Anonymous Referee #2

General comments:

This manuscript makes the point that that isoprene emission model parameters are likely to be different for stressed plants than for unstressed plants. This is a good point to make.

> **Reply:**
> Thank you.

However, I found the manuscript to be problematic. Temperature of the individual leaves could not be controlled and so the temperature response curves of the control and heat or heat-drought treatments were almost non-overlapping.

> **Reply:**
> We understand the reviewer's concern which reflects a component of our study design: It was not our intent to directly control the temperature of individual leaves as the study was meant to mimic emission differences when the entire tree is exposed to a different temperature. Because we were interested in how trees responded to heat wave scenarios, we purposely mimicked diurnal temperature cycles and day-to-day variations. Since temperature responses are known to critically depend on how they were achieved (Niinemets et al., 2010) we intentionally choose this experimental design to mimic conditions how they could potentially occur during heat wave scenarios under ambient conditions (Boeck et al., 2010). The heat wave scenario was implemented on ambient temperatures with + 10°C on average – which, as the reviewer is correct, led to very different temperature range on the measured leaves. The reviewer is correct that this will need to be better highlighted and we will ensure that this becomes much clearer in a revised manuscript.

I found the description of the methods to be difficult. It is not clear to me whether leaves not currently being measured had an air flow of if the airflow only occurred during a measurement.

> **Reply:**
> Thank you for pointing us to this shortcoming of our methods description. The chambers (n=9 + 1 empty chamber), each permanently installed at one leaf petiole (see Fig. S3), were kept open all the time expect during the 10 min measurement, before which the chamber lids automatically closed. To ensure well mixing, the fan inside the chamber remained on at all times. Air flow (VOC-free) through the chamber, however, was only generated during measurements, while during the remaining time ambient air was mixed into the chamber. The permanent installation of the chambers enabled automation and excluded the risk of leaf wounding. We will make this much clearer in a revised version of the manuscript.

A great deal of variation in isoprene emission rates was observed. I was not convinced that the statistical treatments accurately reflected the variability. Isoprene emission is exceedingly difficult to predict, a point made by this lab that affects how these data need to be interpreted.

Reply:
As the reviewer states there is high variability in isoprene emission. This is due to changes in environmental drivers and tree-to-tree variability. However, besides this variability, differences between treatments were statistically significant as seen using linear-mixed effect models and uncertainty bounds for temperature response curves. Does the referee's comment further refer to the uncertainty bounds of the temperature and light fit, which might seem to be too low when compared to the variability of single measurement points? If this was the concern: as commonly done (see Seco et al., 2015) we used bin averaged data (each point weighted by the inverse of its standard deviation) to determine the fit for calculating the temperature and light response curves. The resulting confidence intervals of the fit (as shown in Figure 5) reflect that the fitted curves are statistically different above 25°C for heat trees and above 30°C for heat-drought trees (due to the higher variability in the heat-drought data). However, to clearly show that also the bin averaged isoprene emissions E_{iso} are significantly different between stress and control treatments for T > 28°C we provide Table S1.

While a lot of work has gone into this report, I have significant concerns including that leaf temperature is not known and measurement temperature during isoprene emission measurement was almost non-overlapping.

Reply:
We can understand the reviewer's concern about the missing leaf temperature measurements. We purposely decided against controlling leaf temperature. Instead of leaf temperature we measured air temperature within the leaf cuvette because we were worried that attaching a thermocouple to a black locust leaf will provide us with data that might be difficult to interpret. This is (a) because black locust is known for leaf movement (paraheliotropism), during night-time leaves are generally folded and during daytime leaves fold when heat stress becomes severe (a hairy underside is exposed which helps to protect the leaves from excessive heating), and (b) which part of the pinnate leaf would be suited in order to measure average leaf temperature values? Thus, we could not be certain that a thermocouple attached to the underside would stay in place and deliver representable leaf temperature data. Air temperature is often used for the Guenther equations simply because leaf temperature is much harder to assess and often not at hand (Seco et al., 2015; Vanzo et al., 2015).
However, in order to add further information on this aspect we analyzed data of additional leaf temperature measurements (measured with an infrared camera, PI450 Optris GmbH, Berlin, Germany) during the second heat wave and compared air temperature to leaf temperature. Independent of treatment and temperature range, differences between air temperature and leaf temperature were found to be small and statistically not significant (see Table S2). Thus we are confident that in our study, air temperature is a good proxy for leaf temperature and plan to present Table S2 within the supplementary of a revised version of the manuscript.
A remark regarding the non-overlapping temperatures: The temperature curves of the control and heat-treated trees do not overlap because the control trees were not exposed to heat waves. We make sure that this becomes much clearer in the revised version of the manuscript.

The authors have an important point to make but the manuscript as written will not make that point very strongly. I made a number of comments on the pdf that I hope will be helpful to the authors.

Reply:
Thank you. We will modify a revised version of the manuscript to better highlight the differences in the temperature-light response equations as found for stressed black locust trees. We plan on including additional Figures (S1 and S1, see below) that clearly show that isoprene emissions during the heat waves would be overestimated (>50%) when parametrized with the control model. These figures together with an improved discussion would make our point much stronger in a revised version of the manuscript.

Detailed Comments:

Throughout the manuscript:

Reply:
All grammatical and style changes will be implemented in a revised version of the manuscript.

Line 151: Does this mean that the air flow through the chamber was turned off when it wasn't being measured?

Reply:
When the chamber was not measured it was open and thus circulated with ambient air (see detailed comment above and Fig. S3). This will be explained in more detail in a revised version of the manuscript.

Line 275: Is this the same as Es?

Reply:
No. We explicitly introduced a parameter EF (emission factor) for the temperature fit. In the original parametrization of the temperature response function for isoprene standard temperature was set to 27.8°C. Es (the standardized emission factor at 30°C) was thus not explicitly fitted in our case as we used the Guenther et al., (1991, 1993) over the Guenther (1997) equations as one parameter less was required for the fit. We will add Es to Table 3 in a revised version of the manuscript to improve comparability between literature results.

Line 296: How was leaf water potential 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.

Line 300: This sounds as though the heat-drought trees were subject to a different experiment the previous year than were the other trees.

Reply:
Thank you for pointing us to this. We re-word this sentence to make clear that the trees of the heat and heat-drought treatment had been subjected to two heat waves in the previous year, which resulted in a larger decline of basal area in the heat-drought (-43%) than heat-treated trees (-27%) (Ruehr et al., 2016). This reduction in biomass (reduced basal area and lower leaf biomass) may have

caused the relatively small differences in relative soil water content between the heat and the heat-drought treatment.

Line 317: It is hard to see this in 3c. The overwhelming impression is the variability.

Reply:
In this Figure, each data point reflects one measurement made with the automated leaf chamber, thus the variability includes diurnal variations and differences between the individual trees measured. The trees were exposed to ambient temperature changes and light fluctuations causing the pronounced variability in isoprene emissions the reviewer is referring to. We purposely decided to plot each single data point to highlight the difficulty of modeling isoprene emissions. However, alongside with the single data points we are also providing daily treatment averages, which clearly show the large increases in isoprene emissions. The significance of the treatment effects have also been confirmed by a linear mixed-effects model as given in Table 1 of the manuscript.
In a revised version of the manuscript we could omit the single data points and show instead the SE of the daily treatment averages - if this is preferred.

Line 355: Isoprene is very temperature dependent while photosynthesis is not but both a very light dependent. By restricting the analysis to less than 30oC the data would have been mostly in the light liming range for photosynthesis and so also for isoprene. Above 30°C light was probably mostly limiting and so the very different temperature responses of isoprene emission and photosynthesis would become dominant.

Reply:
We are not sure if we fully understood the reviewer comment. We assume that he/she refers to the differences in temperature responses of isoprene and photosynthesis which become more dominant for higher temperatures. Both, isoprene and photosynthesis are light dependent and temperature dependent. According to Ruehr et al., (2016) black locust trees in the control treatment were close to the temperature optimum (which was between 20°C and 30°C). Beyond this temperature optimum photosynthesis decreases with increasing temperatures. Since the amount of photosynthetic active radiation, to which trees were exposed, remained unchanged for heat-treated trees we conclude that the different temperature responses of photosynthesis and isoprene become more dominant for higher temperatures as photosynthesis already decreases while isoprene is still increasing. In a revised version of the manuscript this will be clarified in the Discussion.

Line 348: It isn't clear why this should be normalized to 500 when Es is normalized to 1000.

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 photosynthetically 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.

Line 353: I am not good at statistics, but I don't feel these numbers represent what I would intuitively take from Fig 5. The highest rate of the bin averaged control seems to exceed any control measurements. The table confirms that there was only one measurement above 32°C. The extreme variability, especially of the heat-drought treatment, make it difficult to draw specific conclusions. I would only conclude that very high rates are possible in heat-drought but low rates are also possible, possibly reflecting dying leaves.

Reply:

The bin-averages 32–35°C and 35-40°C in the control treatment consist of one measurement point each. Since these points did pass the quality control, we did not exclude them from the fit, but used a lower weight which was calculated for each bin average using the inverse standard deviation (in case of n=1, SD was artificially set to 100; we will add this to the Methods section in a revised manuscript)

In this case SE refers to the fit of EF, since the uncertainty of this parameter in the fit was relatively low (at least for the control and heat data) we got a relatively low standard error. Please consider that we did fit the curves to bin averaged data points and not to single measurement points (which are shown for reasons of transparency). We prepared Table S1 with bin averages and corresponding standard errors to show that bin averaged isoprene emissions for T > 28°C are significantly different (based on a t-test) between the control and the stress treatments as well. In case it is required, we can include this table also into the supplementary of a revised version of the manuscript.

Line 354: %a is a temperature response and not normalized data

Reply:

We apologize for this mistake: we refer to Fig 5b here and will change that in a revised version of the manuscript.

Line 375: Both isoprene synthase and DMAPP availability affect this as recent papers have shown.

Reply:

Thank you for pointing us to this. We will change that accordingly in a revised version of the manuscript.

Line 386: First seen by LoretoSharkey TD, Loreto F (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia95, 328-333.

Reply:

We will add this reference in a revised version of the manuscript.

Line 397: I would also cite the work of Delwiche Delwiche CF, Sharkey TD (1993) Rapid appearance of 13C in biogenic isoprene when $^{13}CO_2$ is fed to intact leaves. Plant, Cell & Environment16, 587-591

Reply:

We will add this reference in a revised version of the manuscript.

Line 404: Sharkey and Loreto saw 67% Sharkey TD, Loreto F (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia 95, 328-333.

Reply:
Thank you. In a revised version of the manuscript we will change that accordingly.

Line 436: I am not convinced of this

Reply:
We have taken this information from the 95% confidence intervals of the fit derived for the control and the heat and heat-drought treatment (these do not overlap completely). In a revised version of the manuscript we will add Fig S1 and Fig S2 which illustrate differences between measured data and the treatments fitted curves in in a better way. Thus, we will change this part of the discussion accordingly to make our point much clearer.

Line 461: This is new and likely to well accepted but the current manuscript does not make a strong enough case for it.

Reply:
Thank you. As mentioned before, we will make this much stronger in a revised version of the manuscript by including new Figures (Fig S1 and S2) and a revised Results and Discussion section accordingly.

References:

Boeck, H. J. De, Dreesen, F. E., Janssens, I. A. and Nijs, I.: Climatic characteristics of heat waves and their simulation in plant experiments, Glob. Chang. Biol., 16, 1992–2000, doi:10.1111/j.1365-2486.2009.02049.x, 2010.

Niinemets, Ü., Monson, R. K., Arneth, a., Ciccioli, P., Kesselmeier, J., Kuhn, U., Noe, S. M., Peñuelas, J. and Staudt, M.: The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling, Biogeosciences, 7(6), 1809–1832, doi:10.5194/bg-7-1809-2010, 2010.

Seco, R., Karl, T., Guenther, A., Hosman, K. P., Pallardy, S. G., Gu, L., Geron, C., Harley, P. and Kim, S.: Ecosystem-scale volatile organic compound fluxes during an extreme drought in a broadleaf temperate forest of the Missouri Ozarks (central USA), Glob. Chang. Biol., 21, 3657–3674, doi:10.1111/gcb.12980, 2015.

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 S1: Bin averaged isoprene emissions (E_{iso}) for different temperature classes including the corresponding standard errors (if n>1). Average values highlighted with * denote that E_{iso} is significantly different (p<<0.05 based on a t-test if the number of measurements exceeded three) to the average in the control group ($^{(*)}$ denotes that E_{iso} between both stress treatments are significantly different).

Treatment	**Control**		**Heat**		**Heat-drought**	
Temperature range (°C)	**E_{iso} (nmol $m^{-2}s^{-1}$)**	**SE**	**E_{iso} (nmol $m^{-2}s^{-1}$)**	**SE**	**E_{iso} (nmol $m^{-2}s^{-1}$)**	**SE**
15-20	1.52	0.01	-	-	-	-
20-24	3.81	0.42	-	-	-	-
24-28	7.33	0.76	4.95	1.23	9.83	2.31
28-32	**16.14**	**1.81**	**9.13***	**0.81**	**10.50***	**1.17**
32-35	23.12	-	16.62	0.82	18.83	1.82
35-40	40.41	-	26.26	1.03	24.86	1.83
40-45	-	-	**37.43$^{(*)}$**	**2.58**	**23.36$^{(*)}$**	**2.60**
>45	-	-	32.35	-	13.54	4.24

Table S2: Air temperature and corresponding leaf temperature including standard errors measured for n tree leafs in the control, heat, and heat-drought treatment. Leaf temperature was measured with an infrared camera on two days during the second heat wave. Differences between leaf and air temperature were not significant (pairwise t-test p >0.05).

Treatment	**T_{air} ± SE (°C)**	**T_{leaf} ± SE (°C)**	**T_{leaf} - T_{air} (°C)**	**p < 0.05**	**n**
Control	21.8 ± 1.9	21.1 ± 1.9	0.7 ± 0.5	n.s.	3
Heat	34.7 ± 1.2	34.4 ± 1.5	-0.3 ± 0.5	n.s.	5
Heat-drought	36.0 ± 0.5	35.2 ± 0.8	-0.9 ± 0.5	n.s.	10

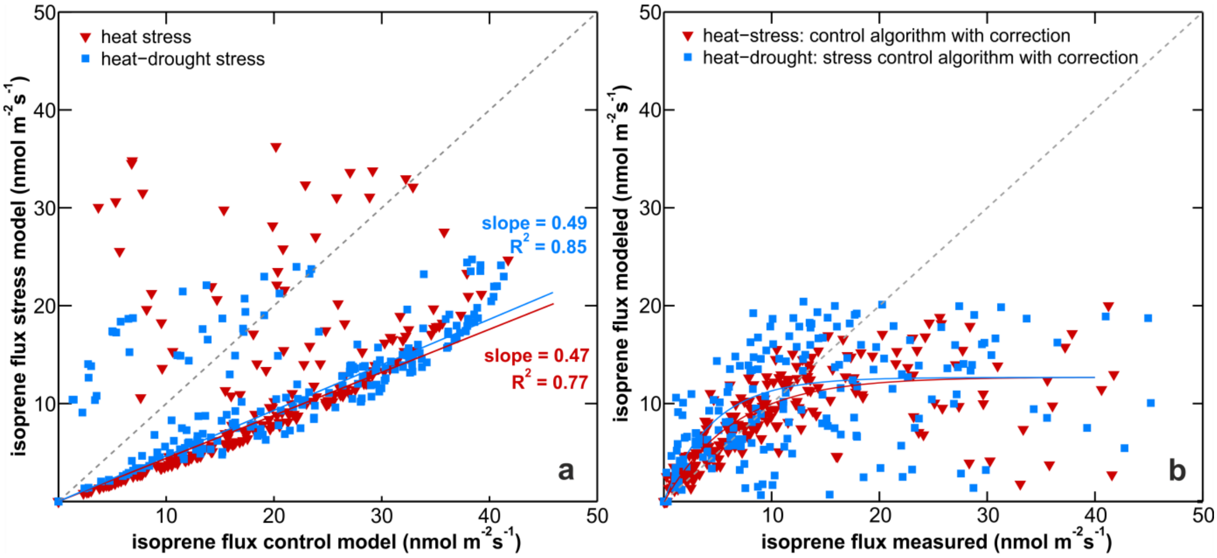

Figure S1: a) Isoprene fluxes of heat and heat-drought stressed trees modeled with the stress algorithm against fluxes modeled with the control algorithm including a linear least-square fit showing the slope which would bring fluxes calculated with the control algorithm in line with fluxes calculated 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.

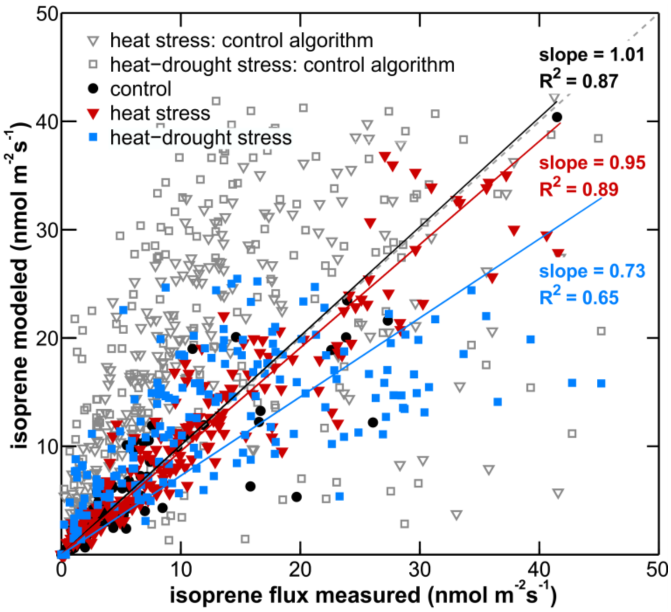

Figure S2: Modelled versus measured isoprene fluxes for trees exposed to control conditions (black circles), heat stress (red triangles), and heat-drought stress (blue squares) including a linear least square fit. Open grey symbols show isoprene fluxes modeled with the control algorithm instead of the 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.