

***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 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-

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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 18O data. I found it useful that for many applications, the direct inference approach was justifiable.

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? 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? 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.

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).

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

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the added utility of having this Tanikon in this manuscript.

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?

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.

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

4. The responses in Fig. 1c,d are hard for me to interpret. How/why did the $\delta^{18}O$ 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.

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

6. Line 27, page 4167 – the work by Nippert and Knapp and by Asbjornsen et al. was

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conducted in temperate mesic grasslands. Not in 'arid systems' as this text states.

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).

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).

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.

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