Review of Witter et al. paper

This is a well written paper describing the dynamics of $\delta^{13}C$ allocation to respiratory paths for beech and spruce trees subjected to long-term chronic O_3 exposure. As a result, I have very few comments to make that could substantively improve the paper. The use of ISOFACE to label trees with ^{13}C-depleted CO_2 is a unique system that has great potential to yield important new information regarding the movement and fate of recently assimilated CO_2 in large, mature trees. Although there is considerable discussion in the literature about the merits of a variety of techniques for assessing the fate of recently assimilated photosynthates (girdling, pulse labeling with enriched or depleted $^{13}CO_2$ or $^{14}CO_2$ – see also Dannoura et al. (2011)) as well as whether it is possible to follow the speed of photosynthates using isotopic labeling alone (see letter by Kayler et al. (2010) and reply by Mencuccini and Hölttä (2010)), the use of pulse labeling in this study seems justified and the results appear to be correctly interpreted.

Given the current discussion in the literature about technical limitations for determining the fate of recently assimilated carbon, I might ask if the conclusions expressed by Mencuccini and Hölttä regarding the suitability of pulse labeling for studying soil respiration are of any concern here? Certainly the conclusions reached by Witter et al. in this paper concerning CO_2 efflux from trees and the fraction of newly assimilated ^{13}C in respired CO_2 are bolstered by the detailed and thorough description of the ISOFACE system provided in Grams et al. (2011) as well as the discussion of the potential sources of error in this paper, and the data are consistent with that reported in other papers coming from this research group (i.e. Kuptz et al. 2011a, 2011b, Nikolova et al. 2009).

In the methods section of previous papers from this group, where repeated measures analyses of variance were used, those authors made mention of that fact, but it is omitted here. I would suggest that a sentence or two be inserted in the Statistical Methods section (2.11) regarding these analyses (see Figures 2 and 3). Also, according to Table 3, paired t-tests were used to detect differences among stem positions and coarse roots. However, it is not clear what was paired. Was upper stem compared to coarse roots, and then also to lower stem? Such comparisons run the risk of elevating the experimental wide error rate. Of course, the differences are so large that this will not change the conclusions, but I thought the authors might justify their statistical approach here perhaps. A similar mention of paired t-tests in Figure 4 leaves the reader confused as to which two items are being compared, so maybe the authors could clear that up.

There is no mention made of the potential impacts (or lack thereof) on photosynthesis from elevating the CO_2 concentration during the stable isotope labeling period. The elevation amounts to almost a 30% increase in concentration over ambient levels prior to labeling, which should certainly affect instantaneous rates, even if briefly. The authors do note that stomatal conductance was most likely not affected, and that C_i to C_a ratios were altered only slightly, but rates of net photosynthesis must have increased somewhat. Perhaps a sentence or two regarding this could be made in the Discussion section. High CO_2 can ameliorate O_3 effects in some species, so would the conclusions drawn from this study be affected if the actual O_3 effect was slightly attenuated during the labeling period?

The authors were careful to account for most sources of variation during the labeling period, such as atmospheric and soil CO_2 concentration and isotopic composition, and therefore I feel confident that the patterns elucidated represent actual changes in tree physiology due to species, organ and O_3 treatments and are not artifacts of the pulse labeling procedure.

Considerable effort has been made to utilize appropriate mixing models to explain transitory changes in $\delta^{13}C$ and the fraction of $^{13}CO_2$ efflux arising from newly fixed photosynthate. Furthermore, it appears that the data have been properly interpreted with respect to sources, pools and storage within the tree, and with regard to the purported effects of O_3 on these patterns.

One item that has not been addressed is the fact that the high O_3 treatment was 2X ambient, which is fairly high relative to current O_3 levels. Perhaps some statement could be made about the relevance of the treatment effects found at 2X ambient, given the known current and projected ambient O_3 conditions for that region.

Finally, I think the paper would benefit from some brief speculation on why beech and spruce differ in their carbon allocation responses after O_3 exposure, perhaps by using the model proposed by Kuptz et al. (2011a) in their paper on seasonal respiratory carbon allocation patterns in these trees.

I have made a few minor suggestions to correct or improve the English and they are given after the references cited below.

In conclusion, this paper uses an innovative labeling procedure that clearly shows an O_3 effect on carbon allocation and respiratory patterns, and in particular how O_3 can negatively affect the C sink strength in trees. In addition, it shows the utility of ^{13}C pulse labeling for understanding C translocation patterns in large, mature forest trees as well as C fluxes through forested ecosystems.

References

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Typos and Suggested Grammatical Changes to Text

Abstract, line 21 – “decreased” should be “decrease”

Section 2.3, line 21 – insert “a” before “combined”; line 22 – insert “a” before “standard”

Section 2.4, line 12 – change “was” to “were”; line 13 – insert a comma after 2 x O_3; line 14 – move “was” to before “increased” on the next line; line 16 – change “similar each” to “each similar”

Section 2.8, line 8 – should it be “12,500” with a comma rather than a period?

Section 3.1 – The authors say that “both species displayed 1 to 4 times higher (beech) and 1 to 2 times higher…” However, 1 X to me would be no change so to say it is higher is technically incorrect. Perhaps this sentence could be reworded to avoid this dilemma.

Section 3.2, line 12 – change “was” to “were”

Section 3.3, line 8 – change “was” to “were”

Discussion, page 4146, line 5 – change “fits” to “fit”; same line - change “increased fine-root” to “increases in fine-root”

Table 2 title – Insert “for” before “each species” in line two of title.

Tables 3 and 4 should include the sample sizes used to determine the means.