Anonymous Referee #1

Isoprene emissions in relationship to plant carbon cycling during drought and high temperature stress is an important and active area of research with numerous papers on this subject in recent years. Isoprene production protects carbon assimilation processes including stabilizing photosynthetic membranes during high temperature stress through numerous potential mechanisms including excess photosynthetic energy consumption, direct antioxidant activity, physical membrane stabilization, and signaling activities of oxidation products. The present study by Bamberger et al. investigated isoprene emissions and net photosynthesis responses in black locust trees growing under controlled environmental conditions before, during, and after drought and heat treatments. As observed in numerous other studies (e.g. Seco et al., 2015), net photosynthesis and isoprene emissions were coupled during non-stress conditions but became strongly uncoupled during heat and drought stress with substantial decreases in net photosynthesis but a stimulation of isoprene emissions.

General Comments The paper generally lacks any new biochemical and physiological mechanistic description of how isoprene and net photosynthesis can become uncoupled at high temperatures and drought. Thus, it is not clear what new information the new study adds other than reporting these expected results in a new tree species.

> **Reply:**
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> We can understand the reviewer's concern regarding the comment on the novelty of our study, 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. Our study goes beyond usual leaf level measurements in that entire trees are exposed to elevated temperatures instead of controlling single leaves and in that ambient temperature variations were used to derive temperature response curves instead of switching between concrete temperature levels. 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. To further strengthen this point and highlight the novelty of our study, we plan on adding two additional figures to a revised version of the manuscript (see Fig S1 and S2 at the end of the document).

However, some novel aspects of the work include a characterization of the light and temperature responses of isoprene emissions during stress. However, the very low light saturation of isoprene emissions of both control and stressed trees (200-300 micromol/m2/s) indicates that the plants were not adapted to normal high light conditions of plants in natural ecosystems during the growing season). As only leaves from the lower canopy were measured, it is difficult to understand how these results can be used for modeling of natural isoprene emissions from nature. Studies show that the majority of photosynthesis and isoprene emissions from natural ecosystems occur in the upper canopy leaves exposed to full sunlight.

> **Reply:**
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> We can understand the referee’s concern but lower light levels under controlled compared to field conditions are a common phenomenon. However, there is no reason to think that this should have affected the different temperature responses of isoprene emissions as found in control versus stressed trees.
> The lower canopy measurements owe to the fast growth of black locust trees. Thus, the branch chambers which were initially installed in the mid to upper canopy (about 1.5 in height excluding pots) turned into lower canopy after some weeks of vigorous growth (trees were up to 5 m in height). Moreover, during prolonged stress preferentially the top-canopy leaves were shed, which would contradict top-of-canopy measurements.

Specific Comments: Take care when refereeing to photosynthesis; the measurements are of net photosynthesis not of gross rates of photosynthesis, which can be drastically different under high temperatures.

> **Reply:**
> We are aware of the difference between net and gross photosynthesis. The reviewer is correct that one needs to be specific in language used and clarify that we always refer to the net photosynthesis rate.

PTR-MS signals at m/z 69 are not necessarily unique to isoprene, especially under drought or high temperature where C5 green leaf volatiles can significantly contribute to their signal (Fall et al. 2001). Since GC measurements were not performed, the results cannot be considered quantitative.

> **Reply:**
> We disagree. Fall et al. (2001) showed that during drying of previously wounded leaves of non-isoprene emitters, C5 green leaf volatiles were identified at m/z ratio 69. Such compounds can add to the isoprene signal under certain circumstances which we can exclude in our study. We did not detect any leaf wounding, and also did not see an increase in acetaldehyde emissions which would be expected in case leaf wounding occurred. Moreover, in a similar study, Vanzo et al. (2015) reasoned that artificial cutting of leaves and the subsequent fast dehydration (for example after cutting grass) are not comparable to natural drought progression. In addition, we found isoprene emissions to quickly recover to the control treatment after stress release, which would not be the case if leaves were substantially harmed. For clarity to the reader we will add reference to these studies in a revised version of the manuscript.

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

> **Reply:**
> We will add the indicated reference to the revised manuscript.

References:

Fall, R., Karl, T., Jordan, A. and Lindinger, W.: Biogenic C5 VOCs: release from leaves after freeze–thaw wounding and occurrence in air at a high mountain observatory, Atmos. Environ., 35, 3905–3916, doi:10.1016/S1352-2310(01)00141-8, 2001.

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.

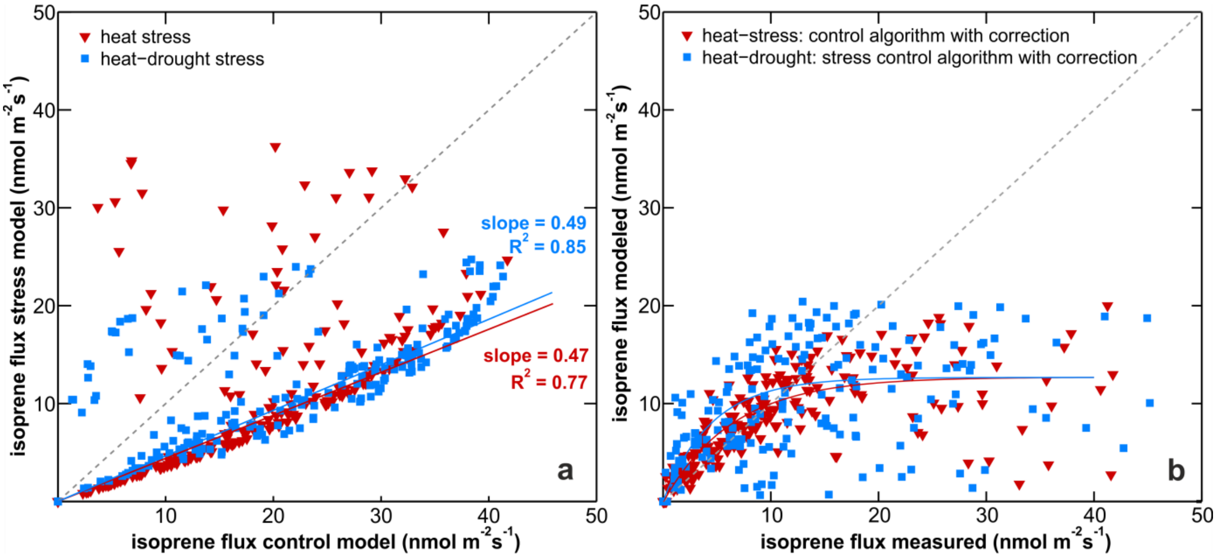

Figure S1: a) Isoprene fluxes of heat and heat-drought stressed trees modeled with the stress algorithm against fluxes modeled with the control algorithm including a linear least-square fit showing the slope which would bring fluxes calculated with the control algorithm in line with fluxes calculated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

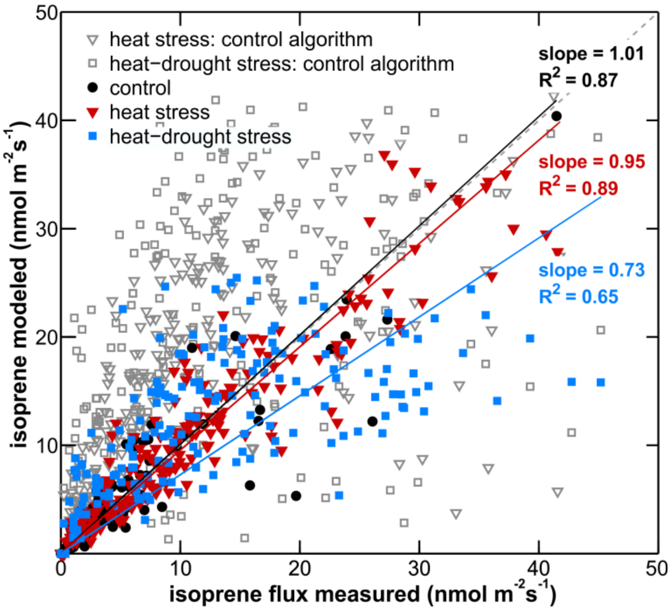

Figure S2: Modelled versus measured isoprene fluxes for trees exposed to control conditions (black circles), heat stress (red triangles), and heat-drought stress (blue squares) including a linear least square fit. Open grey symbols show isoprene fluxes modeled with the control algorithm instead of the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