

# ***Interactive comment on “Differences in instantaneous water use efficiency derived from post-carboxylation fractionation respond to the interaction of CO<sub>2</sub> concentrations and water stress in semi-arid areas” by Na Zhao et al.***

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## REFeree COMMENT-DISCUSSION

Differences in instantaneous water use efficiency derived from post-carboxylation fractionation respond to the interaction of CO<sub>2</sub> concentrations and water stress in semi-arid areas

by Na Zhao, Ping Meng, Yabing He, and Xinxiao Yu

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## General comments

In this work, Zhao et al. present an experimental study on the interactive effects of CO<sub>2</sub> and water availability on instantaneous water-use efficiency (iWUE) and the carbon isotope composition (d13C) of leaf water-soluble organic matter (LWSOM). Although the study of the interaction between CO<sub>2</sub> and drought and its effects on d13C and iWUE is not new (Picon, Ferhi, & Guehl 1997), there is no clear consensus on the interpretation of d13C changes in response to increasing CO<sub>2</sub> (Schubert & Jahren 2012). In this context, the comprehensive dataset here presented may contribute to understand the limitations of d13C as a surrogate for iWUE, and to better predict the response of tree species to increasing CO<sub>2</sub>, particularly in drought-prone environments. This is particularly relevant for the proper interpretation of long-term trends in d13C in relation to changes in water use efficiency, particularly in drought-prone environments, e.g. based on tree-ring records (Duquesnay et al. 1998; Saurer, Siegwolf, & Schweingruber 2004; Voltas et al. 2013), or from herbarium and sub-fossil material (Peñuelas & Azcón-Bieto 1992; Beerling 1996; Köhler et al. 2010).

The experiment is well-designed and the data is generally well presented, although some details on the methodology are missing (see technical corrections). However, the manuscript requires some improvements, particularly on the interpretation of results.

## Specific comments

My main concern about the manuscript is that it relies on the assumption that the only source of divergence between gas-exchange iWUE and d13C of recent assimilates could be post-photosynthetic fractionation. Although this is likely to play a role, the authors should consider that what actually defines carbon isotope discrimination (D13C) is the CO<sub>2</sub> concentration in the chloroplast (C<sub>c</sub>), not in the intercellular space, as used in the simplified equation of the Farquar's model (Evans et al. 1986; Farquhar, Ehleringer, & Hubick 1989). Indeed, the difference between gas-exchange derived values and online measurements of D13C has been widely used to estimate C<sub>i</sub>-C<sub>c</sub> and

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mesophyll conductance for CO<sub>2</sub> (Le Roux et al. 2001;Warren & Adams 2006;Flexas et al. 2006;Evans et al. 2009;Flexas et al. 2012;Evans & von Caemmerer 2013). In this regard, changes in mesophyll conductance could be partly responsible for the observed variations, as it generally increases in the short term in response to elevated CO<sub>2</sub> (Flexas et al. 2007;Flexas et al. 2014), whereas it tends to decrease under drought (Flexas et al. 2004;Ferrio et al. 2012;Hommel et al. 2014;Thérroux-Rancourt, Éthier, & Pepin 2014). Hence, the manuscript would be greatly improved by considering both post-photosynthetic fractionation and mesophyll conductance as potential sources of variation. With the data available, the authors may be able to estimate changes in mesophyll conductance, based on the Evans method, which can be adapted to recent assimilates (Pons et al. 2009). Even without alternative estimates for mesophyll conductance, this would provide an useful ground for a deeper discussion.

#### Technical corrections

In its present form, the title may suggest that instantaneous water use efficiency is changing because of post-carboxylation fractionation, which is clearly not the case. Besides, after considering the role of mesophyll conductance, post-carboxylation fractionation should not play such a major role in the title. An alternative might be "The interaction of CO<sub>2</sub> concentrations and water stress in semi-arid areas causes diverging response in instantaneous water use efficiency and carbon isotope composition". This leaves open the possibility to discuss both post-photosynthetic fractionation and mesophyll conductance as potential causes for the observed divergence.

In the abstract, lines 11-14: it seems that several concepts are mixed together here, trying to summarize everything in one sentence, but the result is unclear. I would recommend to split the ideas in shorter lines, and to try to go step by step in the argumentation line of the abstract.

The number of replicates (saplings) per treatment is not given in the methods (however it is shown in the figures, n=32). Please add, and also specify the number of leaves

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measured/sampled per tree, number of gas-exchange measurements per leaf, etc.

In line 263 an attempt to quantify the so-called 'post-carboxylation fractionation' is given, but the methodology used is not described. As it is written, the sentence "When comparing WUE<sub>g</sub> and WUE<sub>c</sub>, the <sup>13</sup>C-depletion" is misleading, since it is not WUE calculated by the two methods what is compared here, but observed and modelled <sup>13</sup>C. I guess the value results from the difference between observed <sup>13</sup>C and modelled <sup>13</sup>C calculated from gas-exchange data, i.e. by reverting equations 3 and 4, however this is not explained in the methods.

Text in the legends of Figs. 2-5 could be larger. Since each panel is associated to one single species, they could be simplified by including the name of the species elsewhere in the figure, and using the symbols only for the CO<sub>2</sub> levels. The symbols for a given CO<sub>2</sub> level could be the same in all panels, regardless of the species (in this way, one legend would be enough for all the panels).

In Figure 6 I would use the symbols to indicate CO<sub>2</sub> levels, as in the rest of figures. This would be useful to see whether the positive association between "fractionation" and *g<sub>s</sub>* is linked with CO<sub>2</sub> or water availability.

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