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## Reply on RC1

Michael Staudt et al.

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Author comment on "Growth and actual leaf temperature modulate CO<sub>2</sub> responsiveness of monoterpene emissions from holm oak in opposite ways" by Michael Staudt et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-142-AC1>, 2022

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*Dear referee 1*

*First of all, I wish to thank you for the fast and thorough revision of our manuscript. I am aware that the manuscript is quite long and a bit complicated, hence not easy to review. I appreciate and greatly acknowledge all comments made by both referees that will help me to improve the manuscript. In the following I will just respond to the points in order to keep the discussion going (answers are in italics).*

### **Growth and actual leaf temperature modulate CO<sub>2</sub> -responsiveness of monoterpene emissions from Holm oak in opposite ways**

Staudt et al.

General Comment:

The authors did a very thorough investigation on a specific scientific question that certainly is of relevance for the evaluation of climate change impacts on biogenic emissions and feedbacks on air chemistry. In my opinion the experiment has been well set up and carried out. The interpretation is supported by a number of ancillary measurements so that some interesting ideas about the potential underlying mechanisms could be developed. Also, the authors revealed a well-founded knowledge about the topic and the relevant literature.

*Answer: I am very pleased about these very positive and encouraging comments.*

On the downside, I noticed that wording and style could be improved. Many sentences are inconveniently complicated or long and selected expression are often unfamiliar or imprecise. I would recommend to check, shorten, and involve an English native to improve the text.

*Answer: I apologize for my poor English. Unfortunately, I do not have a native speaker available for language proofreading. I suggest that the final manuscript version (if accepted) be reviewed by a professional language editor. Perhaps Copernicus and the associate editor can advise me on this. I will also carefully re-examine the text myself, for example, by doing multiple forward and backward translations with free Internet*

*translators. I usually use DeepL, but perhaps there are better ones.*

Also some shifts between results and discussion sections and a better description of the equations used for sensitivity analysis should be considered at the appropriate places.

*Answer: The MEGAN equation will be placed in the main text.*

Specific Comments:

Abstract

It seems unclear to me, what the cool and warm growth regimes look like. Indicating only the 5-degree difference is not sufficient. Compared with the quite extensively discussed results and conclusion, the description of the outcome is relatively meager.

*Answer: I understand that the description of the results in the ABSTRACT may seem meagre compared to the relatively long DISCUSSION. However, in my opinion, it contains all the results presented in the RESULTS and the DISCUSSION. I also believe that an abstract should be concise and avoid speculative, very far-reaching conclusions, especially if they must include an additional contextual introduction. However, if the referee feels that a particular result of the study is missing in the abstract, I will try to include it.*

*Regarding the temperature-growth regimes, I will add details on the day/night temperatures that were applied.*

Introduction

L65: I assume that MTs are not synthesized but only stored in resin ducts.

*Answer: The terpenes in the resin ducts are synthesised in the glandular epithelium surrounding the cavity of the resin duct into which they are secreted (see e.g. <https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.15984> and references therein). Generally, the synthesis is particularly intense during resin duct development. Hence stored resin MTs are not synthesized in the photosynthetic parenchyma (photosynthetic source tissues) of leaf/needles and then transported into the resin ducts. However, MT synthesis in the resin duct epithelium may rely on photosynthates (essentially sucrose) provided by the photosynthetic parenchyma of source leaves (i.e. leaves that produce more photosynthates than they use for their own respiration and maintenance).*

L76: superfluous 'very' (remove)

*Answer: ok*

L85: superfluous 'before' (remove)

*Answer: ok*

Description

There is a bit of a mix between description and discussion, check (e.g. L200-203)

*Answer: Yes, I suggest removing the sentence "Previous studies showed that ...".*

Could you please indicate the equation used for emission factor reduction in MEGAN here (and not in the results as a caption text)?

Answer: yes, it will be included in the section M&M

Define G400, A400

*Answer: The definition of G and A is found in L130 and the meaning of the subscript number 400 is explained in L205. Briefly, a variable with the subscript 400 is the value that resulted from the first measurement of the CO<sub>2</sub> response curve made at 400 ppm CO<sub>2</sub>, 1000 PPFD and at an assay temperature of 30°C or 35°C. Accordingly, in our study, the term "emission factor" corresponds to the temperature-normalized E400 values. I can add an explanatory example if the reviewer finds the explanation given on L205 unclear or insufficient.*

Results

Figure 1: It is a bit irritating that the emission factor (per unit m<sup>2</sup>) should increase with the number of leaves. I see that the latter is meant as a growth indicator, which should, however, be better illustrated (e.g. final number of leaves? Number of leaves in the end of the growth period?)

*Answer: Yes the total of leaves per plant is taken as a measure of the plant's growth performance. To better understand this it should be noted that young potted QI plants in greenhouse culture do not exhibit a fixed period of leaf growth as it occurs in the field. Under field conditions QI trees show typically only one leaf flush lasting from late spring to early summer (onset of drought), though under certain circumstances there can be a second one in the same year either from the buds formed in the spring and/or from dormant buds. In our experiment, the QI saplings kept under none-stress conditions (well watered, no extreme temperatures) continued more or less growing in repeated cycles or even in indeterminate growth manner (central apex) until the end of the experiment. The acorns were potted at the same time and the number of leaves (plus few other morphological features) were determined at the end of the experiment in September. Also, there was no apparent difference in leaf size among the four growth treatments with only moderate differences in specific leaf weight (LMA). These facts allowed us to consider the number of leaves as a proxy for foliage growth. The plants were not immediately harvested after the experiment, because we wanted keeping them alive for eventual additional measurements. Finally these were not made, due to the lack of time, manpower and because the plants had to leave rapidly the greenhouse compartments. Regarding the results: Growth at elevated CO<sub>2</sub> had a fertilizing effect on leaf growth, while growth under the warmer conditions had rather a negative effect. However, individual plants in each population differed appreciably in plant size and leaf mass, even though they were always maintained under the same growth conditions. This variability was positively related to the emission factor, A400 and ETR400, measured on a single leaf of each plant. This observation is (although not completely novel), in my opinion, one of the most interesting findings that deserved to be considered in the discussion (L400 ff).*

Figure 2: Better use the same design for C<sub>i</sub> in each of the graphs (i.e. that which shows relative NPQ)

*Answer: Yes of course, all figure panels should show the same C<sub>i</sub> scaling. This bug is probably due to a copy-paste error of the Excel figures that we overlooked during the final check of the manuscript and for which I apologize.*

Figure 3: You probably mean key relations instead of key correlations. Actually, I have difficulties to see understand both, the explanations of how this is calculated and the

reason why it has been done.

*Answer: Yes key relations might be more appropriate term than key correlations. Pearson analysis is the analysis of the linear relationship between two quantitative variables. The result is given as Pearson correlation coefficient  $R$ , which ranges between -1 and +1 and provides information about the direction and strength of the linear relationship, or as determination coefficients  $R^2$  (0-1), which is a measure of goodness of fit explaining the proportion of variance explained by the model (linear relationship in case of Pearson). I consider Pearson correlation analyses as a simple mean to check relationships between the key variables of interest (that are 3 in the present study: emission factor, relative emission at low CO<sub>2</sub>, relative emission at high CO<sub>2</sub>) to other variables, thus providing indications of the determinants of variations and mechanisms behind. For example at 35°C assay temperature we found that the emission response to low CO<sub>2</sub> ( $E_{<400}/E_{400}$ ) was positively correlated with the ETR response to low CO<sub>2</sub> ( $ETR_{<400}/ETR_{400}$ , Fig. 3a) and with the leaf's initial photosynthesis rate (A<sub>400</sub>, Fig 3b). The leaf's initial photosynthesis depended much on the leaf's initial stomatal opening G<sub>400</sub> ( $R^2$  between A<sub>400</sub> & G<sub>400</sub> = 0.924) and hence  $ETR_{<400}/ETR_{400}$  also correlated with G<sub>400</sub> (Fig.3c). However, neither A<sub>400</sub> nor G<sub>400</sub> correlated with  $ETR_{<400}/ETR_{400}$ , suggesting that the emissions response to low CO<sub>2</sub> levels is determined by two independent factors (cf.), which could therefore together explain more than 80% of its variability ( $R^2$ : 0.420 and 0.445; Figs 3a, b). I strongly prefer to present such results in scatter plots as in Figs. 1 and 3 because they show the input data in its original form and its distribution (possible presence of outliers, clusters, tendencies for non-linear relationships). However, when the Pearson analyses involve a large number of variables, as in the present study, two-dimensional scatter plots are less suitable for illustrating correlation networks in a clear and concise way. For this reason, I created the diagrams shown in Figure 5.*

L364-366: The difference between the explanations for the two different responses to temperature are unclear. Rephrase and consider to elaborate the arguments.

*Answer: I will revise this part.*

L371ff: Should this really be one figure caption? Generally, I expect a short, clear and consistent description of what I see. This is violated at least since line 376. Instead, take care that the abbreviations are all clear (e.g. chloro, growth?). It could also be considered to use this figure as a basis for discussion and put into chapter 4, possibly in several stages in order to better support the reasoning in the different chapter.

*Answer: This figure was thought to provide readers an overview and summary of the outcome of the Pearson correlation analyses. The caption is indeed very long and it possible to remove caption text from "The results can be summarized..." . I found it also difficult to find the right placement of this figure in the text. Referee 2 suggests removing this figure since it is hard to read. I may suggest moving this figure (after few corrections) to the supplement 2 near the corresponding correlation matrices (Table S3).*

## Discussion

What I am missing is a discussion in how far the results can be assumed general findings or are specific for *Quercus ilex*? Is it likely that conifers, evergreens, broadleaves or Mediterranean plants react similar? Do you think the BVOC emission groups should then be differentiated by their degree of genetic relatedness or to site conditions typical for the species?

*Answer: This is indeed an important point with respect to emission modeling and inventories. As mentioned in the manuscript, our results show a strong similarity with isoprene emissions. Therefore, I might be tempted to conclude that all monoterpene*

*emissions directly linked to their de-novo synthesis in photosynthetic tissues might behave similarly. However, I prefer to be very cautious about such generalizations. Even for isoprene emissions, considerable interspecies differences in CO<sub>2</sub> responses have been observed. There is a recent paper by Niinemets et al. 2021 that specifically addresses this question (<https://onlinelibrary.wiley.com/doi/abs/10.1111/pce.14131>).*

L513ff: With the summary here, the paragraph tends to be lengthy and repetitive. I would suggest to take the essence from this paragraph to the conclusions (and delete it here).

*Answer: I will revise this part and see whether how it could be placed in the section CONCLUSIONS. This paragraph at the end of the discussion was thought to provide readers a brief summary of the multitude of metabolic responses that could have interacted and affected the emissions during CO<sub>2</sub> ramping including others not mentioned before in the discussion. Generally, experimental studies on this topic are focusing on particular processes according their hypothesis (which is understandable) thus neglecting a bit the complexity that in reality exists.*

L550ff: Here, for the first time if I am not mistaken, the authors declare that they also run some simulations to test the sensitivity of the found mechanisms. While I am not against such exercises, this comes as a surprise and should have been mentioned and described before (and shorten it here). Also Fig. 6 is a result and only part of its description belongs into discussions.

*Answer: I fully understand that this part of the DISCUSSION is surprising after the previous part. Originally, when I wrote the first draft, I just wanted to make some general statements here about the degree of emission inhibition observed in our study and the known emission response to temperature (roughly what is written on LL 529-539). The temperature dependence of emission inhibition at high CO<sub>2</sub> levels then led me to run simulations based on climate data collected at the flux tower of our forest station, combining different scenarios for maximum CO<sub>2</sub> inhibition, warming and seasonality. In fact, on the annual scale, it is not easy to predict the extent to which a given emission inhibition could compensate for the emission increase due to X °C global warming, as many different factors interact in a non-linear way. I find the results of the simulations quite informative and have therefore retained this section in the paper in the hope of increasing its impact. It might be possible to move large parts of it to the end of the RESULTS as a small extra chapter.*

Conclusion

L599: concentrations instead of variations; "hardly effect emissions" or "affect emissions only marginally" or similar instead of "affect little emissions". (good example for wrong wording)

*Answer: Thank you very much for the concrete examples, which will help me to improve the wording.*

L615ff: Missing knowledge as well as stating additional references is not something, that should be put into a conclusion. Please consider to shift it towards the discussion.

*Answer: The missing knowledge cited here is meant as a kind of outlook (what should be done next...?), which is a conclusion in a broader sense. Nevertheless, as aforementioned I will revise this part.*

*Thanks again for the helpful comments. I look forward to the next round of discussion and manuscript review.*

*With kind regards,*

*Michael Staudt*