Anonymous Referee #3

Summary:

This work examines the changes in foliar isoprene emission and photosynthesis of isoprene emitting black locust to periods of drought and drought plus heat stress. The isoprene and photosynthesis responses are compared to existing literature and the response of isoprene emission was then compared to that which would be calculated using the Guenther et al algorithm under the same stress conditions. In general the study is well written, although some aspects could be clearer (outlined in the comments sections below). There are a few typographical errors and the within text references need to be looked at as they should be presented either alphabetically or by date order. This study does appear to contain a solid body of work which is worth publishing to add to our understanding of isoprene responses to complex stresses and to help improve modelled emission estimates. However, as the manuscript is currently written I struggle to find the novelty in the work. I have a few concerns and believe the manuscript should be improved as outlined below before it could be accepted for publication.

> **Reply:**
> We like to thank the reviewer for considering the quality of our study. Regarding the comment on the novelty of our study, we can understand the reviewer's concern because we apparently did not highlight it well enough in the current version of the manuscript.
> We will do that in a revised version of the manuscript. So far, there is only one study (Vanzo et al., 2015) which evaluates isoprene emissions in response to prolonged combined and repeated heat-drought stress, we are thus addressing a poorly explored research area – despite of combined heat and drought being a feature of typical extreme episodic weather events which are likely to increase in future. In this manuscript we evaluated the stress-response of leaf-level emissions of four-year old black locust saplings and evaluated the change of temperature and light response functions of isoprene emissions, in view of alerting the modelling community to the complexity of the response patterns. See also our reply to major comment # 4, below, for more detail.

Major comments:

1) Materials and Methods, Experimental set up, line 96. I am concerned that the trees in the stress treatments had previously been exposed to two experimental heat waves and were showing a difference in basal area. Previous work has shown that VOC emissions differ based on exposure to previous environmental conditions (e.g. Sharkey et al, 1999 and citing references). Could the authors provide some reassurance that after pruning and over wintering the development and growth rates were then equivalent and could be fairly compared to one another? If they were not equivalent as suggested in the results section 3.1, were the data normalised?

Reply:

The data were collected as part of a full three-years experiment which sought to evaluate the response to prolonged and repeated stress. During the first year it was unfortunately not possible to collect VOC data, but information from the first year of the experiment showed that black locust leaves recovered its photosynthesis 3 weeks after the last heat wave ended and that basal growth rates were close to control trees (Ruehr et al. 2016). Trees were pruned due to height constraints in greenhouse facility and overwintered outside. Before leaf-out in spring, the trees were returned inside the greenhouse and equipped with sensors. Branch chambers were installed in June. Statistical analysis showed no differences in leaf gas exchange (photosynthesis and isoprene emission) before the heat-waves were imposed in the second year of the experiment (see Table 2 of the current manuscript). Therefore we do not think it would be necessary to normalize the data and we are confident that leaf level emissions did not carry a substantial signal as a consequence of the stress during the first year of the experiment. We will include these aspects to a revised version of the manuscript.

2) Could the authors give an explanation as to why the trees were not randomly selected for the work included in the current study? This would have given a mixture of previously stressed and unstressed trees in each treatment group and removed any concern that the prior treatment of these trees was affecting the current results.

Reply:

This is an important point, and reflects the study design over the entire duration. The purpose was to evaluate how the trees will response to re-occurring heat waves over subsequent years – which made it necessary to maintain trees within one treatment. Studies on heat waves occurring over more than one growing season are scarce and to our knowledge have not been done yet with woody species. Although we found a slightly reduced basal area of previously heat and heat-drought stressed trees in the second year of the experiment, we detected no change in leaf-level emissions of newly grown leaves of stressed trees compared to the control prior to the second year heat waves' (see LME results in Table 2 of the manuscript).

3) I would also like to see a clear description of the growth conditions and number of trees used per treatment and per measurement. Could the authors give a full description of the growth conditions of the trees (temperature, light, CO2, RH) in the description of the experimental setup? Did the greenhouse have supplemental lighting, where was average PAR recorded, what was the day length? How many replicates were used per measurement? At the moment it is not clear to me how many replicates were used for what.

Reply:

The reviewer is correct in that we did not provide all this information in the Methods section, but instead referred the reader to a publication that describes the experimental set-up in great detail. In order to facilitate reading of the manuscript, we plan to add more detail on the methods into a revised manuscript:

In total we had six trees per treatment, however, leaf chambers were installed at three trees per treatment. Although the major component of the photosynthetic active radiation was the sunlight, the greenhouse had supplemental lighting (Philips SON-T Agro 400 W, Philips, Amsterdam, NL). Daylight-length was not artificially modulated, and thus varied according to season. Growth conditions were monitored by two sensors per greenhouse compartment measuring photosynthetic active radiation (PQS 1, Kipp & Zonen, Delft, The Netherlands), air temperature, and relative humidity (CS215, Campbell Scientific Inc., Logan, UT, USA). Photosynthetically active radiation as used for the light response curves was recorded alongside temperature in each leaf chamber. Average growth conditions (PAR, VPD, and temperatures) for the trees within the two compartments of the greenhouse are presented in Duarte et al. (2016). However, for clarity we plan to include a table presenting average CO_2 concentrations, temperatures, relative humidity, and photosynthetic active radiation (Table S3) monitored in the greenhouse compartments in a revised version of the manuscript. In general none of the drivers differed by more than 2 % between the two greenhouse compartments before the heat waves.

4) In general I cannot currently see the novelty of this work. However, this might be improved if the authors could use their data to suggest a new algorithm or an amendment to the existing algorithm to bring modelled isoprene emissions more in line with that which is observed. At the moment the authors highlight the difference between the observed and modelled emissions but don't go any further.

Reply:
Regarding the novelty of our study, please see also our answer above and comments to reviewer 1 and 2. More specifically, to highlight the complexity of modelling isoprene emissions under combined stress and recovery with simple algorithms we prepared two additional figures (Fig S1 and S2) showing much clearer the differences between the stress and control model in estimating isoprene emission in black locust. When using the model parameterized based on data from the control trees, heat and heat-drought isoprene emissions would be overestimated by approximately 50 %. While past environmental conditions are known to alter the isoprene emission factor (Niinemets et al., 2010), we found indication that stressful conditions will as well alter the shape of the temperature response function (e.g. temperature maximum of the response curves moves towards a higher temperature). Thus it is not possible to apply a simple correction factor (based on the slope in Fig S1a) to adjust the standardized emission rate (compare Fig S1b) and bring measured emissions in line with emissions modeled with the control model including a correction factor.
This together with our reasoning before will be added to a revised version of the manuscript.

Minor comments
1) Abstract line 12 – mentions assessing the impact of stress on BVOC emissions but only isoprene is presented in the manuscript. Either remove the reference to general BVOC or include other emitted compounds.

2) Intro, line 38 – include ref to more recent Wyche et al, ACP 2014 which gives positive and negative effects of isoprene emission on secondary aerosol formation.

3) Into, line 65 and line 71 – include ref to more recent Ryan et al, New Phyt 2014 and remove older references unless they are seminal /original work.

4) Mat and Methods, Paragraph starting line 155 – description is not clear. Is the automatic switching of the measurements or the air flow? If air flow does this mean the chambers were clamped on the plants with no air flow for a period of time?

5) Section VOC Line 200 – the PTR-MS only counts set masses and cannot give compound identification. Could the authors include information on any mass identification that was performed (e.g. GC-MS) to confirm that it was only isoprene at m/z 69

6) Line 231 – 500 PAR seems quite low for trees in the summer. Top of canopy PAR in

northern Europe during the summer is more likely to be between 1000 and 2000 PAR. Could the authors give a reason for choosing 500 PAR.

Reply:
Correct. In most studies Es is parameterized for light-saturation at 1000 µmol m^{-2}s^{-1}, however, the value used for standardization is an arbitrary value. In principle it does not matter to which light conditions Es is normalized as long as this value is above the light saturation for isoprene emissions. As in our study the photosynthetic active radiation hardly exceeded 500 µmol m^{-2}s^{-1} and isoprene emissions reached its light saturation at values lower than 500 µmol m^{-2}s^{-1} we used this value for normalization. We will explain our considerations in a revised version of the manuscript.

7) Mat & Methods Line 267 - Formatting error

Reply:
This will be corrected in a revised version of the manuscript.

8) Results 3.1 line 295. Could the authors include a description of how midday leaf water potential was measured?

Reply:
Mid-day leaf water potential was measured by determining the pressure necessary to cause water to exude from a freshly-cut leaf inserted in a Scholander pressure chamber (Model 1000, PMS Instrument Company, Albany, Oregon, USA,). We will add this information to a revised version of the manuscript.

9) Results 3.1 line 299 – typo "relative" should be "relatively"

Reply:
Thank you for catching this. The typo will be corrected in a revised version of the manuscript.

10) Results 3.2 line 307 – I don't understand why " (PAR > 50 umol m-2 s-1)" is included in this sentence, when the sentence is referencing stomatal conductance – please clarify.

Reply:
To clarify that we explicitly calculated daytime averages, since stomatal conductance is nearly zero during the night the averaging period makes a difference in the results. This will be explained in a revised version of the manuscript.

11) Line 316 Daytime (PAR > 50 umol m-2 s-1) – I am assuming this means the authors collated any data collected when PAR readings were over this value to be "daytime"

values. If this is correct please include a clarification at first use to make it easier for the reader to understand.

> **Reply:**
> We will add a sentence to the revised version of the manuscript to make this clear.

12) Line 322 – It may be over-stretching the results to include "marginally significant (p value around 0.1)" results as significant differences. This is not common practice but is perhaps personal preference.

> **Reply:**
> We wanted to indicate that the p-value suggests that these values tend to be higher compared to the control even if the change is not significant based on the p<0.05 criterion. We change our wording accordingly.

13) Results 3.3, line 338 "significantly different to control trees" and "no significant differences. . ." please give p values.

> **Reply:**
> Agreed. We will add the corresponding p-values in a revised version of the manuscript. The decision was based on the criteria p<0.05 (which is common practice).

14) Discussion Line 380 – references you should include more recent ref e.g. Ryan et al New Phyt 2014 who used genetically modified tobacco specifically to study the impact of drought on isoprene emission and protection.

> **Reply:**
> Thank you. We will make sure to include more recent literature in a revised version of the manuscript.

15) Line 385 "A quick recovery of isoprene emissions after periods of drought stress seems to emerge as a 385 common feature that has also been observed in previous studies (Brilli et al., 2013; Pegoraro et al., 2004; Velikova and Loreto, 2005)" and line 288 "The observed faster recovery of isoprene emissions than photosynthesis may be a common pattern following stress release (Brilli et al., 2013; Pegoraro et al., 2004)." This appears to be a repeated point – please remove one of the sentences.

> **Reply:**
> We will critically re-assess our wording and make sure that the intended differences between the sentences become clear.

16) Line 390 – "this is the first study that considers dynamics of isoprene emissions during and following combined heat–drought stress. . ." Unfortunately this claim is untrue – please remove and see Vanzo et al, 2015 and references therein.

Reply:
We apologize for this mistake and will change the sentence accordingly following our reasoning given above and of course include this reference in a revised version of the manuscript.

17) Paragraph beginning line 415 – including reference to Ryan et al, 2014, New Phytologist, who studied isoprene emitting and non-emitting plant responses to drought, would be appropriate here. Most likely with the Vickers et al, 2009 reference. 18) Table 2–could the authors explain why there is such a variation in group sizes (n values from 0–49)?

Reply:
This has two reasons:
(1) As we did not randomize temperatures in the heat and heat-drought treatment but simulated high temperatures using ambient +10°C we do not have an equal number of points in all temperature levels. Thus in some temperature regimes there is a dense distribution of points while in others there are less points.
(2) Since we wanted to use the same temperature bins for all three treatments (which makes the bin averages more comparable between treatments) it was not possible to set bins in such a way that we have in every temperature range a similar group size. Especially for the highest and lowest temperatures of each treatment we thus have a lower number of points within the bin. To account for this we weighted the bin averages by standard deviation. We will take care that this information will become clearer in the Methods section.

References

Duarte, A. G., Katata, G., Hoshika, Y. and Hossain, M.: Immediate and potential long-term effects of consecutive heat waves on the photosynthetic performance and water balance in Douglas-fir, J. Plant Physiol., 205, 57–66, doi:10.1016/j.jplph.2016.08.012, 2016.

Niinemets, Ü., Arneth, A., Kuhn, U., Monson, R. K., Penuelas, J. and Staudt, M.: The emission factor of volatile isoprenoids: stress, acclimation, and developmental responses, Biogeosciences, 7, 2203–2223, doi:10.5194/bg-7-2203-2010, 2010.

Ruehr, N. K., Gast, A., Weber, C., Daub, B. and Arneth, A.: Water availability as dominant control of heat stress responses in two contrasting tree species, Tree Physiol., 36(2), 164–178, doi:10.1093/treephys/tpv102, 2016.

Vanzo, E., Jud, W., Li, Z., Albert, A., Domagalska, M. A., Ghirardo, A., Niederbacher, B., Frenzel, J., Beemster, G. T. S., Asard, H., Rennenberg, H., Sharkey, T. D., Hansel, A. and Schnitzler, J.: Facing the Future: Effects of Short-Term Climate Extremes on Isoprene-Emitting and Nonemitting Poplar, Plant Physiol., 169, 560–575, doi:10.1104/pp.15.00871, 2015.

Table S3: Average CO_2 concentration, temperature, relative humidity (RH), and daytime photosynthetically active radiation (PAR>100 µmol m^{-2}s^{-1}) including the corresponding standard deviation in the two greenhouse compartments between 7 May and 13 June 2014, before the start of the first heat wave. Difference in growth conditions between the average values of environmental drivers are given in percent.

growth conditions 07.05.14 – 13.06.14	compartment 1		compartment 2		
	average	standard deviation	average	standard deviation	difference (%)
CO_2 (ppm)	409	39	404	36	1.2
Temperature (°C)	15.6	5.4	15.6	5.2	0
RH (%)	80.8	13.8	82.1	12.8	1.6
daytime PAR (µmol m^{-2}s^{-1})	419	286	412	248	1.9
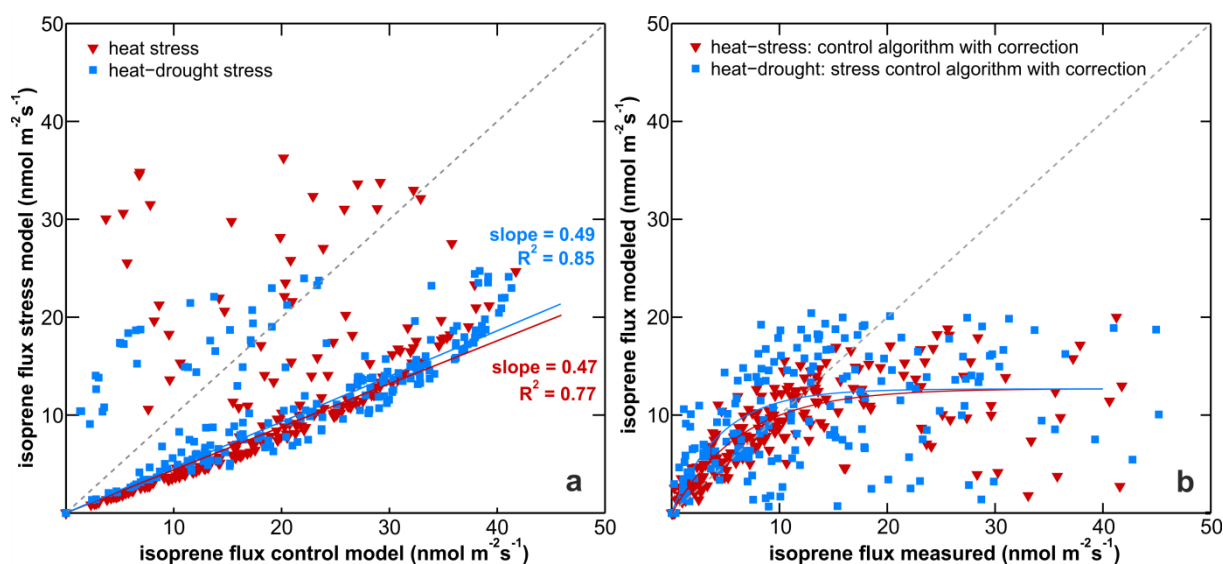
Figure S1: a) Isoprene fluxes of heat and heat-drought stressed trees modeled with the stress algorithm against fluxes modeled with the control algorithm including a linear least-square fit showing the slope which would bring fluxes calculated with the control algorithm in line with fluxes calculated with the stress algorithm; b) Isoprene fluxes modeled with the control algorithm and corrected with the slope denoted in S1a to account for changes in the standard isoprene emission rate during stress.
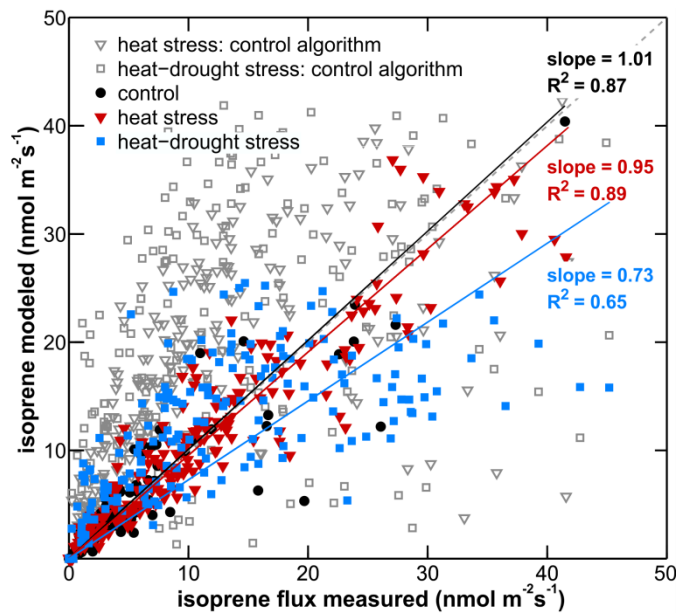
Figure S2: Modelled versus measured isoprene fluxes for trees exposed to control conditions (black circles), heat stress (red triangles), and heat-drought stress (blue squares) including a linear least square fit. Open grey symbols show isoprene fluxes modeled with the control algorithm instead of the corresponding algorithm for heat and heat-drought stressed trees.

Fig S3: Picture of a leaf chamber enclosing a black locust leaf. The lid closed automatically during measurements for about 10 minutes. Between measurements the lid remained open and a fan was circulating air constantly.