Editor of *Ecohydrology*

Sydney, 3rd May, 2016

Dear Editor of *Ecohydrology*,

Please find enclosed our manuscript **“Interactive effects of waterlogging and atmospheric CO2 concentration on gas exchange, growth and functional traits of Australian riparian tree seedlings”**, which we have revised and resubmitted for consideration as a Research Article in *Ecohydrology*.

I hope you find the revised article satisfactory. Please find a detailed description of changes to the article below.

Yours sincerely,

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Responses to Reviewer 1:

Comment: The manuscript is well written, but author needs to discuss about the deficiency of replications because the experiment was conducted under no environmental controlled glasshouse at only one time, this means other background environments can affect the interaction. At least, authors need to show the data of weather environments as figure, temp, radiation, humidity, and please discuss about this point.

Response: I have added information about the environmental conditions under which the experiment was conducted in the Methods (lines 184-189).   
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Comment: The question is what if you repeat this next year, do you believe same results will come out? The treatment started after 90d fro 24d, if you change this timing and this period, how do you think?

Response: Of the environmental variables which were not controlled in this experiment, irradiance is likely to have the strongest influence on the results. I have also added a section in the Discussion which address the reviewer’s comment (lines 395-417).

Comment: You may review the literature compared to your results in terms of weather and also the treatment method.

Response: I have added the lines “As in previous studies of flooding – CO2 interactions, plants were exposed to ambient light conditions (Megonigal et al. 2005; Shimono et al. 2012; Arenque et al. 2014)” (line 187) and “This waterlogging period lies between that of Shimono et al. (2012) (14 days of waterlogging beginning on 14 day old plants) and Arenque et al. (2014) (45 days of waterlogging beginning on 90 day old plants).” (lines 195-196 ) to address this comment.

Comment: axis of several figures are too thin not uniform, fug2 and fig3.

I have fixed the figure axes.

Responses to Reviewer 2:

Comment: My only concern with the study is that the experiment was conducted on juvenile individuals in a greenhouse with very small chambers.

Response: While I acknowledge that the pots used to grow the plants were fairly small, the emphasis in this study was purposely on seedling growth, due to the importance of regeneration to community structure and functioning.

Comment: Plants in riparian zones, especially perennial and woody plants, are acclimated to field conditions. The authors did use similar soils found in the field, which is plausible. Furthermore, woody plants in the field have acclimated rooting systems that surround microbial communities, nutrients, and, more importantly, the plant community as a whole. As a result, the physiological responses of woody plants in the field will not be the same as those from in this study. So I am not too convinced that the results found in this study reflect the actual values in a riparian zone. In addition, flooding in a riparian zone has seasonal/intra-annual variations. These events occur during a specific stage of the plant’s life cycle, suggesting that more focus is needed on the physiological responses during different periods.   
While this problem applies to all indoor experiments, I’d like the authors to address this issue in the discussion, nevertheless, by stating the applicable conditions for their results.

Response: I have tried to address the reviewer’s concerns by discussing the limitations of the experimental design in the Discussion, and comparing the role of manipulative experiments with more field-realistic designs (lines 400-422). I specifically address the reviewer’s comment about cyclical waterlogging (lines 411-413).

Responses to Reviewer 3:  
  
  
Comment: I don’t quite understand the significance and importance to understand the interactive effect of eCO2 and waterlogging. Why do authors focus on these two drivers/stresses? The mechanistic connection between these two is not clear. A small number of publications do not justify the importance.

Response: The rationale for investigating interactive effects of waterlogging and eCO2 on riparian plants is outlined in the Introduction. Waterlogging is one of the major stresses imposed on riparian plants and its impact is mediated strongly through reduction in plant nutrient uptake, metabolism and photosynthesis. In the future, elevated ambient concentrations of CO2 are predicted to also have an important influence on these processes. As such, I believe that understanding potential interactions with waterlogging stress is key to predicting the influence of elevated atmospheric CO2 in riparian plant communities.

Comments: From the FACE experiments (outdoor CO2 experiments), we know that the CO2 effect on plants is not well understood, mostly related to photosynthesis, respiration, temperature, carbon allocation, and nutrient supplies. The connection to waterlogging is secondary. So, the FACE results and knowledge gaps should be well described in this manuscript.

Response: I do acknowledge that FACE studies are powerful tools for investigating responses to elevated CO2 and other environmental conditions at the level of ecosystem processes and community-level functioning, but they are often not as useful for running manipulative experiments. Glasshouse experiments focused more on individual plants grown in pots are able to provide data which would not be feasible to obtain using FACE experiments where the measurements required are too destructive (i.e. harvesting whole plants), manipulations are difficult or impossible to make (i.e. waterlogging), or because a FACE experiment does not exist in the system of interest. In the case of this experiment, we have been able to use destructive biomass measurements to address hypotheses about the mechanisms underlying our observations.  
  
Comment: 2. I have a major concern on the short duration of the experiment, last 90 + 24 +23 days. Within the treatment period, one may see the effect on photosynthesis, but not much on root biomass and stem density. The other effect may be involved such as the difference of the individual plant, species variation, etc.

Response: The treatment period was chosen to simulate a relatively large flood event in south-eastern Australia. The reviewer may be correct that a longer waterlogging period would have a more dramatic effect on biomass allocation and functional traits, although this effect may not be as realistic. This waterlogging period lies between that of Shimono et al. (2012) (14 days of waterlogging beginning on 14 day old plants) and Arenque et al. (2014) (45 days of waterlogging beginning on 90 day old plants). **We have added a comment to this effect in the Methods (lines 195 to 196) Both of these studies measured biomass, although neither made measurements of functional traits.**   
  
3. The temperature varied 16-28 degree C. How did temperature affect the treatment effect?

Air temperature in the glasshouses was maintained within this range, and there was no significant difference in monthly mean, minimum or maximum temperature between glasshouses. A comment has been added in the Methods to clarify this point (lines 184-187).   
  
4. As with all indoor experiments, how do experiment results explain the real, national condition? This is the reason why people designed outdoor CO2 experiments and rainfall experiments to reflect more realistic environmental drivers.

Unfortunately this is an issue common to all manipulative experiments, whereby the ability to tightly control and manipulate the environment comes at the cost of realism and generality. While more realistic outdoor experiments will eventually be needed to understand how plant communities respond in the field, building and maintaining infrastructure required to run these experiments is a large undertaking. Smaller glasshouse experiments are useful as a prerequisite to such studies in order to help pinpoint mechanisms of interest and generate interesting research questions.

Other changes:

Added comments on two further references in the Introduction, which describe investigations of interactions between elevated CO2 and inundation in tidal marshes (lines 126 – 130).