Anonymous Referee #1
Received and published: 24 August 2020

We thank reviewer 1 for the constructive comments. Below is our point by point reply to specific comments.

Reviewer Comment 1 Line 20: please specify to what levels
Reply->Line 19: **We exposed plants with daily ozone concentrations of 100 ppb for one hour for seven days, which resulted in faster stomatal closure (e.g. a mean value -31.3% at an average stem water potential of -1 MPa) partially mitigating drought stress effects**.

Reviewer Comment 2 Line 26: "," should be placed after "Plants" and not after "in".
Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 3 Line 48 "it has be shown" – please fix.
Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 4 Line 49: Remove ",".
Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 5 Line 51: "and longer growing longer seasons" – please rephrase.
Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 6 Lines 51-54: However, drought can also lead to the opposite effect, as you mention below, which seems to be in conflict with this statement.
Reply: Here we refer to "enhanced" as an enhancement of the abiotic stress events, not of the BVOC emissions.
Change-> Line 38: **Future climate scenarios with expected temperature increases between 1.8 and 4°C (IPCC, 2007) suggest an additional enhancement of global BVOC emissions between 30 to 45 % (Peñuelas and Lluisiá, 2003). An enhancement of abiotic stress events, due to an indirect effect of a temperature increase (e.g. via ozone or drought episodes) can also alter BVOC emissions (EEA, 2017; Müller et al., 2008; Loreto and Schnitzler, 2010; Dai, 2013; Unger et al., 2013; Sindelarova et al., 2014).**

Reviewer Comment 7 Line 87 - The hypothesis of this study is based on the research question whether drought and ozone stressors are additives. It would be good to expand more about
1) previous study of these two stressors in a broader scope (e.g., (Pollastrini et al., 2014; Wittig et al., 2007)) and
2) the importance of currently addressing this specific research question (e.g., how it can help scientists to cope with current assessment/research questions).
3) the state of knowledge with respect to BVOC emission (e.g., (Holopainen and Gershenzon, 2010)).
Line 87 - "different abiotic stresses" – be specific about whether you refer here only to ozone and drought stressors and/or to additional stressors.
Introduction- it will be good to provide information about the role of BVOCs in the troposphere.
Reply: We added the following clarifications:
1) Line 77: **Pollastrini et al. (2014) consider a change in sensitivity of the plants to ozone (different poplar clones) under severe drought conditions. In their case, ozone and drought produced a synergistic effect for CO_2 exchange and chlorophyll fluorescence when applied together. Witting at al. (2007) found a dependency on ozone effects under different levels of drought stress. In fact, Witting et al. (2007) report a dependency of the damage in the photosynthetic apparatus depending on the cumulative ozone flux into the leaf, thus in relation with the stomatal conductance.**

2 and 3) Line 82: In this work, our hypothesis was that **ozone and drought stress** in plants is not necessarily additive, and that the plant's response to drought and ozone exposure can result in an alteration of characteristic BVOC emission strengths. **Changing BVOC emissions have an important impact on climate through atmospheric chemistry (Claeys et al., 2004, Paulot et al., 2009; Hallquist et al., 2009). The presence of BVOCs in atmosphere contribute to the formation of tropospheric ozone and growth of secondary organic aerosol (SOAs), and radicals (Griffin et al., 1999; Orlando et al., 2000; Atkinson and Arey, 2003).**
Line 92: **Understanding how BVOC emissions respond to climate change is therefore essential to understand what direct or indirect effects the biosphere can exert on atmospheric chemistry and climate. A better understanding will also help developing strategies necessary to mitigate the effects of climate change itself (Kulmala et al., 2004; Yuan et al., 2009).**

Reviewer Comment 8 Lines 93-94 – " its fast-regulated transpiration rates "- This is not clear. I'm not sure that these two factors are sufficient to result in high tolerance to drought.
Reply: We agree, in particular when comparing with other more drought resistant species. We changed the text as following.
Change->Line 99: According to Ellenberg (1988), **the defensive actions of *Q. robur* against drought stress are caused by fast regulation of transpiration rates and stomatal conductance**, and a low susceptibility of water embolism in the xylem (Van Hees, 1997).

Reviewer Comment 9 Line 106- "air gas exchange" – Do you mean CO2 and H2O exchange- please be specific in this definition throughout the text (e.g., line 119 and elsewhere).
Reply: OK, we now add CO2 and H2O where necessary.
Changes: e.g. Line 110: **were used for BVOC emission measurements, CO2 and H2O gas exchange measurements and biochemical** …
Line 132: Throughout the increasing drought stress, tree leaf gas exchange **(CO_2 and H_2O)** and BVOC emissions were measured for two sets, DS and…
Line 133: Instruments GmbH, Alland, Austria) for 2-3 hours each day in order to measure their **CO_2 and H_2O exchange** along with key…
Line 272: Statistics Toolbox Release 2017a, The MatWorks, Inc., Natick, MA, United States). All leaf gas exchange **(CO_2 and H_2O)** and BVOC flux

Reviewer Comment 10 Can you elaborate on how did you measured this gas exchange?
Reply: We explain the basic measurement setup on Line 161: CO_2 and H_2O mixing ratios in the air leaving the enclosures were measured using a CIRAS-3 SC PP System (Amesbury, MA, USA**), which was factory calibrated three months before the measurement campaign**.

Reviewer Comment 11 I recommend adding the schematic of the experimental design (Fig. A1) to the Materials and Methods. It will help in following your experiments.
Reply: This has been fixed in an updated version of the manuscript

Reviewer Comment 12 Lines 120-121 – please specify if this part still take place in the fitotron.
Change-> Line 116: **The plants were moved from the greenhouse to an indoor climate chamber** (Fitotron Weiss Gallenkamp, UK) 24h hours before the experiment started. **Thereafter trees were kept in the climate chamber for the remainder of the experiment and were only placed into the branch enclosures during the gas exchange measurements. The branch enclosures were situated next to the climate chamber in a climatized laboratory exhibiting the same environmental conditions as in the climate chamber.** The climate conditions during the first day of experiment were kept at 25°C, ~60 % of **relative humidity (rH)** and ~1000 µmol m^{-2} s^{-1} PAR at canopy top, to

adapt to constant air temperature. To continuously increase the drought stress, the plants were not watered and the humidity in the climate chamber was decreased to 40 % rH and temperature was increased to 30°C after the first day. **The same temperature conditions were also present in the climatized laboratory, where the plants were placed in the enclosures at an rH of 32 % and 30°C. Overall light conditions remained constant during the day, with lights of during the night.**

Reviewer Comment 12 Line 131- Please define "rH".
Change-> Line 120: which was kept at 25°C, ~60 % of **relative humidity (rH)** and ~1000 µmol m^{-2} s^{-1} PAR at canopy top, to adapt to constant air temperature.

Reviewer Comment 13 Line 132- "assured" – can you explain in detail why it assures so?
Change-> Line 147: The flow rate of 10 l min^{-1}, **tested during the experiment set-up prior to the actual experiments**, assured that no…

Reviewer Comment 14 Lines 135-136- Irradiation took place also in nighttime? Please specify.
Change-> Line 150: Trees inside the enclosure were LED-irradiated with a mean PAR value of 1374 µmol m^{-2} s^{-1} at canopy top (Eckel Electronics, Trofaiach, Austria**)** **during daytime when the exchange measurements were performed. During night, trees were kept in the dark**.

Reviewer Comment 15: Can you provide details about Background (zero) calibration? e.g., if and what frequency and under what conditions such calibration was performed using the PTRTOF6000X2?
Reply: The PTR-TOF-MS was calibrated daily, this has already been mentioned in the manuscript on line 173. We added some more specificity to describe the background measurements.
Change-> Line 169: The **instrument background was characterized daily during calibrations and in the third empty enclosure that was flushed with background air. Backgrounds were measured every 20 minutes for 5 minutes.**

Reviewer Comment 16 Line 157 – it is not clear to me what do you mean by "combined calibration uncertainties" and by "a compound specific average experiment sensitivity"
Change-> Line 174: **Daily measured sensitivities based on compounds in a calibration standard varied on the order of 8-20 % depending on the compound. This lies within the combined calibration uncertainties of the gas standard and dilution setup using two flow controllers. Whenever a compound was not contained in the calibration standard, we applied a compound specific sensitivity using procedures described by Cappelin et al. (2012).**

Reviewer Comment 16 Line 158 – "40-800 pptv" – it is not clear to me why providing this information is important. It may be more useful to specify specific limits of detection to individual compounds (possibly in Table A3).
Reply: We take this suggestions and omitted this statement in the revised manuscript.

Reviewer Comment 17 Lines 161-168 – I suggest to include each individual compound acronym together with its specific m/z (e.g., in parenthesis) and the specific reference, either in the text or in a table.
Reply: This has been fixed in an updated version of the manuscript

Reviewer Comment 18 Line 179 – Why don't you include each of the abbreviations in parenthesis?
Reply: This has been fixed in an updated version of the manuscript

Reviewer Comment 19 Line 189 – Can you elaborate on how the stomatal resistance was measured? Was it measured for each specific leaf or using another approach?

Reply: Since the experimental setup consisted of a branch enclosure, stomatal resistance values reflect the bulk average of all leaves enclosed in the enclosure. It was calculated by the application of formula 10.

Reviewer Comment 20 Lines 189-190 – Can you explain why assuming that the boundary resistance is zero is justified?

Reply: The high flow rate of 10l/min through our chambers created enough turbulence that the boundary resistance is assumed to be small compared to the stomatal resistance. This can alternatively also be achieved by fans inside the chamber, which we wanted to avoid due to the potential of artifacts from the lubricants that a fan inside the enclosure would cause.

Reviewer Comment 21 Line 197 – " (see below)" – it would be better to specify the specific section number.

Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 22 Lines 203-204 – "peroxidase and antioxidant capacity, and phenol content" – it is the first time you mention these properties. It would be good to expand on them and why they were measured.

Change-> Line 225: **Using foliar materials collected after the seven day period of emission measurements (section 2.2) and stored at -80°C until analysis, peroxidase and antioxidant capacity, and phenol content (TPhe) were measured. These properties provide additional insights in the response of GLV and Shikimate emissions as products of the metabolic process of the enzymatic activity (Betz et al., 2009).**

Reviewer Comment 22 Line 216 – What do you mean by "linear range of: : :" ?

Change->Line 241: The activity was calculated **from the slope in the initial linear portion of the reaction progress curved** using an extinction coefficient of $1.13 \times 10^4\ M^{-1}\ cm^{-1}$ for oxidized *o*-dianisidine (Worthington manual, 1972).

Reviewer Comment 23 Line 256 – no need for multiple definition in the main text.

Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 24 Line 260 – " significant." – can you add a P-value?

Change-> Line 286: R1 and R4 was significant **(p-value 0.02 and 0.05 for DS and DS×OS respectively)**. R1, shown in Fig. 1(a), includes values of trees fumigated with ozone (DS×OS) from the first and

Reviewer Comment 25Line 274 - no need for multiple definition in the main text.

Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 26 Line 285 – " (averagely 96 % of the total emissions" – it would be better to provide this information earlier, so the reader will have this in mind when reading the second paragraph in this section.

Reply: We moved this portion to Line 303: where we now state: The ratio of CBVOCs and C*A* is shown in Fig. 3. **IS, the dominant BVOC (on average 96 % of the total emissions), mean standardized IS emissions of DS×OS treated plants were consistently higher in all SWP ranges compared to DS alone (Figure 3), thus showing the difference between DS and DS×OS in CBVOCs/C*A* in the highest SWP ratio range**

Reviewer Comment 27 Line 287 – "carbon loss ratio" - Be more accurate in definition. Do you mean->CIS/CA?

Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 28 Line 288 –" high drought stress" - Can you specify this in terms of "R"?

Change->Line 315: At very high drought stress **(R4)** this ratio decreased again to 0.4 in DS and 0.8 in DS×OS.

Reviewer Comment 29 Line 304 – Please add "." at the end of the sentence.
Reply: This has been fixed in an updated version of the manuscript

Reviewer Comment 30 Lines 340-341 – "a decrease of MT emissions" – under what conditions?
Change-> Line 375: These observations contrast those by Lluisá and Peñuelas (1998) for *Q. coccifera* reporting a decrease of MT emissions **under severe drought conditions**.

Reviewer Comment 31 Line 366- Is this a new paragraph (if so, make it clear and consistent with the rest of the manuscript)?
Reply: This has been fixed in an updated version of the manuscript

Reviewer Comment 32 Lines 385-386 – " by stimulating the phenylpropanoid pathway" – what about the lipoxygenase and hydroperoxide systems?
Reply: We added a sentence to this section:
Change-> Line 422: On the other hand, DS×OS, showed a small increase of GLV only at the highest stress level. We take this to indicate that ozone has the potential to inhibit drought stress damage and therefore the emissions of GLV, by stimulating the phenylpropanoid pathway to form an antioxidant protection for chloroplasts (Pellegrini et al., 2019**). The GLV emissions in DS×OS are initially inhibited during of the onset of drought. While ozone fumigation initially inhibits the activation of the lipoxygenase and the hydroperoxide lyase pathway indirectly, these pathways are clearly triggered during the progression of severe drought stress (R4) (Heiden et al., 2003; Matzui, 2006).**

Reviewer Comment 33 Line 396 – " well-watered and severe drought condition" – can you specify these also in terms of R?
Change->Line 437: The results of our study showed no significant decrease in TPhen due to ozone fumigation both in well-watered and severe drought condition **(R4)** (OS, DS×OS).

Reviewer Comment 33 Tables 1 and 2 – it is recommended to include the comments below the table.
Reply: This has been fixed in an updated version of the manuscript.

Reviewer Comment 33 A1 – Can you include the thermocouple in the figure?
Reply: We modified the figure and included the thermocouple.

Reviewer Comment 34 Table A3 – add the compound acronyms/names
Reply: We added the table in the manuscript.