical diversity that has rarely been reported in the marine realm.” This sentence is to be taken with a grain of salt as only few studies have looked at the full BVOC signature of marine species.

Our intention was not to suggest that the observed chemical diversity is extraordinary, but rather that macrophytes have very diverse emissions. We have revised the wording accordingly (LINE 711):

At the species-level, we found that the individual macrophytes emit hundreds of distinct compounds, revealing extensive chemical diversity of BVOC emissions.

Supplementary Material

Table S1 & S2: it is essential to add the ‘m/z’ to these tables

Added.

Excel “supporting information”: check this spreadsheet for typos (e.g. “potential”, “unidentified”)

Thank you for noticing; material checked and fixed.

Table S3: “protonated mass” is m/z. You could write it as “protonated mass (m/z)”

Done.

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 repartition. 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 $\text{ng g}^{-1} \text{h}^{-1}$ 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.