Reply to Referees' Comments

The authors would like to thank the referee for the detailed review of the manuscript and the helpful comments that will help us to provide an improved version of the manuscript. We address all the comments below and we will further integrate the corresponding changes in a revised article.

Reviewer 1

General comments:

The authors investigated the BVOC emissions from Sitka spruce under laboratory conditions. Sitka spruce is an important tree species and is commonly used as part of afforestation programs. Using a combination of TOF-CIMS and TD-GC-MS techniques, the authors were able to detect many compounds that have not previously been reported in the emissions from Sitka spruce. The authors also investigated the temperature/PPFD response and emission pathways of the different BVOCs to determine if they originated from storage pools, biosynthesis, or both. The authors then extrapolated their results to estimate the annual BVOC emission fluxes for all Sitka spruce plantations in Ireland.

The main weakness of this study is the low number of plant replicates used. Only 3 seedlings were sampled in this study, and one of them (Spruce 3) was unhealthy and its emissions are not reported. Furthermore, there is a lot of variability in the BVOC emission profiles, temperature/PPFD response, and CO_2 fluxes of the two remaining seedlings (Spruce 1 and 2). For example, isoprene was the second highest emission from Spruce 1, but was only emitted at trace levels from Spruce 2. The authors also note that Spruce 2 might have experienced mild stress, which may be why its emission behaviour was different from Spruce 1. It is hard to obtain meaningful/convincing statistics and to draw generalized conclusions about the emission behaviour of Sitka spruce from these results. I would strongly recommend that the authors conduct measurements on additional Sitka spruce seedlings. There should be at least 3 healthy plants to obtain meaningful statistics and to account for intraspecific variability. There is only one healthy plant (Spruce 1) and one mildly-stressed plant (Spruce 2) in the current study. And if the authors wish to compare the emission behaviour of healthy vs. stressed Sitka spruces, then ideally, they should include 3 stressed plants in addition to the 3 healthy plants.

Author reply (AR): The authors understand the referee's point of view because this study initially intended to replicate all measurements on three healthy plants. Although the aim of the study was not fully realised, we do believe that the data included in the present paper are robust and provide valuable new information for the scientific community that has not been published elsewhere. In particular, the authors would like to highlight the following aspects of this work:

- *The novel experimental set up combining complementary online ToF-CIMS and offline TD-GC-MS for non-target analysis of the emissions.*
- *Measurements performed for seven days on diurnal cycles for the three modalities (temperature, light, and daily cycles) to make the results robust.*
- *Identification of 74 BVOCs in the emissions, while less than 10 were reported in previous studies.*
- *Piperitone identified as the main emitted BVOC while it was only reported as a minor emission previously*
- *First study to obtain a comprehensive understanding of the emissions pathways (pooled and de novo) for Sitka spruce*
- *New evidence to show that the BVOC emissions are best modelled using a combination of pooled and de novo pathways. This combined model is currently not included in large scale models.*

Considering this study represents a very large amount of experimental work and data analysis, it is impossible to conduct additional measurements on other spruce plants within a short timeframe. In addition, it would not be possible to find spruce saplings with the same genetic origin as the ones used in the present study, which would introduce a bias. However, we accept that using emissions from one healthy tree to provide estimates of emissions for scaling up purposes and carbon balance is quite uncertain. As a result, we will significantly reduce this part of the article in a revised manuscript.

Line-by-line comments:

L79: Since these are not mature trees, I would suggest replacing the word "tree" with "seedling". Please also provide the height of the 3 seedlings used in this study.

AR: These are young trees with a height of 60 - 75 cm. We will replace the word "tree" by "sapling", which we believe to be more appropriate than "seedling".

L89: Please provide the manufacturer and model of the lamps, if available.

AR: The lamps were Panasonic FL40SSW/37-PRF3 Fluorescent Lamps (added to the manuscript).

L90: PPFD is usually expressed in units of µmol m^{-2} s^{-1}. Please correct this.

AR: This will be corrected in the revised manuscript.

Figure 1: Recommend replacing "Ball Meter" with "Ball Flowmeter" to make its purpose clearer to the reader.

AR: This will be corrected in the revised manuscript.

L99: Why is there such a large difference in the flow rates into each enclosure? Large differences in flow rates can result in different humidity levels in each enclosure, which in turn may affect the BVOC emission rates. For future experiments, it would be advisable to try to equalize the flow rates into each enclosure using mass flow controllers (or more cost-effective needle valves).

AR: Thank you for the comment. Firstly, the original manuscript contained an error – the flow did not vary between 1.5 and 7 L min^{-1}, but between 4.5 and 7 L min^{-1}. This will be corrected in the revised manuscript. Secondly, we agree that it is better to ensure the same flow when enclosures are identical, but in our case, the volume of the enclosures surrounding the saplings was slightly different. Consequently, we adjusted the flow through the enclosures to compensate for the volumetric variations and to keep the residence time as similar as possible between different enclosures.

L110: The relative humidity inside the miniature enclosure may not be the same as the RH in the other enclosures, as the RH would be dependent on the amount of foliage in each enclosure, the flow rate into the enclosure, and the transpiration rate of the enclosed plant.

AR: True, the relative humidity was probably a little bit higher in tree enclosures, but it is not expected to affect BVOC emissions, as to our no knowledge there is no study showing impact of air humidity on BVOC emissions. In addition we did not observe and condensation on the enclosure walls, which would favour wall deposition, especially for oxygenated BVOCs

Section 2.2: Please state the detection limit and accuracy (measurement uncertainty) of the TOF-CIMS and TD-GC-MS.

AR: Regarding TD-GC-MS, standards were used for qualitative identification purposes only. For ToF-CIMS measurements, the detection limit and accuracy is different for each VOC, but based on the background and calibration tests, the detection limit is around 10s of ppt for monoterpenes, with a measurement uncertainty typically at 30%, in agreement with a previous study using benzene ToF-CIMS (Lavi et al., 2018). To identify an emitted VOC, we used a statistical test (Welch's t-test) to select compounds with ion signals that are 3 times higher than the empty enclosure, meaning that we did not focus on ions that were only present in very small amounts. Taking this into consideration, only compounds with concentrations higher than ca. 0.1 ppb in the enclosures were analysed.

L155: Just to confirm, are you sure that the heated transfer line was connected to a split/splitless injector, and not directly to the GC column?

AR: The transfer line was directly connected to the GC column. This will be amended in the revised manuscript.

L159: The phrase “fire purge” is a bit misleading. I think you mean to say “pre-trap fire purge”, or “pre-desorption purge”.

AR: Thanks. The correct terminology is indeed pre-trap fire purge. This will be corrected in the revised manuscript.

L162: You listed the dimensions of the GC column as 60 m × 0.3 mm × 1.8 µm. 0.3 mm is not a standard column ID. Please specify whether the column inner diameter (ID) was 0.25 mm or 0.32 mm.

AR: The column inner diameter was 0.32 mm. This will be added in the revised manuscript.

L163: For clarity, please specify if the pressure was 23 psig or 23 psia.

AR: The pressure was 23 psig. This will be added in the revised manuscript.

L209-210: These PPFD levels seem quite low. For comparison, can you provide the typical PPFD levels expected in Irish summer conditions?

AR: It is true that these PPFD levels were rather low, but they are representative of the summer daily average PPFD in Ireland, as indicated by Met Éireann, the national meteorological organisation in Ireland. However, peak PPFD levels can sometimes rise to 1000 µmol m^{-2} s^{-1}, see for example figure 1 in Byrne et al. (2005).

L229: How were the Tenax tubes sealed? Did you use Swagelok brass caps?

AR: The Tenax tubes were sealed using Swagelock brass caps fitted with Teflon ferules, which were first sealed by hand and then tightened further using the tool supplied by the manufacturer. This clarification will be added to the revised manuscript.

L232: Is it possible that the plant biomass might have changed during the BVOC measurement period?

AR: Yes, the plants grew slightly during the measurement period, but we believe that the biomass did not increase significantly (compared to total biomass enclosed). Any changes would be hard to quantify, as dry biomass weighing is a destructive method.

L243: The “Flow” term is missing from Eq. 1. Please correct this.

AR: This will be corrected in the revised manuscript.

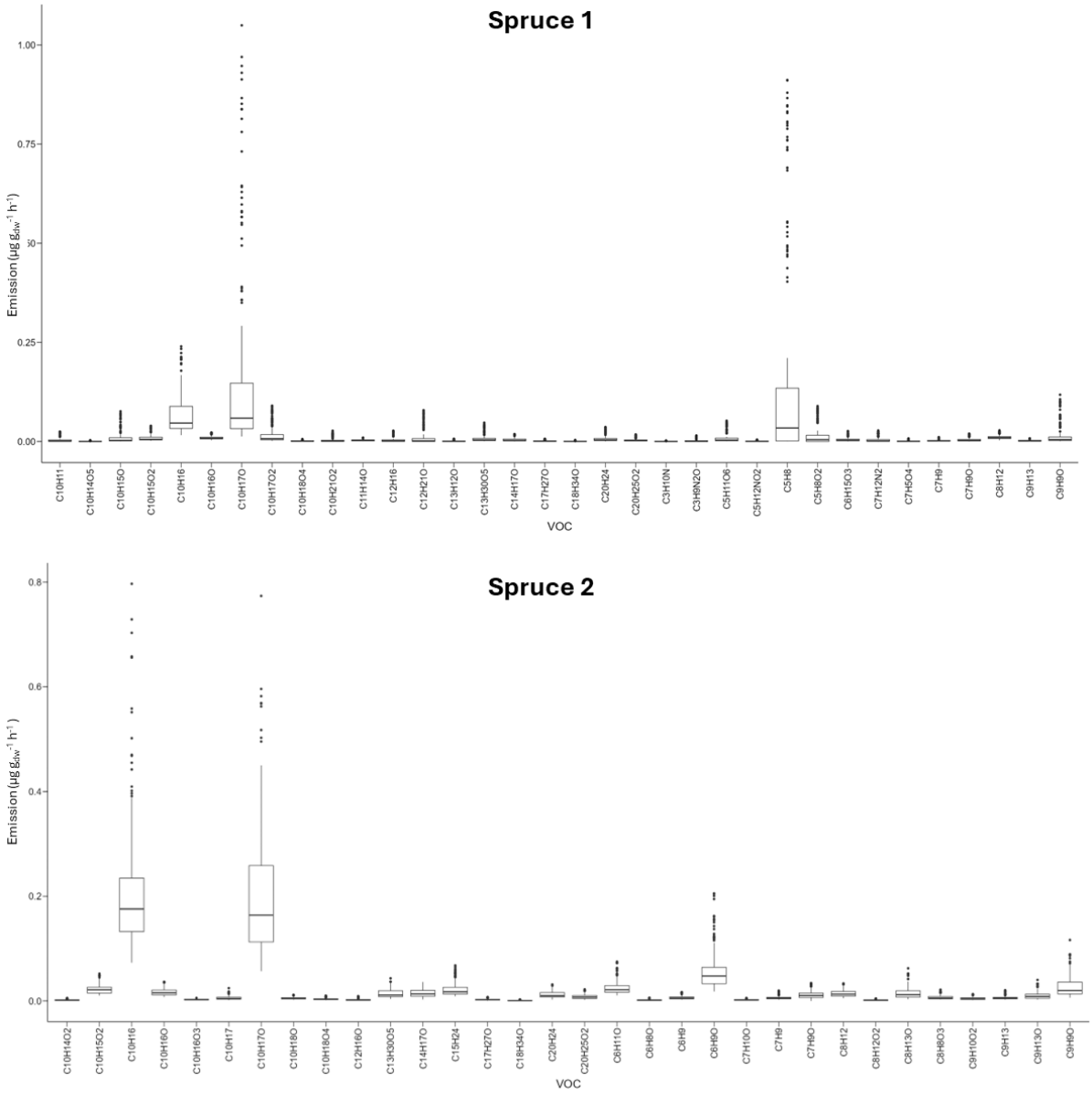
L278: Some monoterpenes originate from de novo biosynthesis and show both temperature- and light-dependency. For example, see study by Ghirardo et al. (2010) on Norway spruce and other species:

https://doi.org/10.1111/j.1365-3040.2009.02104.x

AR: Thank you for this comment, we will add this detail in the revised paper with the reference Ghirardo et al. (2010)

Section 3.1: Can you include a figure that shows the relative contributions of individual compounds to the total BVOC emissions from Spruce 1 and Spruce 2? For example, you mention that myrcene was the dominant monoterpene emitted from both spruces, followed by β-phellandrene, but this is not illustrated in any of your figures/tables.

AR: Thank you for this comment, it is true that this information was not included in a plot in the original manuscript, even if this can be inferred from the table of standardisation emission fluxes in the SI (BVOCs are listed in order of decreasing standardised BVOC emission flux). We will insert a figure in the Supplementary Information to support our statement, in which boxplots representing median emissions and quantiles (5, 25 75 and 95) of each individual VOC are represented (see figure below). Identification of the main isomers (for example, myrcene as the dominant monoterpene) was inferred from chromatogram peak areas in the TD-GC-MS data. This information is available in the dataset associated with the paper.



Box plot for Spruce 1 (top) and Spruce 2 (bottom) BVOC emissions (in µg g_{dw}^{-1} h^{-1}). The bar represents the median, the bottom and top limits of the box are the 25th and 75th quantiles, and the end of the bottom and top bars represent the 5th and 95th quantiles.

L392: Can you specify which BVOC species showed a pronounced increase in emission upon illumination, and which had a more muted response?

AR: Most of the BVOC emissions showed a pronounced increase upon illumination. The ones which had a more muted response are: $C_5H_{10}O_6$, C_9H_8O, $C_{12}H_{20}O$, $C_{10}H_{14}O$, $C_7H_{12}N_2$, $C_6H_{14}O_3$, $C_{12}H_{14}$, $C_{10}H_{10}$, $C_{20}H_{24}O_2$, $C_{10}H_{20}O_2$, $C_3H_8N_2O$, $C_{10}H_{18}O_4$, $C_7H_4O_4$, $C_{18}H_{34}O$. We can add this specific information to the manuscript.

L402: Is it $C_{10}H_{14}O$ or $C_{11}H_{14}O$?

AR: Thanks. It is $C_{11}H_{14}O$ and will be corrected in the revised manuscript.

L404: What is the average lifetime of the post-illumination bursts observed in your study?

AR: In this work, we used a multivalve to switch between each sapling every 7.5 minutes, which did not allow for identifying the exact timing of the spike in emissions. As a consequence, the post-illumination burst was only observed during a single measurement, indicating that it was a short event (less than 30 minutes) and had a time frame similar to that measured by Hayward et al. (2004). The post-illumination burst was reproducible (the duration and ion signal intensity were similar) through the daily cycle for the six compounds that exhibited this behaviour.

L522-524: In the text, the CO_2 flux is reported in units of nmol s^{-1} g^{-1}, but in Figure 9, the units for the CO_2 flux are in nmol h^{-1} g^{-1}. One of these units is wrong, please correct it.

AR: Thanks for catching this. The correct unit is nmol g^{-1} s^{-1}. It will be changed in Figure 9.

L569-571: Furthermore, the value calculated in this work comes from a single Sitka spruce seedling, and therefore may not be an accurate representation.

AR: We agree that more saplings would be required to have a robust statistical evaluation of interindividual variability. Nevertheless, we still believe the estimation (that BVOCs account for ~0.2 of C assimilation) is useful information and worth reporting. We will reduce section 3.4.1 to a single paragraph integrated into the CO_2 flux discussion and place Table 1 in the Supplementary Information.

L582: Fig. 9 shows the time series of CO_2 flux. Please cite the correct figure.

AR: Thanks for spotting this. We will cite the correct figure in the revised manuscript.

L582: Do you mean Table S6 and S7?

AR: Thanks again. Yes, this will now be corrected.

L599-600: Some monoterpenes originate from de novo synthesis and show both temperature- and light-dependency, e.g., see Ghirardo et al. (2010). You have also shown this in your own results.

AR: This is true. We will therefore revise the text in this part as follows:

"This was unexpected, especially for monoterpenes which are usually assumed to be emitted solely from storage pools (Fuentes et al., 2000) and thus entirely temperature dependent with no PPFD dependence (Hayward et al., 2004). A previous study already observed that monoterpene emissions from several boreal species (Pinus sylvestris, Picea abies, Larix decidua and Betula pendula) may also depend on light due to a significant contribution of de novo emissions (Ghirardo et al., 2010)."

L686: It might not be appropriate to scale the emission results from a single seedling to determine the BVOC emission fluxes for all Sitka spruce plantations in Ireland. For a more accurate representation, measurements on additional seedlings would be required to account for intraspecific variability.

L705-710: Furthermore, the BVOC emissions of mature trees can differ from those of younger seedlings.

AR: Taking these two comments together. We agree that BVOC emissions from young and mature trees may differ and that scaling the emission results from one tree has limitations. We will therefore revise the discussion section, by significantly reducing the text describing the scaling effort and placing Table 3 in the supplement. But we do believe it is interesting to report that depending on environmental conditions, the dominant BVOC emitted by Sitka spruce could change, and that this effect is due to the non-linear relationship with temperature and light resulting from the specific combination of pooled + de novo pathways. As a result, we plan to retain the discussion about the effect of environmental drivers on the emissions.

Supplementary Information:

Table S2: What do the different colors (red and yellow) represent?

AR: Yellow and red colours help to visually identify compounds emitted by Spruce 1 and 2 respectively. This will be explained in the table caption.

Figure S7 and S8: Please confirm whether the units for the CO_2 fluxes are supposed to be in nmol s^{-1} g^{-1} or nmol h^{-1} g^{-1}.

AR: The units will be corrected in both figures.

Table S6 and S7: The units for PPFD and emission flux are wrong. Please correct these.

AR: This will be corrected in a revised manuscript.

Technical corrections:
L13: The scientific name (Picea sitchensis) should be italicized.
L25: Replace “Stika spruce” with “Sitka spruce”
L62: Replace “Stika spruce” with “Sitka spruce”
L83: Replace “For identification purposed the trees were named;” with “For identification purposes the trees were named:”
L110: Replace “Viasala” with “Vaisala”
L155: Replace “Agilent 5977B MDS” with “Agilent 5977B MSD”
L297: Replace “Fig 2 and S2” with “Fig 2 and Table S2”
L402: Replace “six BOCs” with “six BVOCs”
L446: Replace “measure data” with “measured data”

L490: Replace “measure data” with “measured data”
Figure 8 caption: Replace “measure data” with “measured data”
L589: Replace “Stika spruce” with “Sitka spruce”
L600: Replace “Stika spruce” with “Sitka spruce”
L729: Replace “Stika spruce” with “Sitka spruce”
L747-751: The units for PPFD are incorrect.
AR: Thanks very much! All of these technical corrections will be made.