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Interactive comment on "^{13}C labelling study of constitutive and stress-induced terpenoide missions from Norway spruce and Scots pine" *by* Cheng Wu et al.

Anonymous Referee #1

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This study investigates de-novo and pool emissions of different terpenes in response to biotic stress. In general terpenoid emissions induced by biotic stress are a very important, timely and interesting topic and it is a good idea to investigate if and how de-novo and pool emissions differ in response to biotic stress. However my major concerns are that there are only three trees per species which are all at different states of healthiness before the start of the measurements. One of each species seemed to be healthy one per species infested with aphids, one of the trees seemed to be disturbed by spiders (which was attributed to stress), and for one of the trees white floccules were attributed to stress. This leads to a n=1 for each treatment and species. This point together with different climatic conditions within the chambers during

measurements (e.g. different temperatures) makes the paper very problematic. Without replicates, information on the severity of stress, and without comparable conditions during the measurements I don't see in which way this paper would increase our understanding how trees respond to biotic stressors with de-novo and pool emissions. There is no information about tree to tree variability, the severity of stress, how the trees behaved before stress, and how different temperatures or seasonality during the measurements (taken between May and October) could have biased the measurements (additionally to biotic stress) and a proper statistical analysis is not possible. Additionally the stress response to biotic stressors is known to be dependent on severity of stress (Niinemets et al., 2013). Although more quantitative information about stress responses due to biotic stress is required to increase the scientific understanding the manuscript in the current state is unfortunately not able to deliver defendable information.

Major comments:

(1) There is no quantitative information about the severity of stress, how long the trees were influenced by biotic stress and how spiders or white floccules would influence stress. This makes it difficult to judge how severe stress was.
(2) In principle the complete information given in this paper is based on one tree n=1 (one healthy tree, one infested with aphids, and one suffering from some other potential biotic stressor) and there are no time-replicates later on or before stress. Consequently it is not known how the infested trees were behaving before they were exposed to aphids (or other potential biotic stressors) and if there were potential differences between trees already before stress.
(3) The experimental description is very short and important information is missing. There is neither information on temperature variations within the climate chambers nor

if and how temperatures within the plant chambers were measured or kept constant. Additionally information about relative humidity or CO2 within the chambers is not given. Also the time of measurement of single trees would be important. According to Table S1 temperatures during the measurements differed considerably (between 18°C and 30°C) for different trees. With the information currently given it is not possible to judge if changes between the trees are, at least partially, due to different climatic conditions, seasonality or history. At least the essential information to understand what was done and how this possibly affected the trees should be transparent.
(4) Between May and October is a quite long time frame which also means that the trees were additionally measured within different seasons. Was a single tree stored outside until the experiment was performed? This in turn would mean that (apart from season) trees possibly experienced different climatic conditions several weeks before they were measured)? Without this information it is not possible to judge if other conditions than biotic stress e.g. different temperatures or dry conditions could have led to changes in emissions.

Specific comments:

(1) Page 1, Line 10-11: I agree that BVOCs are important for atmospheric chemistry but I doubt that it is due to their large source strength since the source strength is rather small compared to the source or sink of CO2, for example. It is rather the high reactivity which matters. . .
(2) Page 1, Line 17-19: It is hard to understand what you want to say with this sentence – consider rewriting.
(3) Page 1, Line 20: 'The comparison allowed the following comprehensive statements. . .' This sentence seems to be redundant.
(4) Page 1, Line 23: You talk about three different carbon sources but you just refer to

two 'assimilated CO2' and 'other alternative carbon sources'. The third carbon source is missing. . . (5) Page 3, Line 8: 'and in some cases stress-induced terpenoids' is a very general statement. It would be better to specify at least that you are talking about biotic stress.
(6) In the abstract it is stated that 'stress induced monoterpene and sesquiterpene emissions are entirely of de-novo nature' which is not reasonable since the storage pools are still present and it is more than likely that stress induced also changes in constitutive emissions which were not de-novo synthesized. I guess you wanted to refer to the compounds which were classified as stress compounds since they are known to be de-novo emitted in response to stress. In this case the argumentation is somewhat circular.
(7) Materials and Methods: see Detailed comments above
(8) Page 6, Line 29: Is it justified to assume that all molecules with excess 13C-atoms are de novo emissions? What if de novo synthesized molecules are incorporated to storage pools?
(9) Page 7, Line 12-13 and Fig 2a: I am not sure if 'stress induced emissions' is the right terminology for the separation you chose. . . constitutive emissions are also very likely to change in response to stress and these changes are also stress induced. At least this needs to be discussed
(10) Fig 2a: It is a bit tricky to draw any conclusions without proper statistics which is unfortunately not possible with one tree per treatment and without time-replicates see also the general comments above
(11) Page 7, Line 19-20: you state 'the detailed emission patters were different for individual plants' why were they different? Due to the stress or due to tree to tree variability? Another unknown factor without proper statistics.
(12) Page 8, Line 12- 13: Why did you choose the constitutive compounds β – pinene and myrcene but not the other constitutive compounds like α-pinene or limonene?
(13) Section 3.2. You stated that you assume that labelled molecules are exclusively de novo emissions on the other hand you find constitutive emissions to be labelled.

Following this argumentation would mean that these constitutive emissions are also de novo and not from storage pools?
(14) Section 3.3.2: Why do you show only (E)-β-farnesene? How do time-series of labelling patterns of other terpenes look like? Similar?
(15) Page 11, Line 14-15: Was this shown in the results?
(16) Page 11, Line 16-17: To my opinion this is also a consequence of your definition of stress-induced monoterpenes.
(17) Page 12, Line 5: '. . .relatively low' Were this nocturnal fluxes above the limit of detection for fluxes or not?
(18) Page 12, Line 5-25: An interesting question to discuss would also be if stress-induced emissions of mono- or sesquiterpenes need to be light dependent or if their light dependency follows the same function as for isoprene (partly discussed in section 4.3). I guess it is not possible to extract light response curves from your measurements?
(19) Section 5: Summary or Conclusion?

Reference:

Niinemets, Ü., Kännaste, A., Copolovici, L. (2013). Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. Frontiers in Plant Science, 4, 1–15. doi:10.3389/fpls.2013.00262

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