

Interactive comment on “Comparison of CO₂ and O₂ fluxes demonstrate retention of respired CO₂ in tree stems from a range of tree species” by Boaz Hilman et al.

Anonymous Referee #2

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This manuscript describes the results of a series of field experiments to measure the Apparent Respiratory Quotient (ARQ) and its influencing factors in 9 different tree species. Sites were located in Peru, Brazil, Panama, Israel, Spain, and the US. The authors sought to obtain estimates of ARQ from trees in different biomes and across seasons, and to test whether ARQ varies with xylem stream characteristics and tree height.

The main points as I understand them are as follows: 1) Theoretically, ARQ should be near 1 in tree stems utilizing carbohydrates as a respiratory substrate. The authors found that ARQ was substantially less than 1 in all trees measured, with a range of

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ARQ from 0.39 to 0.78. Previous studies in tropical trees also found ARQ values less than 1, which suggested retention of respired carbon within the stem. With this paper, the authors extend previous results and show that ARQ values <1 are common in trees across biomes and across seasons.

2) The authors also found that hypothesized explanations for ARQ values <1 related to CO₂ dissolution and transport as the main CO₂ export mechanism were generally insufficient to explain the low ARQ values observed. Specifically, they found that sap flow was not correlated with ARQ, whereas a negative correlation would be expected in the case of CO₂ transport in the stem. Additionally, temperature controls on ARQ are not sufficient to explain the deviation from unity.

This paper has an important result, which is that ARQ values lower than 1 are widespread across biomes, and these low values cannot be explained by dissolution and transport of respired CO₂ in the xylem stream. Overall, while well-written overall, this paper has significant issues with organization and clarity. The introduction is compelling and reads smoothly, but the methods section in particular is difficult to follow. Additionally, the discussion section introduces a new concept to explain the results and dwells on concepts that the introduction stated were not important. There are different methods used and experiments performed at each study site, and this information is not presented logically. Table 1 was very difficult to read due to spacing within the table and it did not contain easily obtainable information about which experiments were performed at which site. I recommend reorganization of these sections. Additionally, I was unsatisfied with the PEPC explanation for the low ARQ values that was only introduced at the very end of the paper. This new concept was introduced without sufficient context, such as 'what is a reasonable value for PEPC fixation?' – there is only 1 true value presented in the table. This concept should be introduced earlier in the paper with proper setup, because as is it feels like a surprise. Finally, I am unconvinced by the authors' assertion that ARQ values from "instantaneous" vs. "steady state" sampling are equivalent because the regression was forced through zero and R² was not

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reported, masking bias that could be present based on previous studies. The authors achieve their original objective to obtain estimates of ARQ in different biomes across seasons, but this important result was obfuscated by a complicated methods section and a scattered discussion section. I recommend significant revisions to this paper that include: reorganization of methods and results sections for clarity, making the discussion mirror the introduction, and improving overall cohesiveness. The authors lay out clear objectives in the introduction, but the discussion has a lot of information in it that isn't set up in the introduction. To make this story more cohesive, the authors should keep their main objectives in mind in the revisions, introduce important concepts earlier in the paper, and make sure the discussion section follows logically from the results presented.

Major comments: I recommend reorganization and attention to consistency in referring to species vs. sites. For example, Figure 4 refers to species, but when presented in the results (lines 260-263), the "Bartlett" and "Harvard" are referenced. The reader should not have to go to Table 1 for reference to understand to which panel in figure 4 the text refers. Sometimes the authors mix species and site names in the same sites, for example in lines 317-318 when they refer to trees in Jerusalem. The reader should again, not have to refer to Table 1 to figure out which species were in Jerusalem.

Site names should be consistent throughout the manuscript. Sometimes sites are referred to by name (e.g. "Hebrew University" or "Carmel Ridge"), sometimes by location (e.g. "Jerusalem" or "Brazil", and sometimes by a more general name like "Israel"). In this example, there is no "Hebrew University" referred to in Table 1 so the reader cannot even be certain which site is being discussed when this term is used. Please be consistent throughout the manuscript with your names for each site.

You assert that ARQ values from "instantaneous" vs. "steady state" sampling are equivalent – however, the regression was forced through zero, and authors only report the slope. Forcing the regression through the origin will mask any main effect bias. There is no reason to assume the regression will go through zero, conversely, Angert et al.



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(2012) showed a large difference in ARQ between these two approaches (Figure 2). You state several times that the methods are equivalent and that there are no measurement effects, but the test used to support the statement is insufficient.

I suggest reorganizing the “measurements made in the site” column of table 1 so it is easier to understand. It would be better if the reader could look at the numbered list of experiments (lines 182-191) and know which experiment was performed at which site. Please have the measurements in the table use the same wording as the subsections in the methods section. For example, the reader should be able to read the section heading 3.3 “Tissue Incubations” and easily discern from Table 1 where these measurements were performed.

The methods section (in particular sections 2.2 – 2.6) is difficult to follow and should be reorganized for clarity. For example, you could try organizing section 2.2 by site, which might make it easier to keep track of which experiment was performed at which site. Or, you could try incorporating the numbered list of experiments (lines 182-191) in the following sections. As is, the section is difficult to follow.

Minor Comments: I found the lengthy calculation in the discussion section (lines 383-393) to be strange. Again, it is nearly the end of the paper, and a new two pool model is introduced and a calculation is performed. Please clarify the purpose of this calculation to answering your overall objectives, and consider how to shorten and make it more conceptual.

The distinction in greenness between Harvard and Bartlett forest is not discernable from Figure 4 (discussed lines 320-322), please clarify.

I was surprised that pre-dawn water potentials were not referred to as a measure of water stress in lines 190-192, especially since stress is stated as a potential explanation for lower ARQ values in the discussion (line 307-308).

Similarly, I was surprised to see the lengthy discussion of lipid storage in stems in lines

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304 to 312, when the authors discount this as a reasonable explanation for lower ARQ values in the introduction in lines 39-41. The introduction made it seem like this was an unlikely explanation anyway, as only a few genera of tree species store lipids in tree stems.

Why does ARQ plateau at 0.7 in the model presented in Figure 1? Shouldn't it plateau closer to 1 if that's what the theory suggests?

Table 1 is very hard to read due to the spacing of text

Figure 3 is graph is good and easy to read, but the two yellow colors on this graph are difficult to distinguish

Figure 4: It is difficult to distinguish colors and symbols on these graphs as many points overlap. Perhaps different symbols for the different heights would help?

Post-hoc comparisons – your methods (lines 249-251) state you did Tukey's post hoc comparisons, but I don't see letters corresponding to the post hoc tests like I'd expect to see on Figures 8 and 5 in particular. Were these tests performed for the corresponding analyses, and if so, why aren't the results on the figure?

Line by line comments: Line 275 – what is the duplicates error?

Please be consistent with the placement of the ARQ measurement type in-text, for example in line 188, "continuous" should be right after "ARQ" instead of at the end of the sentence.

Line 342 should read "must" not "much".

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