

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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Broad comments: In this manuscript, the authors present data from an experiment manipulating summer rainfall to assess changes in source-water uptake of 4 species (2 shallow-, 2 deep-rooted) in monoculture and in mixture. The authors hypothesized that during drought, niche overlap among species would shrink, with deep-rooted species increasing reliance on deep sources, while shallow-rooted species increasing reliance

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on shallow sources. Their results varied from original predictions, as 3 species (1 deep and both shallow-rooted) shifted to deeper sources following drought, while 1 deep-rooted shifted to the shallowest soil layer during drought (in monoculture). Interestingly, shifts in source water use during drought were not related to ‘drought resistance’ [assessed by changes in aboveground productivity between drought and control].

In general, this is a well-written paper on a topic appropriate for Biogeosciences and for a broad audience. Many of us, myself included, have been using natural abundance stable isotopes to quantify changes in source water partitioning in grassland ecosystems for quite some time. I enjoyed the comparison of the two methods to assess ^{18}O data. I found it useful that for many applications, the direct inference approach was justifiable.

Response: We would like to thank the reviewer for his positive evaluation of the manuscript. We have formulated responses to all the comments below, and have made changes to the manuscript where required. #

It is unclear how these results show ‘niche complementarity’ (line 16, and the discussion). Later in the same sentence, the authors note that this response contributed to ‘the diversity effect in mixtures’. What does this mean?

Response: see response below.#

On page 4155, niche complementarity is posited to suggest that a shallow-rooted and deep-rooted species could maximize resource uptake. Couldn’t the same thing happen with a single species with roots throughout the profile?

Response: In functional biodiversity research belowground niche complementarity is an important and frequently invoked potential mechanism / concept to explain the better performance of mixtures compared to monocultures. Of course, if one single species would have high root density throughout the whole soil profile this would be ideal for resource uptake. However, shallow rooted species generally produce a very high root

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length density in the top soil layers (cm root / cm³ soil) but have a limited rooting depth. On the other hand deep rooted species have tap roots with a low root length density in the top soil layer but with the feature to get access to deep soil layers. The concept is that combining these different strengths by combining the respective species results in higher resource uptake in the mixed plant community. We have now re-emphasised the differences between these two functional types in our introduction.#

In addition, it has been shown that for some grassland species, conductive root tissue declines with depth – and thus, the functional uptake of water from deeper soil layers is low regardless of the presence of deep roots. Thus, an assessment of ‘complementarity’ in terms of maximum resource extraction would require an estimate of functional conductivity and specific root length by depth. Neither of these metrics are measured here. Please correct me if you disagree.

Response: In this paper we have already indicated that the presence of roots in itself is not a good indicator for root activity or functional uptake. By using the isotope method, we get a better idea of the relative depth of water uptake, and the different patterns between the species that are expected to be complementary. However, the weakness of the isotope method is the lack of quantitative uptake. Therefore, even though there may be differences in the relative depth of uptake between the species, this is no specific evidence that complementarity was the specific mechanism resulting in increased the total water uptake. Throughout the document we have taken care not to refer to niche complementarity, for which we have no direct proof. However, we are able to test important components of the concept (e.g. do species differ in their proportional uptake from deep layers (our hypothesis 2)? Does proportional uptake shift with growth conditions [drought (our hypothesis 1) and plant community (our hypothesis 3)]? Only in the final part of the discussion, we suggest that the observed diversity effect could have been related to vertical niche complementarity in relation to depth of water uptake based on the differential proportional contribution to water uptake of the different species (in addition to other factors including facilitation). In the discussion we

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have now re-emphasised that there is no direct evidence for this, and this section now reads as follows. “Our results suggest that differences in the depth of water uptake between species may have resulted from vertical niche complementarity in the depth of water uptake between deep-and shallow-rooting species, which may have contributed to this over-yielding. However, due to the lack of data on quantitative water uptake from different soil depths, we cannot provide direct evidence for this. Additionally, other factors, such as vertical soil niche complementarity for nutrients, or interactions between legumes and non-legumes, soil-biotic factors or a combination of factors may have also contributed to the diversity effect.” Additionally, we have removed the sections referring to this from the conclusion and abstract.#

It was difficult for me to extend inference on the role of rooting depth (shallow and deep) broadly, since this experiment used 2 species per category. I would suggest minimizing the inference based on this functional classification (especially since the species compared had varied responses within this classification).

Response: The grouping of the species into deep- and shallow-rooting species was part of the experimental design, and as such at the basis of 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 (we have added some references to the description of species selection in section 1.2). This experimental design was chosen to be able to test whether the species behave as expected from their morphology (deep / shallow rooted). Based on this test and the results obtained we do recognise that the effective rooting depth of species may very much depend on the conditions of the experiment. So indeed a deep-rooting species (*T pratense*) may under certain conditions take up the bulk of its water from a very shallow depth. 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

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from very similar depth as *F. arundinacea*, a renowned deep rooted species.”#

It isn't clear why this experiment was performed at two different sites. There is no comparison of a 'site effect' or a comparison of environments on a drought*rooting depth effect? Since most of the usable plant data from Tanikon was lost, I don't see the added utility of having this Tanikon in this manuscript.

Response:

- The experiment was carried out at two different sites because this is a much more severe test of the concepts, as it refers to two different sets of conditions in terms of soil, climate of the year, and establishment after sowing. Two sites deliver two independent datasets, whereas measuring two years on the same site / the same plots would mean repeated measurements (which are not independent from each other).

- By moving site, ideally we would have two full datasets for a year, which would have allowed site comparison. Because of the loss of data from Tānikon, this site comparison is not an option. However, we do think it is important to keep the Tānikon data in the paper, as it shows the robustness of the results found in Reckenholz. Especially, the unexpected but interesting response of *T. pratense* (see below) was similar in both experiments.#

One of the most interesting aspects of this paper (to me), is the response of *T. pratense*. This species had very little reduction in biomass, shifted its water uptake to surface layers during drought, and constituted the majority of biomass in mixtures. The Discussion section mentions the *T. pratense* results, but I would like to see a bit of extra discussion of the attributes of this species that set it apart from the other 3 compared. Does this species have unique vascular morphology? Isohydric or anisohydric stomatal control? What makes this species so different from the rest? What are the attributes that might lead to the source-water plasticity measured here?

Response: Changes in pre-dawn leaf water potential (unpublished data) in response

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to drought were similar in *T. pratense* compared to *T. repens* so there is nothing there to suggest a difference in stomatal control. We were unable to find any explanation for the different behaviour of *T. pratense* from literature. We have now included these observations in the discussion (section 3.1): “Of all the species, the dry matter yield of *T. pratense* was least affected by the drought treatment (Table 2). Changes in pre-dawn leaf water potential in response to drought were similar for *T. pratense* and *T. repens* and provided no evidence for differences in stomatal control (unpublished data).#

Specific comments:

1. Were the 'stem bases' (page 4158 – line 17) photosynthetic / green? For the water isotope technique to work using herbaceous plants, you have to use crown / non-photosynthetic tissue.

Response: the harvested material consisted partly of root crown and partly of stem bases. The stems of *C. intybus* were white, but the stems of *T. pratense* and *T. repens* were green. However, Barnard et al. (2006) showed that for *T. pratense*, there was no significant difference in the $\delta^{18}\text{O}$ isotopic signal extracted from the root crown and the stem. Therefore, we do not believe that the inclusion of some stem material in the sample for water extraction affected the isotopic signal.#

2. Line 1, page 4166 states that uptake from deeper, wetter soils increased during drought in monocultures for 3 species. But for Fig 2g-i, it appears those bars overlap considerably. Are these statistically significant? Fig. 2b-d doesn't appear to show a shift in source between control-drought, especially for *T. repens* and *C. intybus*.

Response: The significances of the contrasts between drought and control for the individual species are reported in the results section (2.4.2), and show that the difference is significant for *L. perenne*, for *T. repens* only when grown in monoculture and for *C. intybus* there is only a tendency. We have now re-emphasised the difference between the drought response in monocultures and mixtures in discussion section 3.1.#

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3. In figure 2, I presume that the shaded bars are 'control' and open bars are 'drought'? You need a legend.

Response: This information has now been added to the legend.#

4. The responses in Fig. 1c,d are hard for me to interpret. How/why did the $\delta^{18}\text{O}$ values become smaller during drought? Soil drying and evaporative enrichment should produce higher values indicative of drier soils, at least in the upper soil layers (0-10cm). Can you posit a mechanism for these atypical soil isotope results? Were the rainfall inputs in the 'control' of a heavier signature? You need rainfall isotopic data.

Response: This can be attributed to the heavier signature of rainfall inputs in the control plots. We now have included the monthly rainfall $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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). These data have now been included in the supplementary material (Fig. D1) and in the discussion.#

5. Line 14, page 4167 – Nippert and Knapp 2007b has detailed soil moisture info throughout the profile – check Figure 1.

Response: We have now changed the text to reflect this.#

6. Line 27, page 4167 – the work by Nippert and Knapp and by Asbjornsen et al. was conducted in temperate mesic grasslands. Not in 'arid systems' as this text states.

Response: we have removed the reference to 'arid systems'.#

7. Line 23, page 4168 – Please clarify your intention by the statement 'makes sense'. Are you suggesting that uptake from shallower soil layers (compared to deeper) would be beneficial to the plant since there would be a shorter path length for transport, and therefore a reduced gradient in water potential required for movement? If so, this has nothing to do with "convenience" (line 24).

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Response: we have rephrased this sentence to: "uptake from shallower soil layers would be beneficial to the plant since there would be a shorter path length for transport, and therefore a reduced gradient in water potential required for movement."#

8. Line 26, page 4170 – How does nutrient availability affect drought resistance? Responses during drought might be impacted by nutrient availability, but the term 'resistance' implies some morphological or physiological attribute of the individual. I think this statement needs to be rephrased (or at least further elaboration).

Response: 'resistance to drought' has been replaced by 'impact of drought on herbage dry matter yield'.#

9. I may have missed something obvious, but in Fig. 5 how was the category 'mixed' developed? It's unclear to me what this metric refers to.

Response: "Mixed" refers to species-pairs consisting of a shallow- and deep-rooting species. This term was first introduced section 1.5, and we have added an explanation both here and in the caption of Fig. 5.#

On behalf of all authors,

Nyncke Hoekstra

References

Barnard RL, de Bello F, Gilgen AK, Buchmann N (2006) The $\delta^{18}\text{O}$ of root crown water best reflects source water $\delta^{18}\text{O}$ in different types of herbaceous species. *Rapid communications in mass spectrometry* 20: 3799-3802. Schürch M, Kozel R, Schotterer U, Tripet J-P (2003) Observation of isotopes in the water cycle – the Swiss National Network (NISOT). *Environmental Geology* 45: 1-11.

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