

Interactive comment on “The effect of drought and interspecific interactions on the depth of water uptake in deep- and shallow-rooting grassland species as determined by $\delta^{18}\text{O}$ natural abundance” by N. J. Hoekstra et al.

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Main remarks. The paper deals with an essential topic, which refers to the ability of multispecific crops, and in this case, grasslands to sustain more severe droughts compared to pure stands. The choice of species is relevant for all Europe and more. The issue is also about methodology for studying resource sharing, and especially water,

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between species of a community. The choice of natural isotopic abundance of ^{18}O is relevant and the experimental design is sound, using a comparison of control plots (i.e. rainfed) and plots protected by permanent rain out shelters. The duration of the drought studied is long enough to mimic a significant water deficit. The measurements made on biomass produced during the drought period itself are relevant for at least one important issue in drought resistance studies. The replicates number each year and the two site-year experiment provide a significant number of data for sound conclusions. The text is very clear, figures are mostly clear too. All of them if not more are necessary in the main text. Some conclusions are new and important.

I have however two serious concerns with the data itself on the one hand and with the treatment of the data on the other hand. Given the importance of the topic, the quality of the data and the novelty of the science, I really hope that the authors have the resources to work on these points and I therefore suggest that the paper should go through major modifications.

Response:

We would like to thank the reviewer for the positive feedback on the manuscript. We have now responded to all the general and specific comments below, and have made changes to the manuscript as indicated.#

Firstable, the soil water isotopic composition is not clear. Fig B1 should be included as figure in main paper, not as appendix. The difference in soil profiles between the rain fed and rain out shelter is puzzling. No clear explanation is given for a difference as large as 2 ‰ at Tånikon (Fig B1). If the regional waters are close to -8 ‰, how is it possible that we have -11 ‰ at the end of the drought period in Tånikon? Are there any measurements of rainfall isotopic signature? This would be a very useful measurement here. Furthermore, the gradient results from the soil surface evaporation and from the net subsequent diffusion of heavy isotopes downward. Soil evaporation could have been higher under the rainout shelters due to higher temperature but indeed,

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the first and main impact of such superstructure is a reduction of incident radiation. As a consequence and given the small difference in air temperature, ET_c could have been likely 10- 20 % less under rain out shelters. Were there any estimate of such reduction in incident radiation and at least, could the energy interception by the shelter be measured? This is critical to discuss several aspects of the responses (biomass production, water consumption, depth of water extraction, which depends on transpiration (see Boujamlaoui et al 2005) Finally, the soil water profiles clearly indicate a quite important water consumption below 40 cm which is not much addressed in the paper.

Response:

- The difference in soil water d18O composition can clearly be seen in the current Fig. 1, and therefore we do not think including Fig. B1 in the main paper would be beneficial.
- We now have included the monthly rainfall d18O isotopic composition which is available from the Swiss National Network for the Observation of Isotopes in the Water Cycle (ISOT) (Schürch et al., 2003). These show that the d18O of rainwater was less negative during the drought period compared to the preceding months (difference of 2.1 and 2.9 during 2011 and 2012, respectively). Rainfall isotopic composition ranged from -15 (Feb) to -6 (August). These data have now been included in the supplementary material and in the discussion.
- We did measure the photosynthetically active radiation (PAR) and have now included the background readings (taken above the crop canopy) from under the drought shelters compared to the control plots in Table 1. This indeed shows that the incoming PAR underneath the shelters was 11 to 28% lower compared to control plots in 2011 and 2012, respectively. This would have resulted in a decrease in evapo-transpiration, which would contribute to the more negative d18O signal under drought compared to control conditions. We have now addressed this in the discussion.
- We have re-run the IsoSource model with estimated values for the d18O composition of water from 40-50 cm soil depth to get an idea of the effect of including deeper

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soil layers, and have included this in our methodology discussion (for more detail see below).#

Secondly, I strongly recommend to drop all reference to the first direct inference of water extraction from the comparison between the soil delta gradient and the so called "xylem water" signature. (incidentally, only a small fraction of the water extracted from the plant samples is truly xylem water.) It has been shown that such use of comparison is wrong (Durand et al. 2007). Furthermore, it is useless here and therefore unnecessarily weakens the paper a lot. If the question was about the ranking between treatments and species, then there was no need to infer any actual depth from the delta 18O data. The second estimate is clearly much more rigorous, providing an estimate of the average depth. By the way, why was direct comparison of the average depth of water extraction using the Philipps and Gregg methodology with direct inference not made ? This would be much more convincing than a simple correlation. But again, even with the IsoSource computation, such statistical approach is not real evidence for actual depth. IsoSource is certainly an important step forward and provides very interesting insights for interpreting the delta and I find that the use made of that software here is really relevant.

Response:

We consider that the comparison of the two methods is a valuable addition to the paper, 1) because it shows there is a strong connection between the two methods, and 2) because it emphasises the value of the "new" approach using the IsoSource method. Here, we also refer to the review of J. Nippert, who actually expressed his appreciation of presenting and comparing both methods.#

What is worrying is that for the red clover however, there seems to be a contradiction between the conclusions obtained comparing the soil water profiles (drought and control) and the comparisons using the PCWU0-10. This raises the question of the accuracy of the methodology used when there are more sources than markers in this

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case at least.

Response:

Comparison of the direct inference method and the IsoSource method shows that the trends in response to drought are similar, and therefore there is no clear evidence that there was a problem in relation to the number of sources relative to markers resulting in “wrong” estimates. However, the IsoSource model did result in a rather large range in the 1st and 99th percentile of the frequency distribution (See Fig. 2, related to the relatively low gradient in the soil water isotope composition in the top 20 cm) indicating that some care is needed with the interpretation of the results. The fact that the same effect occurred at both sites, strengthens the results.#

Additionally, some more detailed remarks on the manuscript.

1. The species are given relative depth of rooting and water extraction a priori. However, it is very much related to the conditions of the experiments. For instance, Durand et al (1997, 2009) demonstrated that *L perenne* could extract water from very similar depth as *F arundinacea*, a reknown potentially deep rooted species. The qualification of shallow rooted for *L Perenne* is therefore questionable (a mighty reason for doing this experiment indeed). Ascribing any depth of water extraction in the introduction or as a reputation should be made more reluctantly.

Response:

The grouping of the species into deep- and shallow-rooting species was part of the experimental design, and informed our a priori hypotheses and we therefore cannot ignore it. There is substantial evidence to justify the selection of species based on their root morphology (I have added some references to the description of species selection in section 1.2). It is an important part of this study to test whether this morphological distinction between deep- and shallow-rooting species can be translated in terms of depth of root activity (hypothesis 2), and as such we need this a-priori classification.

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Indeed, we show in our own study that the effective rooting depth of species may very much depend on the conditions of the experiment. So a deep-rooting species (*T pratense*) may under certain conditions take up the bulk of its water from a very shallow depth. Similarly, as the reviewer indicates above, shallow-rooting species such as *L perenne* may under certain conditions take up water from deep soil layers. We have added a paragraph at the end of section 3.2 discussing the limitation of grouping species according to rooting depth.

“This research shows that classification of species according to rooting depth may be of limited value, as the “effective” rooting depth depends on the specific conditions. Similarly, Durand et al (1997) demonstrated that *L perenne* could extract water from very similar depth as *F arundinacea*, a renowned deep rooted species.”#

2. That local water extraction depends on local root density is very well established under well watered conditions (both in trees and crops) and is difficult to be introduced as a question.

Response:

We assume that this remark relates to the last sentence of section 3.2 and have deleted this sentence from the discussion.#

3. Similarly, that water is extracted from deeper & wetter horizons when water is scarce near the surface is all but surprising. The water potential distribution in the plant –soil system inevitably leads to that and this has been documented (see literature cited like Sainclair , Garwood, but more recently modeled by Jarrige , Doussan or measured under various conditions by Gonzalez Dugo et al: :)

Response: Even though this may seem “inevitable”, there is quite a large body of evidence indicating that for specific species or under specific conditions, there is no shift in water uptake to deeper soil layers (Prechsl, 2013; Asbjornsen et al., 2008; Nippert and Knapp, 2007a; Nippert and Knapp, 2007b), and in our experiment we found

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a similar result for *T pratense* (see also section 3.1 in the paper). Also, there is not always a gradient in water availability in the rooted soil profile (see paragraph 3.1, and e.g. Kulmatiski and Beard (2013)). Therefore we consider that this hypothesis is not redundant.#

4. The radiation below the shelters was not measured, which is an issue. The irradiative energy balance is likely more important for potential evapotranspiration_ than the air temperature or humidity in that situation. How much could have been ET_ been modified in these conditions ?

Response: As indicated above, we measured the incoming photosynthetically active radiation under the shelters and control plots, and have now included this information in Table 1). The effect of the shelter on ET is likely to have reduced the impact of the drought that could have been expected from rainfall exclusion alone, and we have now added this to the discussion (section 3.6). However, our soil moisture contents were much lower under drought compared to control conditions, resulting in a significant biomass reduction.#

5. The apoplastic water in tiller's base may well not be more than 40 %, out of which, xylem water is even much less.

Response: Barnard et al. (2006) found no significant difference in the $\delta^{18}\text{O}$ isotopic signal extracted from the root crown and the stem base of grassland species (including *T pratense* and *L perenne*). Therefore, we consider that using stem bases (+ root crowns) rather than root crowns alone, is unlikely to have had an impact on the results.#

6. The outer sheath of grass tillers may transpire and therefore enrich the tissues water in heavy isotopes of water. This is not so much related to photosynthesis.

Response: We have rephrased this as: For *L perenne*, the outer sheath, which may be subject to transpiration and therefore have an altered $\delta^{18}\text{O}$ signal (Durand et al., 2007)
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7. The direct inference of depth of water extraction using the soil profile delta 18O is flawed and misleading. The first paragraph of data analysis in material and methods and all paragraphs later on referring to it should be dropped (with absolutely no harm to the strength of the paper in the contrary !).

Response: We are well aware of the caveats relating to the use of the direct inference method (as discussed in section 3.6). However, we feel that the inclusion of the data in this paper is valuable for reasons outlined above.#

8. The use of water from deeper than 40 cm is not discussed. Could that have had some impact on computations of the PCWU0-10 ? It should be discussed somewhere anyway because we have no data on the delta 18O below 40cm.

Response: We reran the IsoSource model with an added 40-50 cm soil interval, estimated as $30-40 \text{ cm} + (30-40 \text{ cm} - 20-30 \text{ cm}) / 2$, assuming that the decline in $\delta^{18}\text{O}$ with increasing soil depth would start to "level out" at this depth (see also Fig 2). The corresponding estimates for PCWU0-10 were highly correlated to the original estimates ($r^2 = 0.99$) and were marginally higher (0.51 instead of 0.49 on average) particularly at low levels of PCWU. As a result this had no material effect on the observed trends in response to drought and diversity.

We have added the following paragraph in the discussion: "For practical reasons, the $\delta^{18}\text{O}$ sampling depth was limited to 40 cm soil depth. However, it is not unlikely that water uptake from below this depth occurred (Skinner, 2008; Pirhofer-Walzl et al., 2013; Garwood and Sinclair, 1979). This would not have affected the mean inferred depth of water uptake, as these values were all well above 40 cm (Fig 2a-e). In order to get an idea of the potential effect of limiting the soil sampling depth to 40 cm on the output of the IsoSource model, we re-ran the model with estimated $\delta^{18}\text{O}$ values for the 40-50 cm soil depth interval. We assumed that the decline in $\delta^{18}\text{O}$ with increasing soil depth would start to "level out" at this depth (see also Fig. 2), and estimated the $\delta^{18}\text{O}$ value for the 40-50 cm soil depth interval as the $\delta^{18}\text{O}$ value for 30-40 cm + (30-40

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cm – 20-30 cm) / 2. The resulting estimates for PCWU0-10 were highly correlated to the original estimates ($r^2 = 0.99$) and were marginally higher (0.51 instead of 0.49), as there was now more support for the relative reliance on shallow soil depths. As a result, adding an extra (estimated) depth to the IsoSource model input had no effect on the observed trends in response to drought and diversity.”#

9. The differences observed between the delta 18O profiles in the deep horizons at the same place are difficult to understand. What could have caused this ? Were the soil sampling conditions similar?

Response: Soils were sampled in two consecutive days under similar sampling conditions and we do not have any other explanation for this.#

10. P12 L 337: no agreement between the two estimates is presented but a correlation.

Response: “agreement” was replaced by “correlation”#

11. Why no estimate of the average depth of water extraction using IsoSource is shown?

Response: The output of the IsoSource model is a proportional contribution of the different source (soil depth intervals) to plant water uptake, and to our knowledge does not allow calculation of the average depth of water uptake.#

12. Drought resistance should be defined here. In this case, drought resistance is defined as production during dry condition relatively to control conditions. The control is always an issue in drought response analysis. All that is needed here is a clear definition.

Response: We have now included this definition in the abstract and the main text.#

We wish to thank the reviewer for their detailed reading of the manuscript, and for helping to improve the manuscript. On behalf of all authors,

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