

Authors' responses to reviewers' comments on:

Physiological responses to ultra-high CO₂ levels in an evergreen tree species

Reviewer 1

I appreciate the authors' efforts in addressing my previous comments and for the comprehensive revision, which has significantly improved the clarity and overall quality of the manuscript. The writing is clear, and the experimental results are thoughtfully presented and discussed. I only have a few remaining suggestions and questions regarding clarity:

[Response:](#) We thank Reviewer 1 (Dr. Manandhar) for the valuable review of our manuscript, which helped improving it. Below please find our responses to each of the remaining issues.

L31: The sentence "In summary, ultra-high CO₂ may partly compensate for water shortage." This summary sentence is a bit broad and lacks content. Consider rephrasing "water shortage" – a broad term with terms specific to plant physiology, such as "stress" or "limited water availability".

[Response:](#) The sentence was revised per the reviewer's suggestion: "In summary, ultra-high CO₂ may partly compensate for limited water availability".

L35: Consider rephrasing "the very fabric of life", as it feels a bit rhetorical.

[Response:](#) Correct. The sentence was revised per the reviewer's suggestion: "Understanding the implications of elevated atmospheric CO₂ levels on plant physiology is paramount, as it has profound consequences for global ecosystems, water economy, and terrestrial carbon cycling (Bazzaz et al. 1990)".

L37: "a response is triggered" this is somewhat broad and redundant

[Response:](#) The sentence was revised per the reviewer's suggestion: "When the level of CO₂ within the plant diminishes, a regulatory response is triggered within the stomatal guard cells, and the stomata open, allowing diffusive uptake of atmospheric CO₂ (Lawson and Morison 2004)".

L50: "adequate nutrient availability": "adequate" seems redundant

[Response:](#) The sentence was revised: "Fluctuations in nutrient availability, particularly potassium, can influence stomatal conductance by affecting the turgor pressure of guard cells, which control stomatal opening and closing (Lebaudy et al. 2008)".

L157, 159: It's not clear if one week is sufficient for plant acclimation though; Poor et al. 2020 doesn't provide an assessment of acclimation. Also, Poorter et al. 2020 is not currently in the Reference list.

Response: We thank the reviewer for catching the missing reference, which was now added. The text is now clarified: "...treatment periods were limited to just over three weeks (23-25 days), so that all treatments were applied on plants at the same developmental stage. This period is sufficient to allow for plant acclimation to the CO₂ treatment (Poorter et al. 2022). The experiment was conducted between 4 June and 27 August 2023. Plants were first exposed to 400 ppm CO₂, followed by 1600 ppm, and then 6000 ppm, i.e., from ambient to high and ultra-high CO₂. Prior to each treatment, plants underwent an additional one-week acclimation period within the mesocosm chamber".

L183: How long did each CO₂ level last?

Response: The information was added to the text: "CO₂ level was adjusted every 3-4 minutes to 400, 100, 50, 0, 150, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 and 400 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, in this order".

L220: "n=10 per treatment" could be further clarified. It's unclear what does "n" denote.

Response: We thank the reviewer for catching this error. Text was changed to: "n=10 plants".

Fig. 1: A couple of questions regarding clarity:

- The colors of 1600ppm and 6000ppm are difficult to distinguish.
- The x-axis minor ticks are not visible, but seems to correspond to individual days. Clarifying this in axis label would be helpful.
- It remains difficult to interpret which data points the P-values refer to. For example, the first p-value ($P=0.411$) seems to compare one 400ppm and one 6000ppm measurement, and it's unclear if the adjacent 1600 measurement was also included.

Response: Points well taken. The 1600 ppm data points were changed to a lighter shade of grey. X-axis minor tick marks were added for clarity, and the caption was revised for clarity: "**Fig. 1. Leaf transpiration and stomatal conductance are decreased at 1600 ppm CO₂ and remain low at 6000 ppm.** Data points are means of 10 guava saplings subjected to different CO₂ concentrations. Measurements were made with a leaf cuvette. Error bars represent standard errors. *P*-values are from ANOVA on transpiration

and stomatal conductance levels at specific dates (± 1 day), and *, **, and *** indicate differences among CO₂ levels at 0.01, 0.001, and 0.0001 significance levels”.

L364: Potential typo “under PAR of $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ”

Response: We thank the reviewer for catching this error. Text was changed to: “under PAR of $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ”.