

## ***Interactive comment on “<sup>13</sup>C labelling study of constitutive and stress-induced terpenoid emissions from Norway spruce and Scots pine” by Cheng Wu et al.***

**Anonymous Referee #2**

Received and published: 24 September 2017

In the present work the authors aimed to contribute to the understanding of the origin carbon ending up in the emission of volatile terpenoids in conifers, i.e. Scots pine and Norway spruce. In particular the authors aimed to separate constitutive emissions coming from storage pools (resin ducts) in needles and bark tissue from constitutive and stress-induced emissions originating from recently fixed carbon intermediates (de novo). Studying the carbon origin of isoprene and monoterpene emission not novel. It started in the 90ties of the last century and was driven by the interest in understanding the link between light-dependent isoprene and monoterpene emission of trees with no distinct storage structure for terpenoids. For understanding the origin of carbon ending up in terpenoid emissions the situation in conifers is rather complex due to the

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presence of these molecules in storage structure in needles and bark tissue where the can be released by temperature-dependent evaporation or mechanical destruction of resin reservoirs. Hence only a few studies on this topic exists. The present work uses <sup>13</sup>CO<sub>2</sub> and <sup>13</sup>C-glucose feeding to determine the amount of <sup>13</sup>C in monoterpenes and sesquiterpenes released from needles (bark) of tree coincidentally infested by insects and pests when grown under natural conditions. This arbitrary selection of plant material resulted in an individual response pattern of each plant reflected in different emission rates and pattern of i.e. stress-induced mono- and sesquiterpenes. Despite this limitation in homogeneity the experiments were performed with high accuracy enabling some conclusions about the carbon origin in terpenoids. Overall, the data are not novel, nevertheless adding new and welcome information on the origin of terpenoid compounds in the emission conifers. Understanding of this trait is still of great importance for the development of new/better emission algorithms to be implemented into BVOC emission models.

Overall the work is technically performed very well and the methods are comprehensively described. In particular the description of the calculation of <sup>13</sup>C incorporation into terpenoid compounds is important to understand and interpret the results. However, due to the uncontrolled treatment /plant cultivation and hence the very individual response of each plant, overall conclusions on the fractions of constitutive and de novo-synthesized terpenoid cannot be drawn seriously, weakening strongly its relevance to the field.

Specific comments:

Concerning night emissions of de-novo synthesized mono- and sesquiterpenes. I agree that is very interesting and not well documented. However, not surprising: E.g. In some floral tissues (see e.g. papers of Dudareva and colleagues) sesquiterpene emissions peak during night, indicative of a highly active MVA pathway, which is circadian regulated. Also monoterpene biosynthesis in roots and during resin duct formation in wood is not depending on light. Moreover, many sesquiterpene synthases are bifunc-

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tional, either using C10 or C15 precursors depending on substrate availability. Gene expression analyses show that MVA pathway genes are more expressed during night while transcription of MEP pathways genes is higher during the light phase. There are many indications that the MVA pathway is more active during darkness, while the MEP pathway mostly works in the light. Your discussion is very general. Please check more actual literature on that issue. I agree that the labeling of sesquiterpenes with  $^{13}\text{C}$  is more variable probably due to the multiple sources of carbon ending up in the cytosol, compared to the situation in the plastids, therefore the light-dependency of sesquiterpene emission cannot be as tightly linked to photosynthesis as is the case for de novo synthesized monoterpenes. The regulation of the MVA pathway is not widely unknown. Please check literature and update your discussion. p.14 line 31: must be . . . . MVA derived IDP (isopentyl pyrophosphate) and DMAPP. . . . P15 line 26/27: In Taipale et al 2011 and Ghirardo et al. 2010, no stress-induced emissions were observed. Therefore, they couldn't be taken into account in these studies. There are a few more studies giving a ration between constitutive and de novo based emissions in conifers. The values in the present work are somehow lower (excluding the stress-induced emissions). Do you think it can be related to your culture conditions and the time analysis? It might be that the expression of terpene synthases responsible for the constitutive de novo emissions are highly variable and therefore the values are more scattering and lower compared to other studies. Please be more precise in your discussion.

Table 4: Please explain in the legend the abbreviation of RC-meas and Riso\_meas  
Figure 1: Please make the legend more explicit describing that the scenarios 1 and 2 reflect the bi-modular overlay of unlabeled (likely from storage or old carbon) and completely de novo synthesized compounds.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-260>, 2017.