

Reviewer 1

General comments: In this manuscript, Ouyang et al. present the results of a precipitation manipulation experiment in which the dry season was exacerbated or lengthened (along with a compensating increase in wet season water supply). The authors report a number of traits for the two dominant tree species at their experimental site, along with species-specific transpiration and water-use patterns. They conclude that the two dominant species show contrasting water-use strategies and their findings have important implications for the survival of these species under a changing climate regime. The experimental setup and amount of data collected is impressive, but the manuscript has some key issues that I believe preclude publication at this time. Below, I have listed some broader feedback about the manuscript, followed by smaller line by line comments.

1) In general, I think the most novel aspect of this manuscript is the water uptake depth data. However, I think there is significant methodological detail missing about how water uptake depth was partitioned and there seems to be no statistical analysis comparing these data across treatments or species. I also think it should be mentioned that water samples were collected during a very limited time frame and that plant water use could have shifted during the experiment, as could precipitation 18O. Further, the authors make claims about these data (primarily differences between species), that look unfounded to me but could potentially be shown statistically. In sum, I don't think the water uptake depth analyses are valid as is. Without the water uptake depth data, I think the manuscript is fairly simple, merely showing the effects of the precipitation manipulation on transpiration, along with a few traits.

2) Related to the above point, I think there are some large gaps in the methods that make a solid interpretation of the results difficult. In particular, I think the manuscript is missing detail on leaf 13C sampling, lacks a discussion on the age of the bulk material they sampled for 13C analysis and what this means for their interpretations, neglects description of their water uptake depth model, and lacks some additional smaller details on sampling that I've highlighted below.

Response (R): We sincerely appreciate that the reviewer pointed out the pertinent problems of our manuscript and detailed modification suggestions. We have thoroughly checked and revised the manuscript by following reviewer's suggestions, and respond as well as explain item-by-item the questions as follows. The methodological detail about how water uptake depth was partitioned was added in the manuscript: "Four rainfall samples from four precipitation events were collected

for the isotope analysis. Previous root sampling analysis has verified that more than 80% of the total root biomass for *S. superba* and 80% of the absorbing roots of *M. macclurei* were distributed in the surface soil layer (0-60 cm) (Hao and Peng 2009; Li 1984). Therefore, soil samples around the sampled trees were collected from the upper soil layers (0-20 cm), middle soil layers (20-40cm) and deeper soil layers (40-60 cm) with soil cores from each experimental plot. Water from a small well near the experimental plots was collected as the groundwater and kept in the laboratory at 0-5 °C for further analyses (Sun et al., 2018). ” (Line 287-296)

In addition, according to reviewer’s suggestion, we recalculate the proportions of water resource use based on the measured isotope data and display the statistical analysis in Figure 4. The corresponding results of statistical analysis are also described in the manuscript: “ According to the statistical results, the utilization of rainwater and soil water of *M. macclurei* trees showed no significant treatment-difference, while the DD and ED treatments significantly decreased its utilization of groundwater (Figure 4). However, the changed precipitation pattern posed a significant influence on the water use proportions of *S. superba* from different water resources ($p < 0.05$)...Furthermore, the two dominant tree species shared similar water use proportion under the control condition, and *M. macclurei* used more soil water (0-60cm) than the *S. superba* under DD treatment. Comparatively, *M. macclurei* utilized more rainwater, while *S. superba* was inclined to make use of more groundwater under ED treatment ($p < 0.05$).” (Line 379-392)

The detailed information about leaf ^{13}C sampling and analysis was also added in the manuscript: “In this study, the obtained fresh mature leaves (the pre-treated details was described in section 2.6) were oven dried (80°C, 48 h), crushed to a powder and sieved through a 100 mesh. The samples passing through the mesh were oxidized with an elemental analyzer (VARIO EL3, Elementar, Germany) and analyzed for $\delta^{13}\text{C}$ by mass spectrometry (DELTA V Advantage, Thermo Scientific, USA) using Pee Dee Belemnite (PDB) limestone as the standards. The $\delta^{13}\text{C}$ value (‰) was calculated from the following equation...” (Line 260-266)

3) In many places throughout the manuscript, the authors claim their results speak to the competitive ability of these species using words like “competition”, “success”, “coexistence”, “tolerance”, etc. I do not think the data support these claims since the manuscript merely shows patterns in water use between the two species and some additional traits. I think this language should be toned down and I’ve highlighted areas where this issue came up.

R: The terminology words such competition, success or tolerance were deleted or revised to avoid high-toned. We think the use of the terminology “coexistence” might be reasonable, because the two dominant species in this area, *M. macclurei* and *S. superba* shared and competed for the resources with different distribution of root biomass, various water use and growth indexes, thus, their coexistence could be a point in this study.

4) Certainly, this is not a big issue at this stage, but I think the manuscript could use some significant editing for grammar and sentence structure.

R: We have read and done our best to make revisions throughout the text to avoid grammar mistakes and long sentences.

Specific comments:

L1-2: I would really recommend altering the title. I do not really think the study pertained to “acclimation traits”, nor were any traits “reshaped”. Further, I think that saying they “altered their coexisting relation” is not supported by the data (my point #3 above).

R: The title was changed to “Species-specific transpiration and water-use patterns of two dominant coexisting tree species under manipulated rainfall in a low-subtropical secondary evergreen forest”

L27: See #3 above about claims of competitiveness.

R: This sentence was changed to “...that *M. macclurei* has more survival and growth advantages in this subtropical forest.” (Line 27-28)

L29-30: How are you defining drought tolerance? It seems like the two species had fairly similar responses to the precipitation manipulation in terms of transpiration and there was no evidence that either species was water stressed (point 3# above).

R: Actually, due to the abundant rainfall, drought events do not happen very often in south China, but previous reports also claimed many tropical areas with rich species have already experienced little or no rain falls during dry seasons and upper soil layers might undergo severe drying (Goldstein et al., 2008; Liu et al., 2010; Gao et al., 2015) (Line 88-91). In this study, *S. superba* allocated much less root biomass on surface soil than *M. macclurei* (47% vs. 72%), and as shown in the statistical results, the *S. superba* was inclined to use more groundwater than *M. macclurei* under ED treatment. Therefore, we think *S. superba* might be drought tolerant under potential drought in the future. To make it less controversial, this sentence was changed to “Therefore, under the seasonal drought caused by uneven distribution of rainfall in the future, *M. macclurei*

that inclines to use shallow soil water would adopt a drought-avoidance strategy, whereas *S. superba* being able to uptake deeper soil water would be drought tolerant. (Line 29-31)”

L31: See#3 above about the coexistence terminology.

R: As we had explained in the previous response, *M. macclurei* and *S. superba* shared and competed for the resources, and had different distribution of root biomass, various water use and growth indexes, thus, the coexistence terminology might be reasonable.

L78-80: I do not think it is well supported in the literature that isohydric species tend to occur in mesic areas. They can and do exist most everywhere.

R: This sentence was changed to “Isohydric species, however, are often regarded as drought avoiders as they can avoid drought-induced hydraulic failure by way of strict stomatal control and relatively constant minimum leaf water potential (McDowell et al., 2008)” (Line 75-78)

L128-135: It seems like #1 and #2 are really similar in the sense that they both describe traits and water-use patterns. Maybe the authors could separate these objectives out into one about tree water use (and water uptake depth), and one about how traits mediate tree water use patterns.

R: Thanks for the suggestion, we have rewritten the objectives: “Therefore, main objectives of this study are 1) to investigate the effects of manipulated precipitation conditions on spatial-temporal water use patterns of *S. superba* and *M. macclurei* in this subtropical forest; 2) to understand the potential mechanism for the varied responses of tree transpiration to the changed precipitation patterns by examining the variations in morphological adjustment, such as Huber values (A_s : A_l), the intrinsic water use efficiency, and the contributions of water resources to the tree transpiration.” (Line 135-141)

L161-164: I think it would be very helpful for the reader if there was a simple diagram (or perhaps labels added on to a time series figure) showing when precipitation was excluded or added back in for each treatment.

R: To describe the manipulated precipitation treatments more clearly, we listed the detailed information of excluding and irrigating water under DD and ED treatment in Table 1. “The exclusion of precipitation was achieved automatically by a tarpaulin covering approximately 67% of the area of the DD and ED plots. To guarantee the equal total annual rainfall, approximately equivalent amounts of excluded water were pumped into these plots several times (4-8 times) during wet seasons (from June to September for DD and ED treatments) (Table 1).” (Line

174-178)

L178: In order to interpret the effects of the precipitation manipulation, there needs to be information about how the experimental year's climate related to average site climate. For example, if this was a really wet year, there may be no reason to expect significant treatment effects in the first place.

R: We have described the long term climate of the study site in section of "Site description", and we also presented the climatic parameters especially the precipitation of the experimental year in section 3.1: "Total precipitation at the research site during the experimental period was 2094 mm. The precipitation was unevenly distributed and occurred mainly between April and September, accounting for approximately 84% of the annual total. It was noticeable that the heaviest precipitation with a value of 498.6 mm occurred in August, while the lightest precipitation occurred in February with only 2.7 mm. (Line 327-332)" Data has obviously shown the unevenly distributed precipitation, which could make our experimental design sense.

Yes, it was really a wet year, as the total precipitation being of 2094 mm during the experimental period, but the rainfall manipulation that excluded 67% of precipitation under DD and ED treatment (the amounts of excluded water were shown in Table 1) still had a non-negligible effect on tree transpiration, especially during the periods of dry and spring drought season (Figure 3).

L216: What depth were these samples collected at?

R: "soil samples (0-30 cm) were periodically collected in the experimental plots to measure the soil water contents (*SWC*) by gravimetric method." (Line 222)

L252-290: I think some text should be added (either in the methods or discussion) that clarifies what the ^{13}C signal would represent. If these leaves had been around prior to the experiment, their bulk tissue ^{13}C would incorporate the ^{13}C signal from climatic conditions at the time of leaf expansion, the carbon used to make those leaves, and any dynamics influencing non-structural carbohydrates since leaf expansion. Any information on the life span of these leaves would help here, or simply a caveat that the ^{13}C signal could be complicated.

R: The detailed information about leaf ^{13}C sampling and analysis was also added in the manuscript: "In this study, the obtained fresh canopy leaves (the pre-treated details was described in section 2.6) were oven dried (80°C, 48 h), crushed to a powder and sieved through a 100 mesh. The samples passing through the mesh were oxidized with an elemental analyzer (VARIO EL3,

Elementar, Germany) and analyzed for $\delta^{13}\text{C}$ by mass spectrometry (DELTA V Advantage, Thermo Scientific, USA) using Pee Dee Belemnite (PDB) limestone as the standards. The $\delta^{13}\text{C}$ value (‰) was calculated from the following equation...” (Line 262-268)

Since the *S. superba* and *M. macclurei* are evergreen tree species with perennial leaves, ^{13}C value of mature leaf is generally considered to be constant after being fixed in the leaves, and thus, the use of mature leaves for ^{13}C sampling and analysis at the end of the experiment are reasonable.

L275: When were rainfall samples collected? Were these multiple samplings or were there 4 replicates of one rainfall event? If it is the latter, I don't think you can assume that this rainfall event is representative of all rainfall.

R: The collected rainfall sample were taken from four precipitation events. “Four rainfall samples from four precipitation events were collected for the isotope analysis.” (Line 287-288)

L286: Please describe in detail what IsoSource is and the methodology behind how it partitions water uptake depth.

R: The IsoSource, a mixing model software, is developed and introduced by Phillips and Gregg (2003). It is designed for situations in which n isotopes are being used and more than $n+1$ sources are likely to contribute to a mixture. IsoSource uses stable isotope data to calculate feasible ranges of source contributions. Detailed information about this software was presented in Phillips and Gregg (2003). In this study, the xylem water was regarded as the mixture, and different water samples for isotope analysis included the rain, soil water from different soil layers, and groundwater. First, all possible combinations of source proportions that sum to 100% are calculated in user-specified increments (2% in our study). Second, the predicted isotope values of the mixture are computed using linear mixing model equations that preserve mass balance (Phillips 2001). Isotope values of computed mixtures are then compared with the observed isotope values; the range of combinations that match within a user-specified tolerance value (0.05% in our study) is then described.

L343-350: What are these percentage reductions in comparison to (i.e., what counted as dry versus wet seasons)?

R: These percentage reductions are in comparison to wet seasons. In the new version of manuscript, we deleted this part to avoid the unnecessary description.

L364-378: As mentioned in point #1, this sections needs some statistical analysis to be able to

draw any conclusions from the data.

R: The results of statistical analysis are also described in the manuscript: “According to the statistical results, the utilization of rainwater and soil water of *M. macclurei* trees showed no significant treatment-difference, while the DD and ED treatments significantly decreased its utilization of groundwater (Figure 4). However, the changed precipitation pattern posed a significant influence on the water use proportions of *S. superba* from different water resources ($p < 0.05$)...Furthermore, the two dominant tree species shared similar water use proportion under the control condition, and *M. macclurei* used more soil water (0-60cm) than the *S. superba* under DD treatment. Comparatively, *M. macclurei* utilized more rainwater, while *S. superba* was inclined to make use of more groundwater under ED treatment ($p < 0.05$).” (Line 379-392)

L390-391: Is this saturation model warranted for the data considering that most of the responses seem to be fairly linear? Is there a first principles reason to expect a saturation relationship?

R: Generally, the response of tree transpiration to *PAR* is generally linear when the *PAR* values are relatively low, while non-linear (saturation) relationships would be observed as *PAR* further increase. Considering the wide range of *PAR* in our study (0-50 mol m⁻² d⁻¹), we thus used the exponential saturation model to explore the response of tree transpiration to *PAR*.

L424: I’m confused as to how Huber value can be used to understand how much water a species has access to.

R: This sentence was revised as “Results indicated that *S. superba* had significantly larger Huber value ($A_s:A_l$) (Table 3), possibly meaning a less investment on leaf biomass but a better efficient transport system (Zhu et al., 2014).” (Line 447-449)

L427: See point #3 above regarding the “drought-tolerant” terminology.

R: As we have explained above, to make this terminology less controversial, we revise this sentence as “This character could lead to restraining of transpiration and better transport efficiency, and thus to drought-tolerant for *S. superba* when severe drought occurs. (Line 451-453)”

L433-434: I’m not sure this is supported by the data (especially since there are no stats). The bars seem to be similar in size and the error bars seem to overlap.

R: The results of statistical analysis are added in the revised manuscript and Figure 4. “According to the statistical results, the utilization of rainwater and soil water of *M. macclurei* trees showed no significant treatment-difference, while the DD and ED treatments significantly decreased its

utilization of groundwater (Figure 4). However, the changed precipitation pattern posed a significant influence on the water use proportions of *S. superba* from different water resources ($p < 0.05$)...Furthermore, the two dominant tree species shared similar water use proportion under the control condition, and *M. macclurei* used more soil water (0-60cm) than the *S. superba* under DD treatment. Comparatively, *M. macclurei* utilized more rainwater, while *S. superba* was inclined to make use of more groundwater under ED treatment ($p < 0.05$).” (Line 379-392)

L489-490: I'm not sure this claim is supported since the treatment effects seemed to be fairly similar in the two species. Perhaps specify or cut this sentence?

R: This sentence was deleted as we actually don't have the experimental data to support that.

All figure and tables: Please specify what the +/- means in the tables (standard error, deviation?), what the lettering notation indicates, and what error bars represent.

R: Done. The +/- means mean values \pm standard deviation. Different small letters indicate differences among the three treatments within the same tree species ($p < 0.05$); Different capital letters indicate differences between tree species for a single treatment ($p < 0.05$).

Fig. 1: During what hours were these daily values calculated? It might be more relevant to present mean daytime PAR and VPD.

R: Yes, we also think that the mean daytime values of *PAR* and *VPD* would be more relevant, however, the values were measured and offered by the Heshan National Ecological Station and only daily values presented.

Fig. 3: I think the clarity of this figure could be improved. In general, it is hard to parse out trends due to the experiment since the points are so close together. It is also hard to interpret the panels for each species since they encompass different and overlapping time points. Perhaps clarify what manipulation was occurring in each panel, or maybe make one longer time series graph with all the treatment times labeled?

R: The Figure 3 was re-plotted according to the suggestion. We used three separate graphs to describe tree transpiration of two species during the whole experimental period under BC, DD and ED treatment, respectively.

In terms of the treatment times, “The exclusion of precipitation was achieved automatically by a tarpaulin covering approximately 67% of the area of the DD and ED plots. To guarantee the equal total annual rainfall, approximately equivalent amounts of excluded water were pumped into

these plots several times (4-8 times) during wet seasons (from June to September for DD and ED treatments) (Table 1).” (Line 174-178)