Author response for "**Multi-stress interaction effects on BVOC emission fingerprints from oak and beech: A cross-investigation using Machine Learning and Positive Matrix Factorization**", Dey et al.

*The authors would like to thank the editor and reviewers for taking the time to read and review the manuscript and for their constructive comments. The reviewer comments are included here in **black**, author responses are in **blue**, and modifications to the text in the revised manuscript are in **purple**. Line numbers in our response relate to the original submitted document (preprint).*

Reply to comments from Reviewer 1

The manuscript describes the effect of heat and elevated ozone exposure treatments on the BVOC emissions from one species of oak and one species of beech. The apply the treatments in series on the same set of individuals. The order of the stress treatment application is different for the two species and, as far as I can tell, they only have one set of experiments for each tree species. They use a couple different analytical tools to identify particular BVOCs that are associated with the stressor/species. The topic is timely and interesting since the effects of stress (including multiple interactive stressors) on BVOC emission rates and composition has been a major challenge in the research community for decades. The use of these new analytical tools is novel and could provide a roadmap for others in this field to pursue. However, there are some flaws in the experimental design and data visualization that need to be addressed. The flaws in the experimental design make it very challenging to use this data to draw many conclusions about stress effects on BVOC emission responses and I wonder if this would be more effectively framed as a proof-of-concept for the novel analytical methods employed – as a measurement techniques paper rather than a science paper. Furthermore, the introduction is missing critical information on a topic highly relevant to the study that needs to be included. I recommend major revisions before this could be accepted for publication and am not convinced that this is the correct journal for the work given the flaws in design.

Response: First, thank you for your time reviewing our work. We addressed all of your constructive suggestions and comments below with the necessary revisions throughout the manuscript.

MAJOR COMMENTS

Introduction – some of the citations in the introduction are not appropriately referenced. For example, they cite the Penuelas & Llusia paper from 2004 to state there are 30,000 identified BVOCs. However, the cited paper is not an original research publication but is actually more of an "opinion" or "letter" about a couple other recently published papers. In the "opinion" piece, the authors do state there are 30,000 identified compounds, but they provide no citation for this statement. I would encourage the authors to reference a paper with actual scientific evidence that supports the statement. Another example is their citation of the Palm et al., 2018 paper to state that hydroxyl radical and ozone are the dominant atmospheric oxidants that react with BVOCs. The referenced paper is about an OFR study conducted in the Amazon where they oxidize BVOCs with OH and/or ozone to study SOA formation; the study is not addressing any science question about which oxidants (out of all atmospheric oxidants) are primarily responsible for reacting with BVOCs. There are papers that address that question and those would be more appropriate to cite in this context. One could even just cite well-known atmospheric chemistry textbooks to make this statement, such as the Pitts & Pitts textbook or Seinfeld & Pandis' well-known reference book on atmospheric chemistry and physics. I will not go through each and every citation in the introduction, but there were a few that stood out to me as a red flag with this type of inappropriate referencing. Please double-check your citations.

Response: We agree with the referee that the statement by Penuelas & Llusia (2004) is just an estimate, although a reasonable one, given that in floral scent of 90 different plant families 1700 compounds had been identified in 2006 already (Knudsen and Gershenzon, 2006) – and modern techniques (e.g. 2D-GC-TOF) easily find thousands of peaks in one BVOC emission sample. However, we cannot find a more recent count of identified compounds, and have therefore revised the text to now say *"encompassing over 1700 identified organic compounds (Knudsen and Gershenzon, 2006)"*.

Line 45, to state that hydroxyl radicals and ozone are the dominant atmospheric oxidants that react with BVOCs, we have updated our reference to Finlayson-Pitts and Pitts (1997). We have also thoroughly revised our references throughout the manuscript, with changes shown more specifically below.

"The primary mechanisms of BVOC reactions involve the oxidation by hydroxyl radicals (OH) and ozone (O3) (Finlayson-Pitts and Pitts, 1997~~Palm et al., 2018~~)."

Introduction – references to the ecological function of terpenes as imparting thermotolerance through membrane stability should be considered carefully. The foundation for this assertion is grounded in old seminar papers out of Tom Sharkey's group, and he has recently published a paper now claiming that isoprene cannot possibly impart thermotolerance by stabilizing membranes because there just isn't enough of it present in membranes to appreciably alter membrane fluidity. However, he has also written a recent review that still claims this is a function of isoprene. Ultimately, it sounds like this is a slightly more controversial function than some of the many others that have more ample support. Either way, I encourage you to cite the more recent works with updated information. The paper stating that isoprene cannot possibly impart membrane stability is #1 below and the more recent review is #2 below.

#1 - https://link.springer.com/article/10.1007/s10863-015-9625-9

#2 - https://www.cell.com/trends/plant-science/abstract/S1360-1385(25)00132-3

Response: Thank you for pointing out this interesting and important detail. We have revised our statement in the manuscript as follows:

"As a defensive mechanism, plants increase isoprenoid production, and it has been proposed that plant thermotolerance can be enhanced by protecting photosynthetic apparatus (Sharkey, 2005). However, this mechanism remains debated: Harvey et al.(2015) showed that physiological concentrations of isoprene are likely too low to directly stabilize thylakoid membranes, while more recent work Zuo et al. (2025) suggests that isoprenoids may instead contribute to thermotolerance through signaling pathways (Ca^{2+}-mediated) that regulate stress-responsive proteins, maintain photosynthetic efficiency, and induce heat shock responses."

Introduction - This intro is missing a summary of the work that has already been done on multiple stressor effects, though. I agree it is more rare than studies of stressors in isolation, but there are some papers out there that are interesting and informative. Since the entire rationale for this study is filling knowledge gaps related to the effect of interacting stressors on BVOC emission rates and composition, it is absolutely critical to include a summary of those findings. There has been work in the Kuopio group (Holopainen and Blande) as well as the Sharkey lab on this topic. Please add this information to your introduction. In my opinion, this is much more important to include than a summary of the biochemistry. If you are worried about space, you could easily cut down the biochemistry review to a couple sentences and focus more on this information related to interacting stressors which is MUCH more relevant and useful. This information is particularly useful for thinking about how competing stressors could alter plant physiology. Essentially, stressors could be additive, one could dominate the response over the other, or there could be some non-linear synergistic effect of both stressors combined. Establishing this sort of framework for the study should be priority #1. Here is a non-comprehensive list of some papers that could be included in this summary and these papers likely have other references within to follow up with additional papers on the topic.

#1 - heat and CO2 combined: https://www.frontiersin.org/journals/forests-and-global-change/articles/10.3389/ffgc.2019.00008/full

#2 - plant responses to multiple air pollutants: https://onlinelibrary.wiley.com/doi/abs/10.1111/plb.12953

#3 - ozone + herbivory: https://royalsocietypublishing.org/doi/full/10.1098/rspb.2022.0963

Response: Thank you for this suggestion. We have added a discussion outlining established interaction frameworks from recent studies, and hope these now better frame our hypotheses.

"Multiple abiotic and biotic stressors can interact in additive, antagonistic, or synergistic ways, modifying plant physiological processes and BVOC composition beyond single-stressor expectations. For instance, Lantz et al. (2019) have shown that the combined effects of elevated temperature and CO_2 on isoprene emission are highly interactive rather than independent: temperature exerts a dominant influence on emission rates, whereas elevated CO_2 can suppress isoprene production even in the absence of triose phosphate utilization limitation. Likewise, exposure to multiple air pollutants or concurrent abiotic–biotic stresses (e.g., O_3 × herbivory) can trigger complex, non-linear responses that may enhance defensive signaling, alter stomatal conductance, and consequently modify volatile uptake and emission dynamics (Papazian and Blande, 2020; Yu et al., 2022). The overall impact of stress-induced changes in BVOC emissions still remains elusive, specifically under multiple stressors (Yang et al., 2025), as the effect of blending two stressors, like heat + O_3 or O_3 + elevated CO_2 is not well-understood (Holopainen et al., 2018), and responses may vary between species, as we discussed."

Figure 1 – First, I really like this figure for describing the experimental design. Very clear and well done. However, I do have some questions about the experimental design. The figure indicates that beech were exposed to ozone stress, then the combined stress, and then just the heat stress while oak had a different sequence (ozone then heat and then combined). It also appears that these stress exposures were conducted just once for each of the tree species (again, with a different sequence of stressors for the different species). Finally, the series of stress treatments appears to have been imposed on the same set of individuals, correct? If all of this is correct, can you comment on the decision to implement these stressors in sequence rather than using new individuals for each of the different treatments? It is a non-traditional approach to this type of study and makes it difficult to interpret results since prior stress exposure can have lingering effects on subsequent stress responses. Furthermore, the decision to change the order of the stressors for the different plant species is incredibly problematic for making comparisons about stress response between the species. I think this design needs some additional context because it is not only studying the impact of combined stressors. It is studying the impact of repeated exposures to different stressors which is a different question than was suggested in the introduction. Again, the authors should conduct a thorough literature review on the topic of repeated stress exposures to provide adequate context for how this would be expected to influence plant responses. It is unclear to me how you would even tease apart any differences in the responses observed between the two different species. Any effect could be related to differences in species-specific responses OR it could be related to the effect of the stress sequence. You won't know!

Response: Thanks for the compliment. Yes, you are correct, we applied the sequence of stress treatments on the same set of beech and oak individuals. While we agree this is a non-traditional experimental design for this type of stress study, our aim was to simulate a more natural phenomenon, where trees in the same landscape may experience heatwaves and ozone pollution either simultaneously or sequentially. We acknowledge that this approach introduces the possibility of lingering effects from previous stress exposures, and we have explicitly noted this as a caveat in the revised version.

Regarding the stress order, we recognize that it differed between the two species. However, despite oak being an isoprene emitter and beech a non-isoprene emitter, both species showed similar responses (in pattern) to heat and ozone stress, although with different magnitudes (Fig.3). In oak, heat was applied after ozone, while in beech, heat followed the combined stress, yet the main stress-induced emission patterns were consistent across species. We have clarified these points in the manuscript (in section 3.2).

" However, despite oak being an isoprene emitter and beech a non-isoprene emitter, both species showed similar responses (in pattern or diel variation) to heat and ozone stress, although with different magnitudes (Fig.3). In oak, heat was applied after ozone, while in beech, heat followed the combined stress, yet the main stress-induced emission patterns were consistent across species."

In the revised manuscript, we changed text to state explicitly that the study investigates sequential stress exposures rather than only isolated or simultaneous ones, with a literature discussion on repetitive stress exposures.

In introduction: "We applied stressors sequentially on the same individuals to simulate realistic environmental stress storylines."

In methods: "The stress treatments were applied sequentially on the same set of individuals to simulate realistic environmental scenarios ("storylines"). This experimental approach was designed to reflect natural conditions, where trees in the same landscape may experience heatwaves and ozone pollution either simultaneously, sequentially, or in varying order. In addition, previous studies (An et al., 2024; He et al., 2022; Musselman and Minnick, 2000), have reported that certain areas can experience relatively high ozone concentrations at night; therefore, ozone stress was also applied nocturnally to assess the influence of nighttime ozone exposure. Plants can also be more susceptible to ozone stress at night than during the daytime (Musselman and Minnick, 2000)."

In results: "Implementing stress sequentially on the same individuals may have carry-over or "lingering" effects from prior stress (Kleist et al. 2012) and represents as such a realistic scenario that a tree may experience in an ecosystem. However, this non-traditional approach makes the results of each stress scenario linked to the previous sequence and thus not generalizable on their own. Recent studies showed that repeated or sequential stress exposure can induce a form of physiological stress memory, wherein plants retain molecular or metabolic imprints that influence subsequent responses (Fleta-Soriano and Munné-Bosch, 2016; Liu et al., 2022). Such memory arises through transient chromatin modifications, persistent activation of defense-related genes, and metabolic reprogramming that can enhance or attenuate volatile production upon re-exposure (Ding et al., 2012; Xin and Browse, 2000). For instance, Blande et al. (2014) highlighted that prior oxidative or thermal stress may reallocate carbon and energy resources, altering precursor availability for VOC synthesis, leading to reduced emissions under prolonged exposure but more rapid or efficient activation during mild re-exposure.

Line 149 – authors state that they selected the six "healthiest" individuals from each set of plants they had. Please elaborate on how this was determined. "healthiest" as determined by what metric?

Response: By "healthiest," we refer to individuals that were disease-free, showed no visible leaf damage or discoloration, had straight stems, and exhibited overall vigorous growth (uniform leaf development and no signs of pest infestation) that we mentioned in line 138.

"Before the experiment, 24 saplings of ~1.4 m in height of beech (12 individuals) and oaks (12 individuals) were selected under conditions ensuring they were disease- and pest-free, had straight stems, and were overall healthy."

Line 155 – some papers suggest that ozone exposure stress responses can recover quite quickly, even within 24 hours. This is another example where having conducted a more thorough literature review on the topic could have informed an improved experimental design. I understand the issue about not wanting to include reaction products in the measurement of emissions, but this is often why it is necessary to have two separate chambers – one chamber for the ozone exposure and a separate one for the BVOC measurement. At the very least, you should discuss the possibility that the ozone response could be missed with this design if the plants recovered quickly.

Response: Yes, you are right, several studies have shown that plants can recover from ozone stress within 24–72 hours, depending on the species (Kanagendran et al., 2018; Velikova et al., 2005), and that stomatal uptake is reduced during the night. We acknowledge that this recovery potential could influence the interpretation of our results, as transient responses might have been missed due to the sequential stress design. However, this gave us the opportunity to assess the influence of nocturnal ozone exposure, a condition increasingly observed in certain polluted regions, on subsequent daytime BVOC emissions and to understand how such nighttime ozone events interact with heat stress under realistic environmental scenarios. We have now discussed this in the revised Discussion, along with suggestions for future studies to include independent exposure and measurement chambers to better capture transient ozone responses.

"The VOC response to ozone alone was relatively low compared to heat or combined stress in both species, likely due to limited stomatal uptake during nighttime exposure, resulting in a weaker trigger of VOC biosynthesis (Table 1), unless combined with additional stressors (e.g., heat). Several studies have shown that plants can recover from ozone stress within 24–72 hours, depending on the species (Kanagendran et al., 2018; Velikova et al., 2005). While this recovery potential may have moderated the observed ozone response, it also provided an opportunity to capture more ecologically realistic post-exposure dynamics."

We have also updated the text to specify "nighttime ozone stress" instead of "ozone stress" in several places.

Line 176 – More recent papers on BVOC emissions typically refer to the measurement as an emission rate measurement rather than a "flux." I think this vocabulary has changed a bit over the decades with the massive expansion of the flux research community. The term, flux, is now often associated with eddy covariance measurements at much larger scales than the leaf, branch, or even a few individuals (as you have here). I understand this is just semantics, but it is something to think about when communicating your science because the term, flux, could be confusing in this context to some of your intended audience.

Response: We have replaced the term "flux" with "emission rate" throughout the manuscript to ensure clarity and consistency.

Line 218-219 – what are these "known contaminant" compounds that you eliminated from analysis?

Response: The known contaminants are mainly siloxanes, which are degradation products from the capillary column. We also removed phthalates since they are used widely in plastics and could have been derived from the storage or transport of the tubes.

"Contaminant compounds that are known to arise from plastics (e.g., phthalates) from storage or transport or from column degradation (siloxanes), were excluded from the dataset"

Line 250 – this line raised a red flag...it reads that the decision was somewhat subjective if something was retained. Perhaps a little more detail on how something could be determined to be "biologically" justified would improve the rationale.

Response: To clarify, compounds showing absolute correlations greater than 0.9 were carefully examined to determine whether the correlation reflected true co-emission or an analytical artifact. For instance, isoprene ($C_5H_9^+$) correlated with an r^2 of 0.93 with $C_3H_5^+$, which represents a known fragment ion rather than a distinct compound. In such cases, the correlated feature was removed. We have clarified this explanation in the revised Methods section.

"Subsequently, correlation was checked between the features (by Pearson correlation). Features showing absolute correlation coefficients greater than 90% were flagged and reviewed individually. Known fragment or water cluster ions were removed. For instance, isoprene ($C_5H_9^+$) correlated with an r^2 of 0.93 with $C_3H_5^+$, which represents a known fragment ion rather than a distinct compound. Subsequently, a logarithmic transformation was applied to reduce skewness, scale down extreme flux magnitudes, and improve distribution symmetry."

Figure 2 - I am struggling with this figure. I think it is trying to do too much. There are a couple different questions one could ask that I think would benefit from separate figures. The first is related to a comparison of the pre-stress emission rates of different types of compound classes and/or the effect of the stressor on the actual emission rate values of different BVOCs. The second question is related to comparisons in the diel profile. The latter seems to be the focus of the text discussion about this figure, but it is not the clearest way to visualize this. One could normalize the emission to maximum and then plot multiple curves on the same graph to more effectively make these comparisons. Other criticisms include - the shaded region is very difficult to see and the shaded regions for "std deviation" are also often very difficult to see. I think Figure 3 actually works great for addressing question 1 in my comment. So I also think Figure 2 could be normalized to "max emission" set to a value of 1 to compare the diel trends between the different tree species and treatments. It would be more effective as a visualization to address that particular question. And I would probably also present this figure 3 first followed by the diel trend info as a more logical flow of information.

Response: We appreciate your suggestions regarding Figure 2. We understand that the original version may have been difficult to interpret. Based on your comments, we normalized the emission data to the maximum emission value to better visualize diel trends across species and treatments (see current document Fig. R1.1). However, we found that this normalization did not substantially improve overall clarity or interpretability. As an alternative, we enhanced the visibility of the shaded standard deviation regions to make variability more apparent (see current document Fig. R1.2). We now add the normalized figure (here R. 1.1) to the Supplement, and keep the updated Fig. R.1.2 in the main manuscript.

We also agree with your suggestion regarding figure order and have rearranged the figures so that the comparison of absolute emission rates (previously Fig. 3) is presented before the diel emission trends, which we believe provides a more logical flow of information.

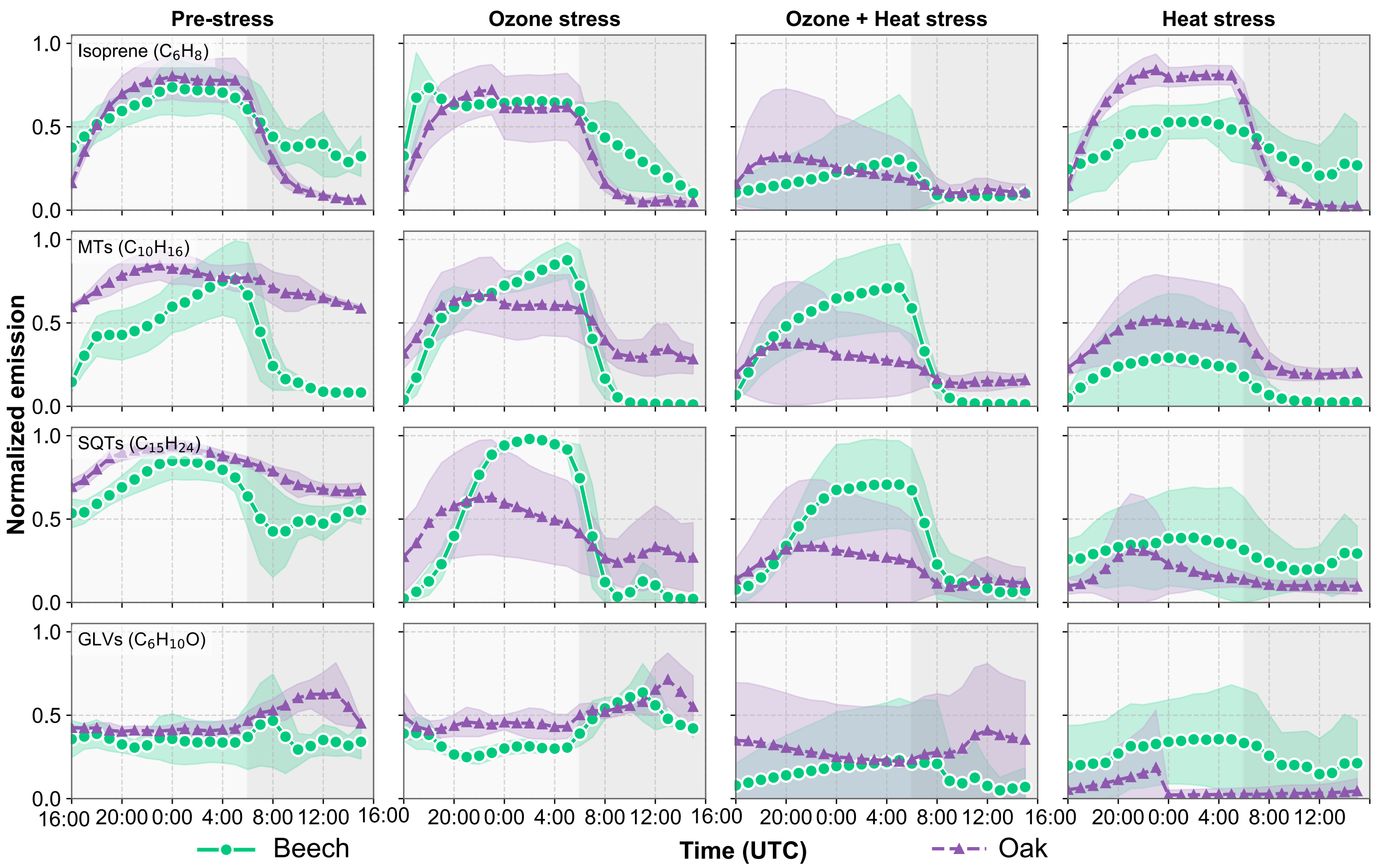


Figure R1.1 Diel variation in isoprene, monoterpene (MTs), sesquiterpene (SQTs), and green leaf volatile (GLVs) emissions from beech (a,c,e,g) and oak (b,d,f,h) under four conditions: pre-stress, O_3 stress, heat stress and O_3 + heat. On the x-axis, the unshaded region (16:00–6:00 UTC) corresponds to the plants' daytime (lights on), while the grey shaded region represents the plants' nighttime (lights off). Data points are normalized diel averages and shaded areas around them represent the standard deviation.

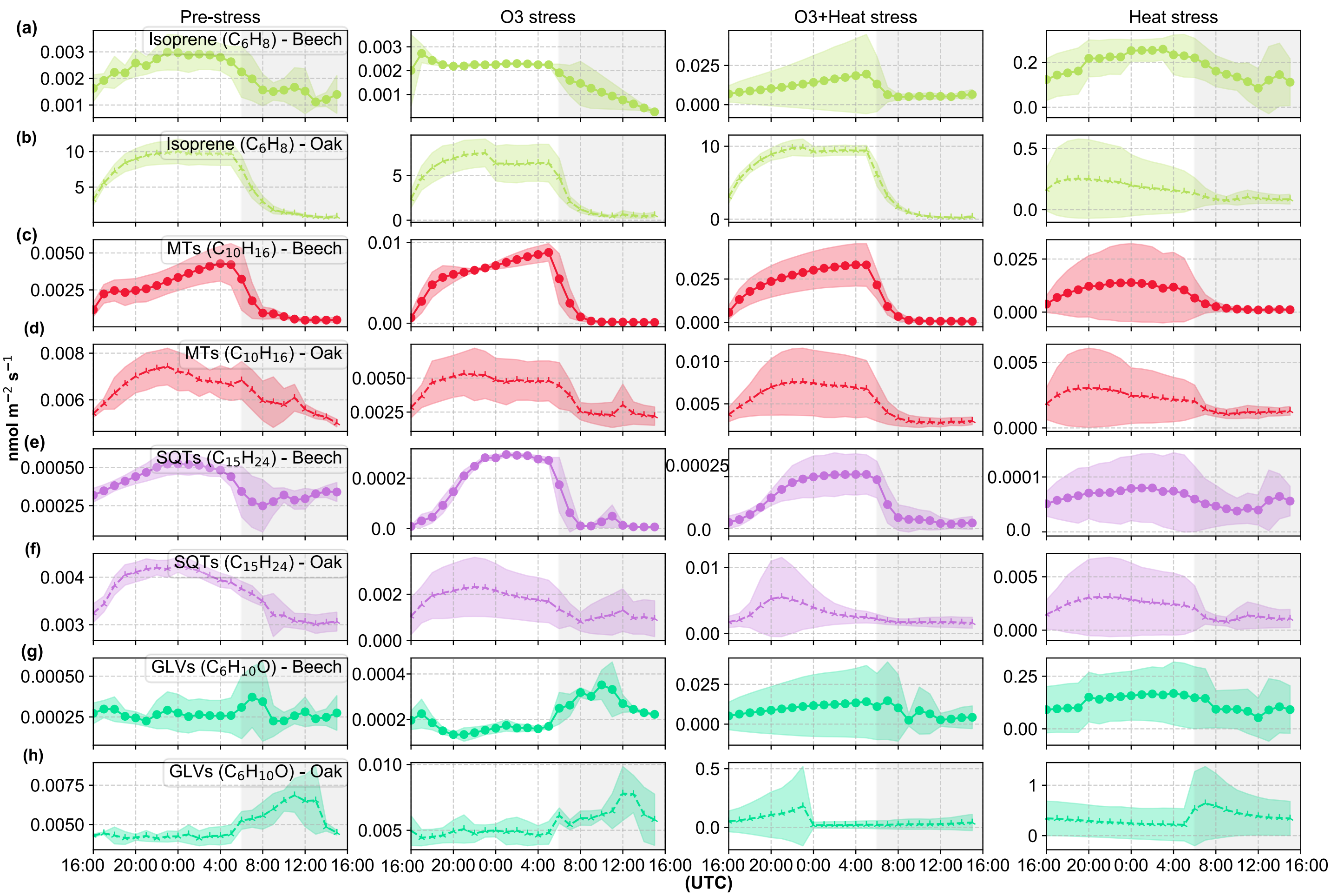


Figure R1.2 Diel variation in isoprene, monoterpene (MTs), sesquiterpene (SQTs), and green leaf volatile (GLVs) emissions from beech (a,c,e,g) and oak (b,d,f,h) under four conditions: pre-stress, O_3 stress, heat stress and O_3 + heat. On the x-axis, the unshaded region (16:00–6:00 UTC) corresponds to the plants' daytime (lights on), while the grey shaded region represents the plants' nighttime (lights off). Data points are diel averages and shaded areas around them represent the standard deviation. A version of this figure that is normalized to the maximum diel emission is presented in the Supplement (Fig. S 7).

Table 1 - This would be a more effective visualization as a figure. Alternatively, some shading of the boxes could help as well - one color for increase and another color for decrease. Otherwise, it is difficult to pull out clear patterns from a table of numbers.

Response: In the revised version, we have reformatted Table 1 into a figure using color shading to indicate the direction and magnitude of change (i.e., one color for increases and another for decreases).

"Table 1 Percentage change in emission fluxes of volatile organic compounds under four biosynthetic pathways, MEP (Methylerythritol Phosphate), LOX (Lipoxygenase), SKP (Shikimate), and MVA (Mevalonate) in beech and oak for three stress conditions. Arrows (▲/▼) indicate increases or decreases in emission relative to the pre-stress baseline (set at 0%). Superscript letters (a–d) denote statistically significant differences within each row, with different letters indicating significant variation among stress events ($p < 0.05$).

	Beech	**Oak**

	Pre-Stress	Ozone Stress	Combined Stress	Heat Stress	Pre-Stress	Ozone Stress	Heat Stress	Combined Stress
MEP	0% [a]	▲55%[b]	▲601% [c]	▲4049% [d]	0% [a]	▼28% [b]	▼18% [b]	▼97% [c]
LOX	0% [a]	▼37% [a]	▲1565% [b]	▲25437% [c]	0% [a]	▼3% [a]	▲4839% [b]	▲2639% [b]
SKP	0% [a]	▲1% [a]	▲30% [c]	▲812% [b]	0% [a]	▼36% [b]	▲373% [c]	▲254% [d]
MVA	0% [a]	▼78% [b]	▲104% [c]	▲4171% [d]	0% [a]	▼57% [b]	▲98% [c]	▲73% [d]

“

Line 389 – Yes, conducting the ozone exposure in the dark when stomatal uptake is substantially reduced does seem to be a major flaw in experimental design. Also, ozone levels tend to be highest in the afternoon so it is a mismatch between a real-world context of what the plants would experience and how the treatment was applied during the experiment.

Response: We acknowledge that daytime ozone typically peaks in the afternoon (due to active photochemistry). However, several studies have reported nocturnal ozone enhancement (Musselman & Minnick, 2000; Cheng et al., 2022; An et al., 2024), where elevated ozone concentrations persist or even increase at night, particularly in areas influenced by residual layer mixing or weak boundary-layer dynamics (An et al., 2024). Especially mountainous areas can suffer from elevated nighttime ozone (Musselman & Minnick, 2000) – and in Germany, most remaining forests are in mountainous areas, as most lowlands are used by agriculture or settlements. These nighttime ozone events can result in considerable ozone flux into leaves, as stomatal conductance, although reduced, is not negligible at night (Musselman & Minnick, 2000), especially in species or ecosystems maintaining partial nocturnal stomatal opening (An et al., 2024; Musselman & Minnick, 2000). Our main goal with the experimental design was to avoid ozone reaction with emitted BVOCs, but also allowed us to study such realistic nocturnal ozone scenarios (we now make this clear in the Methods in the revised version). Moreover, plants may show lower defensive capacity at night, making them more susceptible to oxidative stress (which we also observed in our experiment) (Musselman & Minnick, 2000). Therefore, the nocturnal ozone treatment explores the carry-over and delayed daytime emission responses following nighttime exposure, which may differ mechanistically from acute daytime ozone stress.

However, we still agree that certain limitations exist in our experimental design, as it is not feasible to address all uncertainties within a single study. We have now clearly acknowledged these aspects as caveats in the revised manuscript (in conclusion), and future research can further explore these processes from complementary perspectives.

“**In methods:** The stress treatments were applied sequentially on the same set of individuals to simulate realistic environmental scenarios (“storylines”). This experimental approach was designed to reflect natural conditions, where trees in the same landscape may experience heatwaves and ozone pollution either simultaneously, sequentially, or in varying order.

… ozone was (except for two days for the oak experiment, Fig. 1c) applied during the night cycle. The reason for this approach was twofold: On the one hand, this approach avoided reactions of emitted terpenoids with ozone during the day, when emissions are highest, which would interfere with quantifying primary BVOC emissions because it would produce oxygenated VOC products. For later analysis of BVOC emissions we excluded data from nights and from the two days when ozone was applied, to avoid ozone impacts on the observed VOCs. In addition, this allowed us to study the understudied phenomenon of nighttime ozone exposure of trees. Previous studies (e.g. An et al., 2024; He et al., 2022; Musselman and Minnick, 2000), have reported that certain areas can experience relatively high ozone concentrations at night, while plants can be more susceptible to ozone stress at night than during the daytime because they have lower defenses at night (Musselman and Minnick, 2000).”

Section 3.1 : "The VOC response to nighttime ozone alone was relatively low compared to heat or combined stress in both species, likely due to limited stomatal uptake during nighttime exposure, resulting in a weaker trigger of VOC biosynthesis (Table 1), unless combined with additional stressors (e.g., heat). Several studies have shown that plants can recover from ozone stress within 24–72 hours, depending on the species (Kanagendran et al., 2018; Velikova et al., 2005). While this recovery potential may have moderated the observed ozone response, it also provided an opportunity to capture ecologically realistic post-exposure dynamics. The exposure to nocturnal ozone reflects ecologically relevant conditions, as recent studies have reported frequent nocturnal ozone events, where ozone concentrations remain elevated or even increase at night due to residual layer mixing and limited nighttime deposition (Musselman & Minnick, 2000; An et al., 2024), especially in mountainous areas such as most of German forests. Although stomatal conductance is generally lower at night, it is not negligible, and nocturnal ozone flux into leaves can still occur, potentially leading to oxidative stress when plant defense capacity is reduced (Musselman & Minnick, 2000). It has been reported that trees in regions with high ozone levels can have stomata open at night (Caird et al., 2007)."

Line 401-402 – you do not have the observations to support this statement. Just because the heat stress had the largest effect on both plant species does not mean the plant would have responded to the combined stress differently if the stressors were imposed in a different order. You have ONE set of experiments for each of the plant species and they had a different sequence of stress exposures. You have no idea if the response to heat or combined stress would have been different if the sequence was altered.

Response: Thanks for the critical observation, we fully agree that our current experimental setup and outcomes does not allow disentangling sequence-specific effects from the overall stress responses. Indeed, because each species experienced the stressors in a different order, we cannot conclusively determine how reversing the sequence would have influenced the magnitude or direction of the responses. Our intention, however, was not to infer order-specific causalities, but rather to assess how repeated and combined stress exposures (we acknowledge that this was not clearly stated in the earlier version), as they may naturally occur in forest environments, affect VOC emission patterns. Now, we included those issues as a caveat. Future studies using factorial or randomized stress sequences will be needed to fully resolve the role of exposure order in multi-stress responses.

"Additionally, since stress treatments were applied sequentially and in different orders for the two species, sequence-specific effects cannot be separated from overall stress responses. The outcomes thus represent species-specific responses under the applied sequences rather than generalizable effects of stress order. Since the duration and intensity of stress influence how much it changes plant emissions, our results may not be generalizable for all ozone, heat, and O_3 + heat situations. Future research could validate our findings through integrative approaches (including factorial or randomized stress sequences,…"

Line 410 – I don't understand this unit, "per square millisecond?" I think you need to add a space between the m and the s.

Response: We have now corrected the notation to m s^{-2} .

Figure 4 – Is this mass-based or mole-based? I think it is molar based on Figure 5, but it should be clarified in the Figure 4 caption as well.

Response: Thank you for pointing this out. The data presented in Figure 4 are indeed mole-based. We have clarified this in the revised figure caption.

Section 3.4 - This is a relatively new tool for this particular field so I think you should provide a bit more explanation about what these different metrics mean and how the reader should be interpreting these figures. Very little text is devoted to explaining how to interpret Figure 6, for example. Elaborate please.

Response: Thanks for the suggestion. In the revised version, we have substantially expanded Section 3.4 to explain the meaning and interpretation of the key evaluation metrics and visualization components presented in Figure 6. We also included it here:

The classification matrix for all classes from the trained random forest model for beech and oak shows precision and recall of 0.95 to 1.0 (Figs. 6a-b). It compares predicted versus actual stress categories, where the diagonal elements represent correct predictions (true positives) and off-diagonal elements indicate misclassifications. Precision reflects how many samples predicted for a class were correct, recall measures how many true samples of that class were correctly identified, and the F1-score combines both into a balanced accuracy measure. From the matrix, it is clear that the model can effectively discriminate between the different stress.

Model evaluation was not restricted to standard classification metrics but was extended to explore the classification's consistency, reliability, and uncertainty. Shannon Entropy (uncertainty in prediction) was used to quantify the classification confidence (Figs. 6c–d). Entropy values close to 0 indicate that the model made confident predictions (i.e., one class strongly dominated the probability distribution), whereas higher entropy values reflect greater uncertainty between classes. Most samples showed low entropy values, indicating that the classifier was highly confident across most conditions. A smaller number of predictions have moderate to high entropy (but less than the threshold).

To further assess the relationship between uncertainty and misclassification, entropy distributions were compared between correctly and incorrectly classified samples (Figs. 6e–f). Incorrect predictions were generally associated with comparatively high entropy, confirming that the entropy well captured classification uncertainty rather than random variability. Also, no random entropy spikes were observed across conditions, supporting the model's stability. In addition, the time series entropy (see Figs. S5(d) and S6(d)) showed that most classifications were made with high certainty (entropy < 0.6), though slight increases occurred under combined O_3 + heat stress, potentially reflecting overlapping BVOC patterns and the model's sensitivity to complex stress signals. Performance stability was also checked across classes; bootstrapped distributions of classification scores with low variance (Figs. S5(e-f), S6(e-f)) indicate the model's consistency. These evaluations confirmed that trained models are useful in classifying stress types.

The UpSet plots (Figs. 6g–h) show the dominant BVOC fingerprints (SHAP-derived compounds) that contributed most strongly to classifying each stress type and shared or overlapping compounds between stresses. For example, certain VOC features appeared across both O_3 and combined O_3 + heat stress, suggesting common biochemical pathways or coordinated defense mechanisms.

Line 524-527 - It would be useful to know how much of the total signal these identified "Stress markers" contributed. Are they still tiny components of the overall composition, which would make them difficult to use as an ambient measurement marker of plant stress? Or did they contribute to a substantial portion of the total signal following the stress exposure?

Response: In our dataset, ~21 out of ~82 VOC species were identified as stress markers based on their stress-specific emission patterns. These compounds together accounted for roughly 20–25% of the total emission signal. They are not minor trace components but constitute a substantial fraction of the overall BVOC composition, and these compounds could serve as detectable and meaningful markers for plant stress in ambient measurement applications.

"A limited number of BVOC (20–25% of the total emission signal) were unique to individual stress conditions among the top 15 stress fingerprint compounds in both species."

Line 529 - why are you referencing the Figure 6 Upset plot after discussing Figure 7? This was ordered in a confusing way. Perhaps this would be resolved with some additional discussion about Figure 6 before moving on to Figure 7.

Response: We revised that and elaborated on the discussion regarding Fig.6 before moving to Fig.7.

"~~The interaction was checked to identify stress-specific and their BVOC overlaps across classes.~~ A limited number of BVOC (20–25% of the total emission signal) ~~(Fig. 6g-h)~~ were unique to individual stress conditions among the top 15 BVOC features in both species."

365 Figures 8-9: These figures are really messy and need to be cleaned up. I think you could move the mass spectra of the individual factors to the supplement and focus on the more meaningful info for the context of this study, which is the compounds that comprise the different factors (shown in C). I don't understand what the inset is showing in C, though. Please make that clearer.

Response: Based on your and reviewer 2's suggestions, we revised Figures 8 and 9. Here is our updated version.

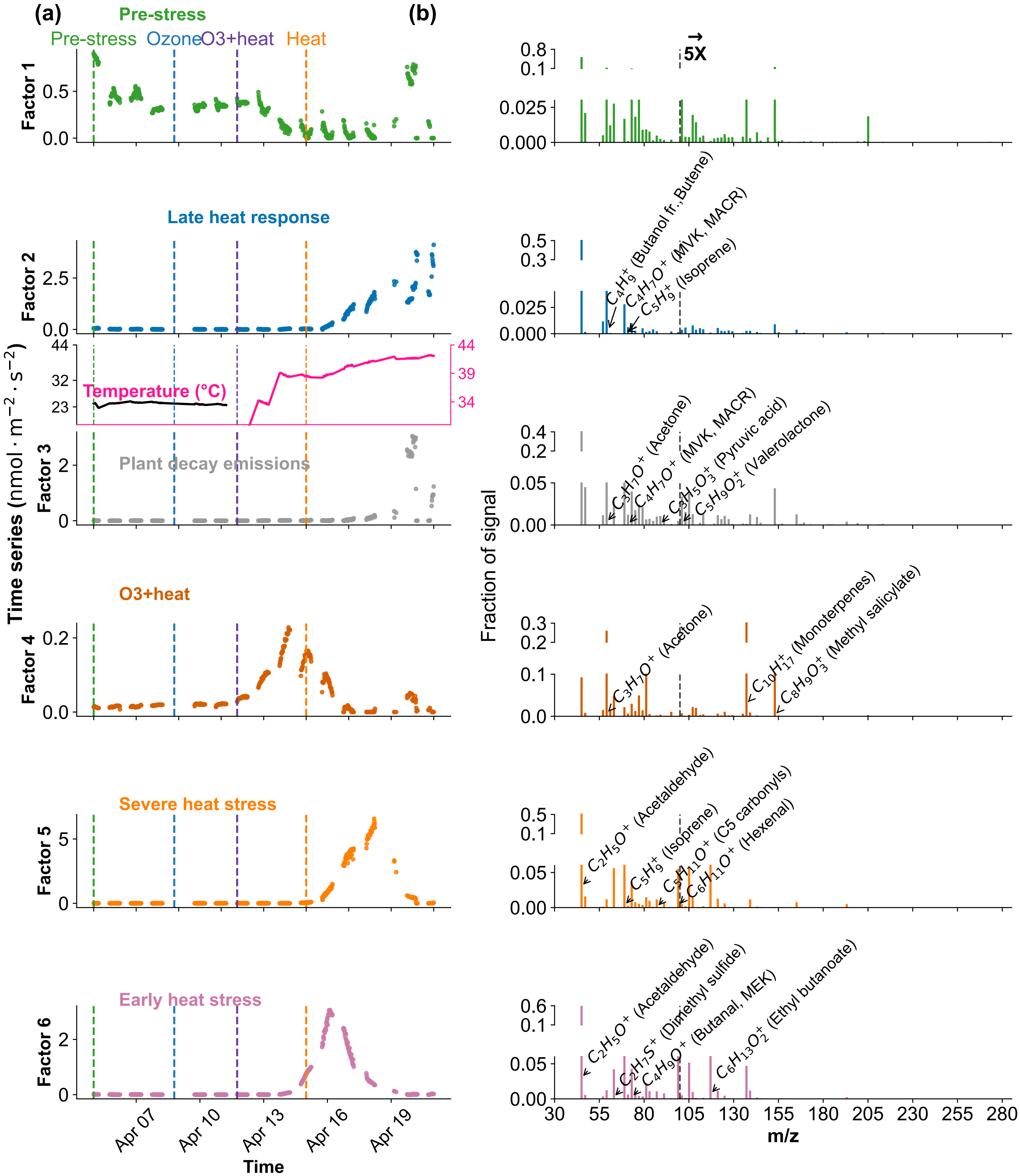
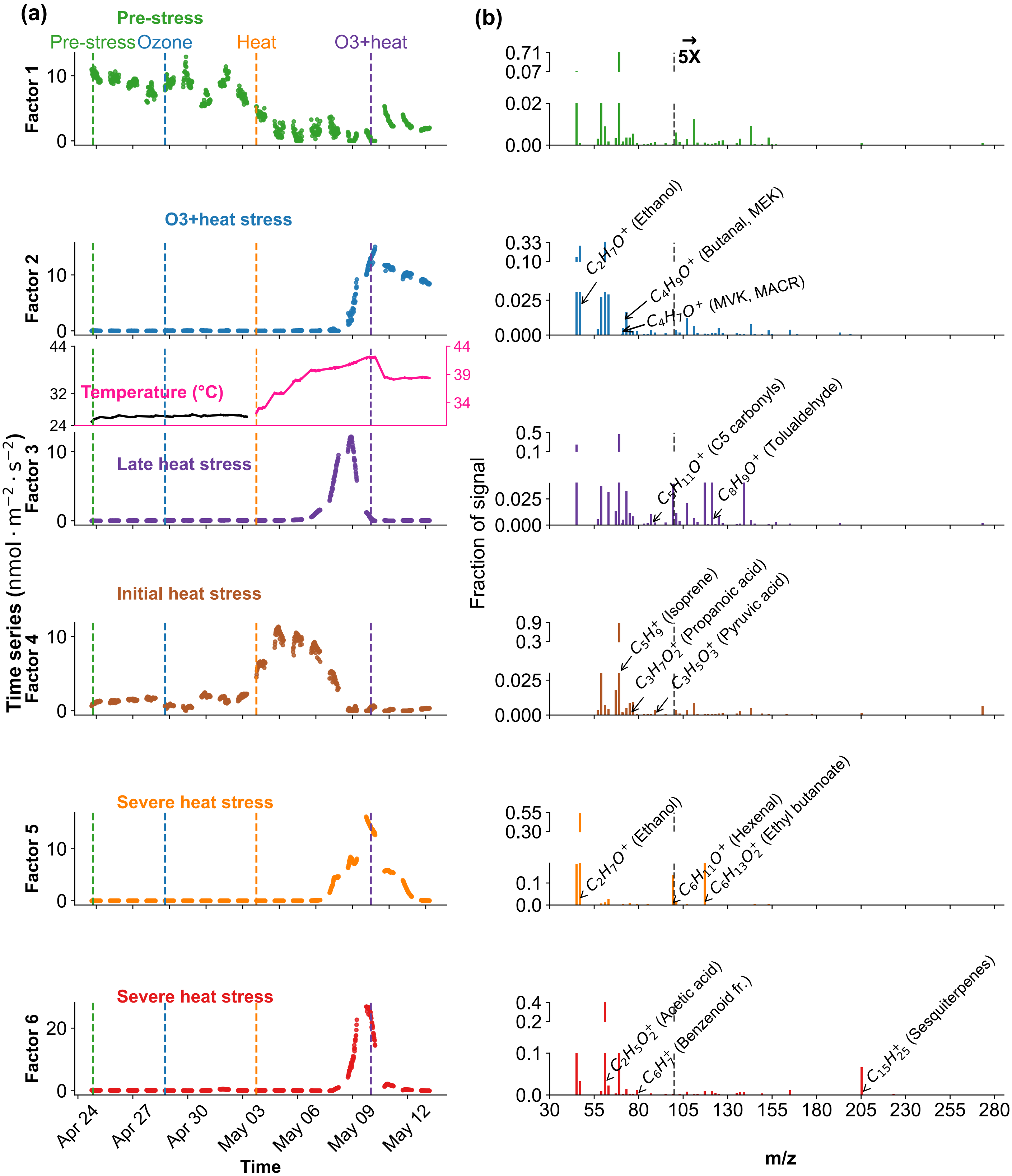


Figure 8. Positive Matrix Factorization (PMF) analysis of VOC emission profiles from beech under different environmental stress conditions. (a) Time series of a six-factor PMF solution. Colored vertical dashed lines indicate the starting of different stress phases. (b) Corresponding mass spectra (m/z profiles) of each factor and their relative signal contributions, m/z 100–280 are scaled by a factor of 5.



375

Figure 9. Positive Matrix Factorization (PMF) analysis of VOC profiles in oak under different environmental stress conditions. (a) Time series of a six-factor PMF solution. Colored vertical dashed lines indicate the starting of different stress phases. (b) Corresponding mass spectra (m/z profiles) of each factor and their relative signal contributions, m/z 100–280 are scaled by a factor of 5.

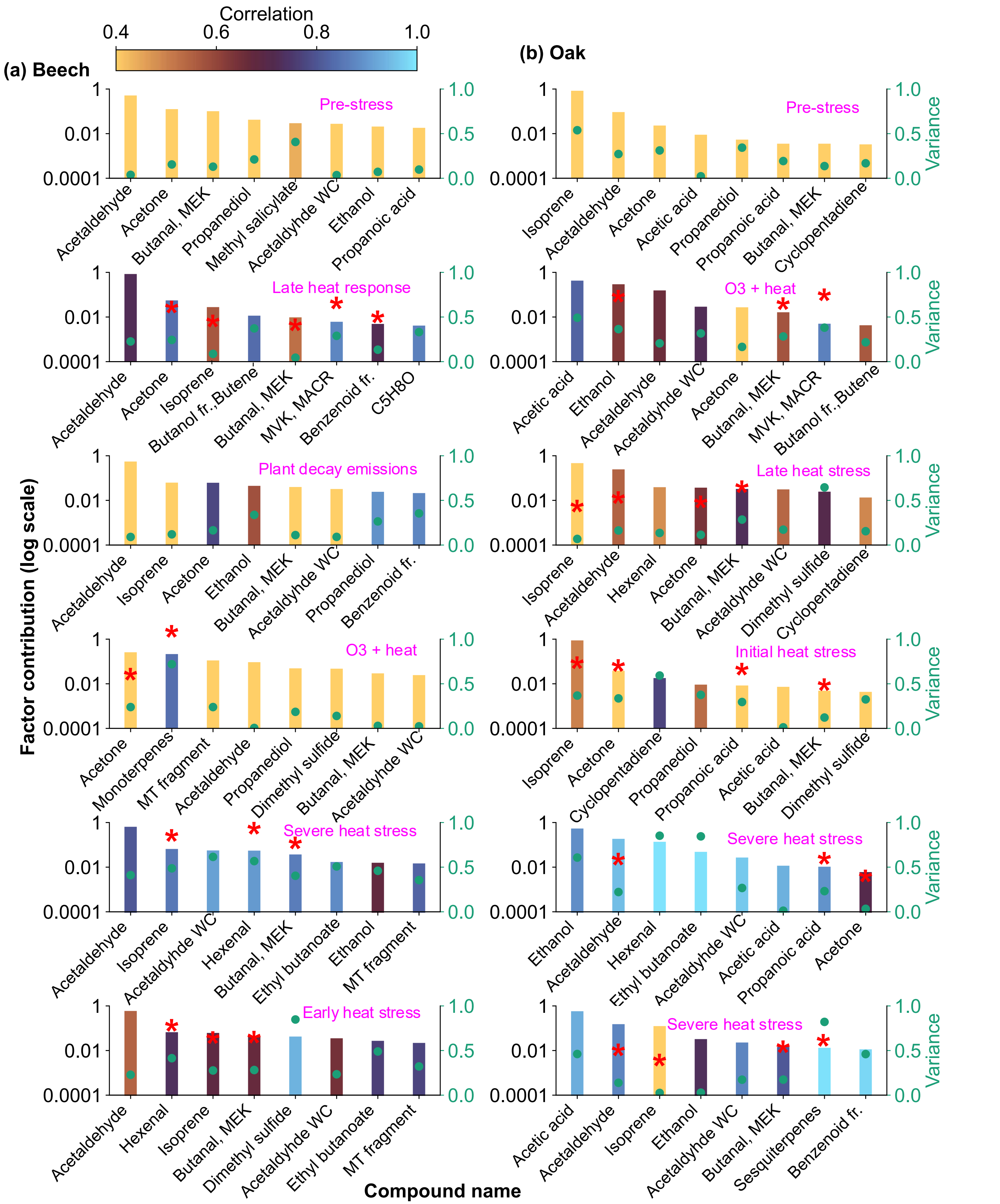


Figure 10. Top 8 stress-BVOC markers for each factor for (a) Beech and (b) Oak. Bar plots show the relative contribution of specific compounds, while green dots represent the correlation coefficient with the respective factor time series. Color

shading indicates their correlation with the corresponding factor timeseries, and asterisks (*) denote compounds that were also identified as fingerprints by the machine learning.

MINOR COMMENTS

Line 159: Do these “heat stress” values represent something meaningful? Values reached during some typical heatwave or something? Please provide additional context.

Response: The applied heat stress values (e.g; ~38 °C) were selected to simulate realistic and physiologically stressful conditions comparable to recent Central European heatwave events, where canopy temperatures frequently exceed 35–40 °C (Schuldt et al., 2020). We added a statement in section 2.1.1 in the revised manuscript.

“During heat stress and combined stress, it was 38 ± 3.3 °C and 38.1 ± 0.4°C, respectively. The applied heat stress was selected to simulate ecologically realistic and physiologically stressful conditions comparable to recent Central European heatwave events, where canopy temperatures frequently exceed 35–40 °C (Schuldt et al., 2020).”

Line 324 – again, please cite more recent Sharkey group papers. The thinking on this is evolving.

Response: We revised our introduction and discussion based on your insightful suggestions and incorporated a literature review based on relevant Sharekey group articles.

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Pastor, F., Paredes-Fortuny, L., and Khodayar, S.: Mediterranean marine heatwaves intensify in the presence of concurrent atmospheric heatwaves, Communications Earth and Environment, 5, 1–13, https://doi.org/10.1038/S43247-024-01982-8, 2024.

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Weraduwage, S. M., Whitten, D., Kulke, M., Sahu, A., Vermaas, J. V., and Sharkey, T. D.: The isoprene-responsive phosphoproteome provides new insights into the putative signalling pathways and novel roles of isoprene, Plant, Cell & Environment, 47, 1099–1117, https://doi.org/10.1111/PCE.14776, 2024.

Table 2 - the symbol in the table doesn't look like the same symbol in the caption for PMF. It is also interesting that PMF never identified a fingerprint compound that ML did not, but ML did identify some ozone stress fingerprints that were not picked up by PMF. Perhaps discuss this a bit more in the text.

Response: In PMF, we presented only the PMF-derived outcomes, and compounds that ML also detected were indicated with asterisks. In Table 2, however, we comprehensively outlined all potential fingerprints and marked them with the symbols ψ (Machine Learning) and Φ (Positive Matrix Factorization) for clarity and easy interpretation for readers. PMF, being an unsupervised approach, identifies hidden factors based on variance structures within the data, whereas ML classification is a supervised method guided by predefined stress labels. Consequently, while both approaches converged on the almost same dominant fingerprints, the ML model identified several additional ozone-specific markers that PMF did not resolve, potentially because the magnitude of emission changes under ozone stress was relatively small and therefore much harder to detect for PMF, while the machine learning approach ignores magnitude. Conversely, PMF successfully differentiated contextual emission patterns (e.g., early, late heat stress). The additional rationale for using these fundamentally different approaches was to evaluate how consistently they capture stress-related features and how effectively they can distinguish overlapping stress events. As shown in Table 2, a substantial proportion of the identified fingerprints were consistent between both methods.

We added a discussion in our revised manuscript.

"PMF, being an unsupervised approach, identifies hidden factors based on variance structures within the data, whereas ML classification is a supervised method guided by predefined stress labels. Consequently, while both approaches converged on the same dominant fingerprints, the ML model identified several additional ozone-specific markers that PMF did not resolve, potentially because of their small magnitude. Conversely, PMF successfully differentiated contextual emission (Fig. 8-9) patterns (e.g., early, late heat stress). The additional rationale for using these fundamentally different approaches was to evaluate how consistently they capture stress-related features and how effectively they can distinguish overlapping stress events. As shown in Table 2, a substantial proportion of the identified fingerprints were consistent between both methods. Collectively, PMF and ML offer complementary perspectives: PMF elucidates temporal emission patterns, whereas ML identifies the most informative features for distinguishing stress types."

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