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 2:

This is a well written and thorough manuscript about the impacts of stressors on two different tree species. There is some imbalance in that certain parts of the manuscript are described in great detail, e.g. the methods, while other parts are left with quite limited discussion, e.g. the impacts of the limited experimental design. I do recommend that this paper be published in ACP, in part also because it provides a well-described observational dataset (with several analytical techniques applied) that can be very useful for future studies. However, it would still benefit from various improvements, as listed below.

Response: First, we would like to thank you for the positive and constructive feedback. Based on your comments and those of Reviewer 1, we have revised the manuscript with an extended and more balanced discussion, particularly limitations of the experimental design. We also appreciate your comment regarding the journal fit, and defer to the editor for this decision. However, ACP specifically focuses on broader atmospheric impact, which in our study is limited to calculated OH reactivity of the VOC emissions. Many BVOC emission studies are published in Biogeosciences. This manuscript specifically focuses on direct plant emissions and not on particle or ozone formation from these emissions, which in our view would be necessary for an ACP study. We see this study at the intersection between biosphere and atmosphere and thus exactly within the scope of Biogeosciences ("interactions between the biological, chemical, and physical processes in terrestrial or extraterrestrial life with the geosphere, hydrosphere, and atmosphere.").

General comments

The experimental design: It is noted that the experiments were conducted in SAPHIR and PLUS, but only data from PLUS are reported here. Based on this, and the way the experiments were conducted, I would guess that the details of the experiment were designed with something else, or broader, in mind than what is presented in this manuscript. If so, it would be worth stating that more clearly. Because if the target was to study effects of combined stressors, running the experiments with the same trees, switching directly from one stressor to another, and running the stressors in different order for the two tree species does not make sense. In particular, the fact that the order was different is not even acknowledged in the text before late in the results section. It is stated also that all plants had wilted by the end of the combined stressor period, although for beech this was not even the last studied period. What does this mean for the interpretation of the data from the final period? The effects of the experimental design require more discussion, for example on how different the heat stress response might have been for beech if it had been the first stressor to be applied for beech rather than the last. In addition, the ozone stress was applied only at night, but only very little (and very late) is there discussion about how this might impact the emissions during the day. How fast is the recovery? The authors can of course not change the experiments anymore, but they can more adequately discuss the limitations of the experiments.

Response: You are correct that the experiments were designed within a broader framework conducted at the SAPHIR and PLUS chambers, aiming to investigate stress fingerprints and simulate atmospheric oxidation under different stress scenarios. However, we focus only on the PLUS chamber data, i.e. direct emissions, in this manuscript.

We clarified this in the text:

"The experiment was carried out in PLUS (Plant Chamber Unit for Simulation) which can be coupled to the atmosphere simulation chamber SAPHIR (Simulation of Atmospheric PHotochemistry In a large Reaction Chamber) at the Forschungszentrum Jülich, Germany, during the summer, 2024. This study focuses on direct plant emission measurements obtained from the PLUS chamber (Fig. 1a). Emissions were introduced to SAPHIR for oxidation experiments that will be part of a future analysis."

We agree that the experimental sequence limits direct comparability because of the non-conventional sequential stress application and the differing order of stressors between species. This approach was intentional, as it aimed to simulate realistic environmental stress where trees in the same landscape may experience heatwaves and (nocturnal) ozone pollution (He et al., 2022; Musselman and Minnick, 2000) either simultaneously or sequentially.

Regarding the wilting observed after the combined stress period, we have clarified that physiological recovery and BVOC emission capacity were likely compromised thereafter. Therefore, the final heat-stress period in beech is interpreted with caution, emphasizing emission trends rather than absolute quantitative changes (although we reported detailed emission rates in SI (Table S3 and S4)).

Regarding the experimental design and timing, we have extended our discussion and acknowledged the limitations in greater depth. We originally aimed to include a recovery period in the measurements; however, as mentioned in the manuscript, some individuals had wilted by the end of the experiment, which made it impossible to reliably assess recovery.

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)."

In 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 (i.e., 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)."

“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.”

In Conclusion: “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), on the one hand going into biomolecular directions for a better understanding of the plant reactions, and on the other hand photochemical oxidation of the plant emissions to investigate their impact on atmospheric chemistry.”

Section 3.4.1 and Figure 6: Perhaps someone more familiar with this type of approach can read out more from this part, but for me it was not clear what, if any, useful findings came out of this analysis. While I could not necessarily follow everything written in section 3.4.2 either, at least there it was clear that this analysis provided insight on fingerprint compounds from different stresses. Still, Fig. 6 is hard to understand, and it remains unclear it if provides any added value in this manuscript.

Response: Thanks for your feedback. We understand that Section 3.4.1 and Figure 6 may not have conveyed the message that we want to express regarding the machine learning consistency and uncertainty. We have now revised the text to explicitly explain what each metric represents and how it contributes to the interpretation of model performance. We have revised Section 3.4.1 accordingly to clarify this interpretation and emphasize how these metrics complement Section 3.4.2, where the dominant stress fingerprints are identified.

“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's clear that the model can effectively discriminate between the different stressors.

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) showed 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."

Figures 8-9: The other reviewer suggested to move the spectra (b panel) to SI, but a time series and mass spectrum is typically what is shown (and expected) for PMF results. I would rather move panel c to the SI, as that would also give more space to panel b, where the largest contributors are anyway seen. In addition, the whole manuscript is based on mass spectra from the PTR-ToF, and therefore it is appropriate to show at least some mass spectra also in the main text. But for me, panel c does not provide critical insights that would necessitate making the figure this compact.

Response: Thanks for the suggestion. Based on your feedback, we moved panel c from the PMF result to a separate plot. Since it shows the PMF fingerprint, we believe it would be better as an additional figure in the main manuscript rather than in the SI.

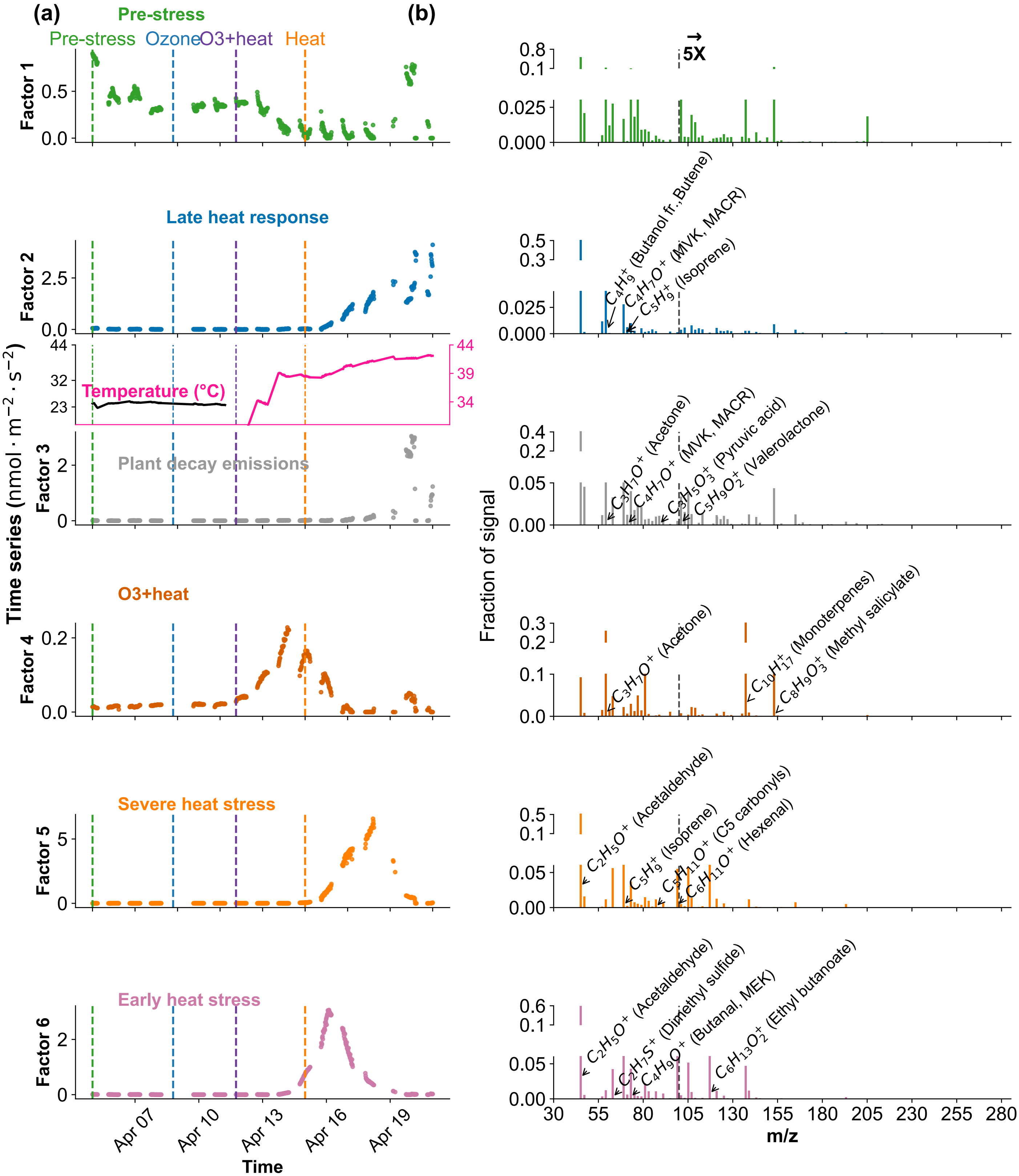

Figure 8. Positive Matrix Factorization (PMF) analysis of VOC emission profiles from beech under different environmental stress conditions. (a) Time series of a six-factor PMF solution. Colored vertical dashed lines indicate the starting of different stress phases. (b) Corresponding mass spectra (m/z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

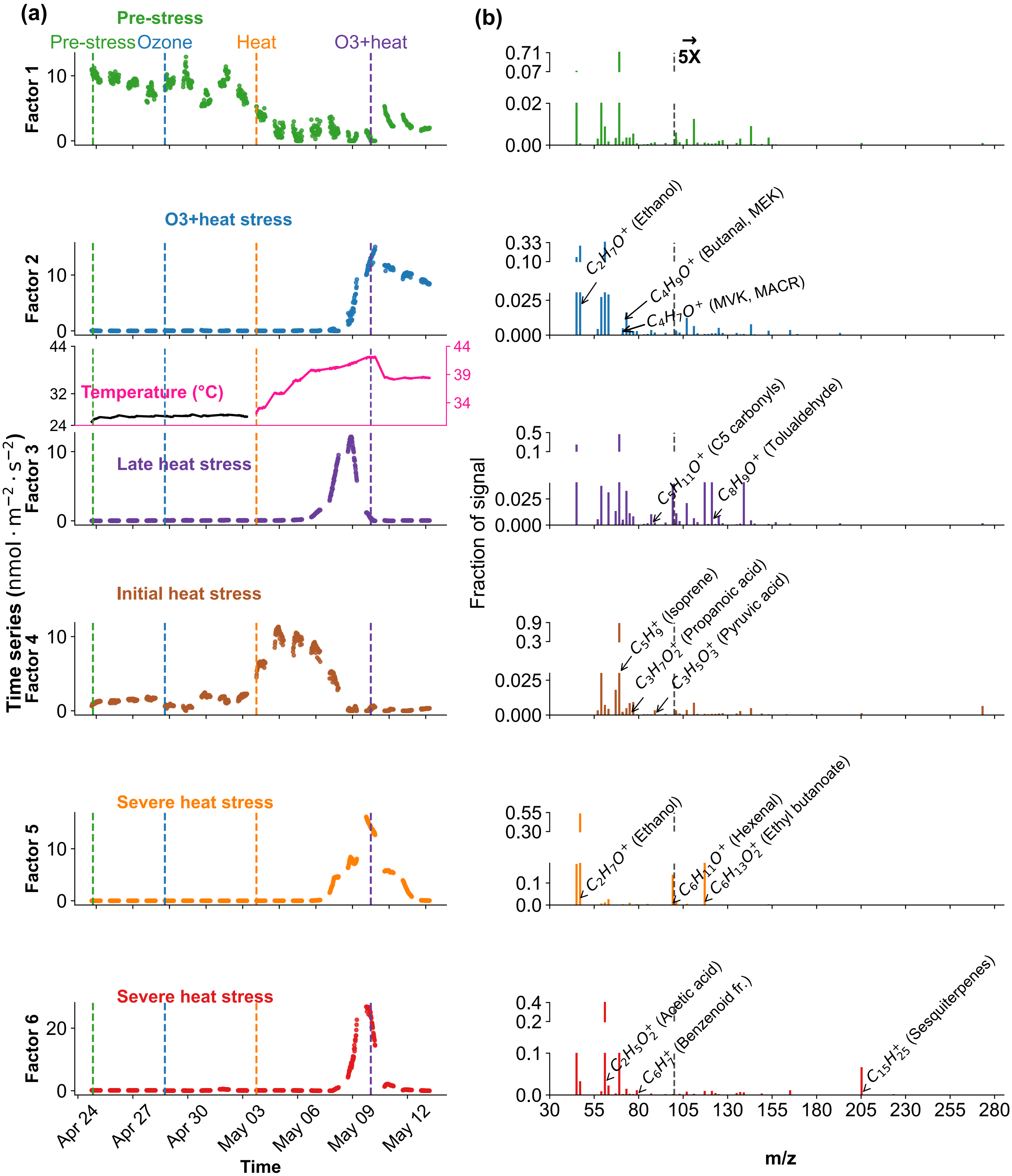

Figure 9. Positive Matrix Factorization (PMF) analysis of VOC profiles in oak under different environmental stress conditions. (a) Time series of a six-factor PMF solution. Colored vertical dashed lines indicate the starting of different stress phases. (b) Corresponding mass spectra (m/z profiles) of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

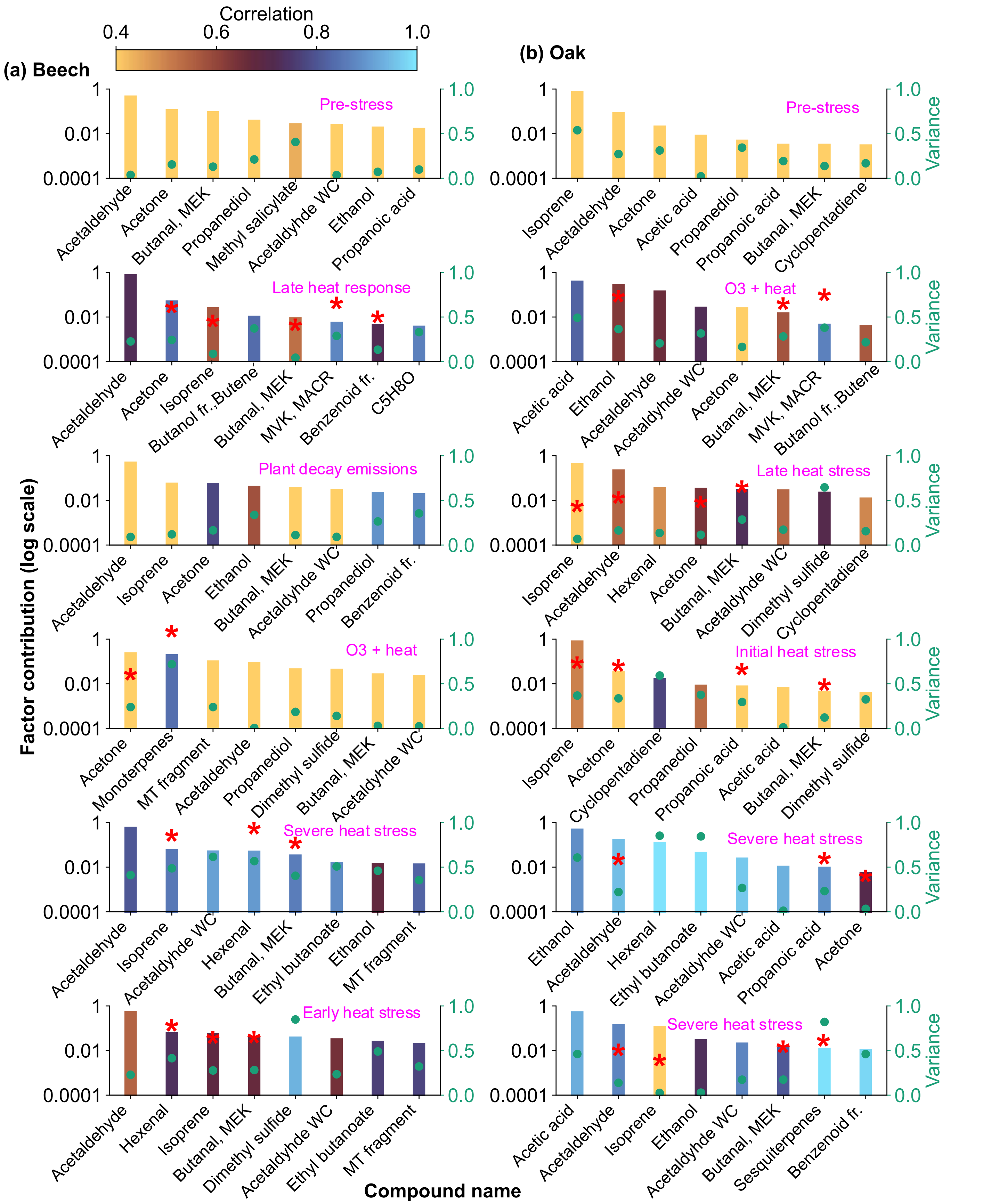

Figure 10. Top 8 stress-BVOC markers for each factor for (a) Beech and (b) Oak. Bar plots show the relative contribution of specific compounds, while green dots represent the correlation coefficient with the respective factor time series. Color

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

Specific comments

Line 44: Under what circumstances would isoprene lifetime be only seconds? Minutes, or even hours, would seems more reasonable.

Response: It can vary from seconds to minutes but we agree that minutes is more common, so we change the text to "minutes".

Lines 99-100: Some word presumably missing.

Response: Thanks, we revised that line. Before, “found that” was missing.

Studying Norway spruce, Kivimäenpää et al. (2013) found that a moderate temperature increase (~1 °C above ambient) significantly enhanced BVOC emissions, especially monoterpenes and sesquiterpenes, but this effect was partially suppressed by elevated ozone levels (~1.5× ambient).

Line 528: Some word missing here too? At least the sentence seems strange to me.

Response: We removed that line.

Line 541: “Factor 1” would be more consistent.

Response: We corrected that.

Table 2: The markers in the caption and in the table are not identical.

Response: We revised the table caption.

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