

## ***Interactive comment on “Coffee and shade trees show complementary use of soil water in a traditional agroforestry ecosystem” by Lyssette E. Muñoz-Villers et al.***

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We thank Matthias Beyer for his positive and constructive comments which allow us to further improve the article. Please find below our response to each of the comments.

[1] Major points: l.249-250: was complete extraction somehow validated? Also note that clay-rich soils need higher extraction temperatures (see recent (Gaj et al., 2017; Orłowski et al., 2016) papers on mineral mediated isotope fractionation). Using a water bath at 100\_C might result in an offset in isotope compositions and lead to errors/uncertainty in the mixing model (the reservoir of water that is extracted would not equal the reservoir that is available to plants). The authors state at one point that there was

C1

an offset of the values towards more depleted – this is exactly what would happen and was observed in other studies when clay was an issue. This issue should be at least discussed.

Reply: Validation of complete extraction. We did not check whether all water was extracted using a gravimetric water content assessment. However, according to the findings of Araguas-Araguas et al. (1995) and West et al. (2006), extractions do not have to reach full completion (i.e., all water extracted) to obtain an unfractionated and, therefore, isotopically consistent value. Experiments have shown that the isotope value of any extracted water increased quickly during the first 20-75 minutes of extraction, after which the isotope value of the extracted water remained constant regardless of further increases in extraction time. The time at which this threshold is reached is the minimum extraction time (Tmin) required to obtain an isotopically unfractionated water sample, and once Tmin is reached, only a very small amount (microliters) of water may remain in the sample. Recently, Orłowski et al. (2013) showed that even if the extraction is conducted until what they claimed was complete, the isotopic signature may not be recovered from different soil types. The Tmin value varied depending on the source material. West et al. (2016) showed that woody stems required the longest extraction times (60–75 min), while values of Tmin were shorter for soil (40 and 30 min for clay and sand soil textures, respectively). Following West et al. (2016), we used the same extraction time for stems and soils (60-70 min).

Clay-rich soils need higher extraction temperatures. Apart from extraction duration, the literature has shown that the extraction temperature “might” have an impact in the soil isotopic composition. Araguas-Araguas et al. (1995) showed that a highly mobile water reservoir that is weakly bound to soil particles can exist (especially in clay-rich soils where interlayered water can be present), and remains largely intact at extraction temperatures < 100°C. More recently, the studies of Orłowski et al. (2016) and Schoonheydt and Johnston (2015) have discussed whether the extraction temperature should be increased. However, there has been no systematic investigation that clearly

C2

identified the driving forces that might cause an isotope effect on the isotopic composition of the extracted soil water. Since it has been shown that soil samples containing a high clay fraction might affect the quality of the soil water extraction, and therefore the isotopic composition of the bound water, several papers have suggested that investigations should now incorporate information of the soil hydro-physical properties, and more importantly for clayey soils, information about the cation exchange capacity (CEC), as Vidal and Dubacq (2009) have pointed out that the effect of this interlayered space/water in clay-rich soils can be indirectly evaluated with CEC. For our study, we did determine other soil physical and chemical properties such as CEC. Therefore, we are going to incorporate this information in the manuscript to show that the contribution of this interlayer water bound in the clay mineral structure was small for our soils, and therefore of little significance for the entire isotopic composition of the extracted soil water, and for the mixing model results.

Importantly, we did state that the values of  $\delta^{2}\text{H}$  and  $\delta^{18}\text{O}$  plant xylem ( $-40.8 \pm 15.0\text{‰}$  and  $-4.6 \pm 1.6\text{‰}$  respectively) were on average more positive in comparison to bulk soil water ( $-46.7 \pm 16.4\text{‰}$  and  $-6.0 \pm 2.3\text{‰}$  respectively) (L386-388); however, the isotopic range of plant xylem water ( $-7.64$  to  $-0.56$  for  $^{18}\text{O}$ , and  $-65.47$  to  $-9.64$  for  $^{2}\text{H}$ ) fell within the bulk soil isotope range ( $-11.10$  to  $-0.87$  for  $^{18}\text{O}$ , and  $-83.35$  to  $-11.86$  for  $^{2}\text{H}$ ), and no statistically significant differences were found ( $p > 0.05$ ). Therefore, instead of reporting the mean and standard deviation values, we will present the isotopic signature ranges to show that overall we had a good isotopic match between the soil pore and xylem water.

[2] Another question (but this is more general) related to the cryogenic extraction is why such long extraction times are needed (I know, West et al. 2006 propose that). I think one part of that is related to the relatively low extraction temperature, but still. The extractable water should be leaving the sample side very fast given the low volumes (even under  $100\text{ }^{\circ}\text{C}$ ) – waiting longer would not evaporate more water from the sample side unless the temperature is increased further.

C3

Reply: Please see our reply to your previous comment.

[3] I. 297-300: These assumptions need to be validated/proven. Why was not the soil water isotope composition of the first 5 cm used directly? I guess in order to account for water that was taken up by the plant before the actual sampling date?

Reply: We have revisited this assumption. Since each isotope sampling campaign was preceded by at least 6 days up to 22 days without or with minimum accumulated rainfall ( $< 5\text{ mm}$ ) (L235-236), we acknowledge the difficulties to consider rainfall as a potential source of near surface soil water. Following the suggestion, we have decided to use the isotopic composition of the soil at 5 cm depth as a source for near surface soil water. Thus, the discretization of the mixing model originally presented is going to be modified in the revised version; the methods and results will reflect this change accordingly. Please also see our reply to the next comment.

[4] How was the classification used for the mixing model decided? Slightly above and below the zero-flux plane, the isotope composition of soils normally changes drastically during dry periods: :for clay this is often in the first 15 cm soil depth. The 30 -120 cm depth were isotopically similar? In my understanding, the discretization used in the mixing model should be done after the isotope depth profiles are evaluated and backed up by statistical measures of differences between different depths. After checking the supplementary data, I'm really doubting the discretization used. There are partially huge differences of the isotope values of the soil profiles between 30 and 120 cm. And how about 15-30 cm? – was the isotope information of this depth not used at all? (in that case, the mixing model is missing a source which violates the mixing model requirements). I refer, once again, to the Rothfuss et al. publication, which might help to address these issues.

Reply: The classification used in the mixing model was based on the changes in the isotopic composition of soil water and the changes in the root and nutrient distributions along the profile. We divided the soil water pool in two compartments: shallow (5-15

C4

cm depth) and deep soil (30-120 cm depth) sources. In each campaign, we sampled the soils for isotopes at the following depths: 5, 15, 30, 60, 90 and 120 cm. Further, we classified the soil isotope data collected at 5 and 15 cm as shallow and those obtained at 30, 60, 90 and 120 cm depth as deep. Thus, the potential tree water sources that we considered were restricted to these categories and data. There are other examples in the literature in which the evaluation of the relative contribution of soil water sources to plant uptake has been restricted to particular groups of soil depth (cf. Barbeta et al. 2019), without violating the mixing model requirements. However, since the isotopic composition at 5 cm depth is going to be used as the near surface water source, we performed some statistical tests to define the new classification of the soil water pool. Based on the results of these tests, the soil water pool will be divided in the following compartments: near surface (5 cm depth), shallow (15 cm depth), intermediate (30 cm depth) and deep (average of 60-120 cm depth) soil water sources. Preliminary runs of the Bayesian mixing model using this new discretization and without or with the informative prior data continue to show a complementary water use strategy between trees and coffee plants during the dry and wet periods investigated.

[5] Minor points: - Since many different analysis were carried out with the soil and plant samples, this could be summarized in a table nicely. - It would have been easy and interesting to check the uptake depths of the large trees separately and not lumping them. (but maybe not of interest for the study)

Reply: Since these analyses are already described in detail in the text, we consider it redundant to add a table. With regard to the uptake depth, we were unable to distinguish between roots of coffee shrubs and shade trees, as well as between the roots of the different species of shade trees.

[6] - I suggest strong discussion of the use of informative priors and putting a more general focus on this aspect, as this is the key scientific/methodological novelty in this paper in my opinion.

C5

Reply: We will improve this section in the discussion to stress the importance of using informative priors in the mixing models.

[7] - (more a comment): It would have been interesting to have water potential measurements in both soils and trees, because those could really constrain the possible uptake depths.

Reply: Yes, we agree that such data would have been interesting. In a follow-up study, we have been doing water potential measurements at the time of sample collection for isotope analysis.

[8] Abstract l.27: Providing the rainfall amounts in addition to the year would be nice; in addition, it would be nice if the authors could state the type of environment of the study (e.g. semi-arid, tropical, : :)

Reply: We will add this information.

[9] ll.35/36: the percentages are the mean? median? I suggest adding a +/- xx % notation accounting for uncertainty

Reply: The percentages are mean values; we will add the +/- % standard deviation.

[10] l.39: short-term wetness status? Do the authors mean that the uptake depth is not influenced by small rain events? This sentence is not easy to understand, I suggest rephrasing

Reply: We will rephrase this sentence for clarification.

[11] ll.39-41: this sentence needs to be rephrased. The terms near surface vs. much shallower are confusing the reader (5 and 15 cm are both shallow). Perhaps 'upper five centimeter'?

Reply: We will use the terms mentioned above (i.e., near surface for 5 cm depth and shallow for 15 cm depth).

C6

[12] l.42-43: the spatial segregation mentioned, is it due to the different rooting depths of the studied plants? Was this validated somehow?

Reply: Please see our reply to a similar previous comment.

[13] l.44: plant-soil water uptake? Confusing phrase. Do the authors mean 'root water uptake patterns/depths'? I feel like a concluding sentence is missing in the abstract. What are the implications of the study? What novel things were found out? Is 120 cm the max. rooting depth??? Uptake depth vs. rooting depth? (coffee shallow, others deep)

Reply: Yes, we mean root water uptake patterns. We will rephrase the sentence. The implications of our study are presented in Section 4.3 (Implications and future direction) in the Discussion. The contribution (novelty) of this research has been argued in the Introduction and the Discussion sections. 120 cm was the deeper potential water source that we examined. Regarding the question about uptake depth vs. rooting depth is hard to answer it in the context of the line 44.

[14] Introduction I really like the way the introduction is written (clear and concise). The Bayesian mixing model needs to be addressed though. The word is only mentioned once, and some readers might not know what it even is. At the end of the introduction, sentence is missing highlighting the importance and novelty of this research.

Reply: We will provide more background information about Bayesian mixing models and highlight the novelty of including priors for the quantification of plant water sources.

[15] l.55 and l.73: 'soil resources' sounds odd: : can the authors specify please?

Reply: We will specify this.

[16] l.87: However,

Reply: Thank you for the suggestion.

[17] l. 90-92: please note that mixing models are also frequently criticized, (Rothfuss

C7

and Javaux, 2016)

Reply: We are aware that mixing models have been criticized; however, they have several advantages over other methods. That is, they allow for determining the likelihood of the different water sources available to plants using a robust statistical approach and they allow for the incorporation of biophysical parameters (e.g., root and nutrient data) as informative priors (Muñoz-Villers et al. 2018; Ogle et al. 2004).

[18] l.92: 'Although rarely implemented' – do the authors have examples where it was implemented? (this is out of interest)

Reply: To our knowledge, Muñoz-Villers et al. (2018) have been the only ones to use nutrient and root distribution data as priors to better inform a Bayesian mixing model. We will add this reference in the text.

[19] l.143: micrometeorological measurements (which)

Reply: We will change this to "microclimatic measurements". The list of the microclimatic variables that were measured are provided in Section 2.2.

[20] l. 146: nice the authors are implementing priors. See related publication where this was suggested (and also MixSIAR was used): (Beyer et al., 2018). You don't have to cite us but maybe it helps for some explanation in the authors manuscript.

Reply: Thank you for the recommendation.

[21] l.151/152: The answer to question no. 2 is not reflected in the abstract Materials/Methods

Reply: We will rephrase our results in the abstract to include the findings of question #2. With regard to the Materials/Methods, in the Section 2.3 we did say that the dry season of 2017 was warmer and drier offering the opportunity to examine the vegetation responses under more pronounced dry conditions.

[22] l. 168: on an; is there no data after 2000 for rainfall? This seems like it's likely to

C8

have changed meanwhile

Reply: Indeed, there are no data after 2000. And we don't have the data ourselves to determine if there have been any changes in rainfall.

[23] l.214: 'carried out' rather than 'performed'?

Reply: We will make this change.

[24] l.218-222: how many replicates per individual were taken? (same later for coffee and the soil samples)

Reply: This information is given in Tables 1, 2 and 3. For the coffee, the number of replicates is also provided in the text (L225-228). For the trees and soil samples, we can add this information in the text.

[25] l.232-233: 'Auger sampling points were located so that each of the sampled shade trees and coffee plants had a total of three soil sampling points within their 3 m radius.' – If it was sampled at only three different locations (see sentence before), so it means that all the trees had the three sampling points in their 3m radius? That seems odd. Can the authors please check if this phrasing is correct here?

Reply: We will rephrase the sentence for clarification.

[26] l.247: refrigerated – was any mold developing on the samples? This can affect isotope ratios

Reply: Some mold had developed on some of the samples of the trees and coffee shrubs; we will add this in the text and discuss its possible effect in the xylem isotope ratios.

[27] l.268/269: What is API – if it is not a common method, it needs to be explained briefly.

Reply: API stands for antecedent precipitation index and it was calculated following the

C9

method of Viessman et al. (1989) (L267-269). It is actually a common hydrological metric used to quantify the antecedent precipitation conditions (7 or 15 days) prior to a rainfall event, sampling date, etc.

[28] l.304/305: It would be very appreciable to the community I believe if the authors explain how the priors were determined and implemented into MixSIAR as this is not something that has been done often.

Reply: The macronutrients (N, P, K) and root biomass data were first grouped (averaged) according to the defined depths to represent different plant water sources (L306-308). Then, each profile was normalized to obtain a distribution with depth that totalized 100%. Finally, the normalized profiles were averaged across depths to obtain a distribution that represents the prior probability for each source. The prior proportions used for the 2014 sampling dates were: rain = 1, shallow soil = 67, deep soil = 32. For 2017, the following proportions were used: precipitation = 1, shallow soil = 57, deep soil = 42. This configuration resulted in sharp proportions for each source contrasting the "uninformative" prior distribution. We will add this information in the Supplementary Material.

[29] Results l.321: I see a point in putting this as result, but this is nothing that belongs to the objectives of the study as such. I suggest including it into the methods chapter. In many hydrologic and soil studies variables such as rainfall and soil moisture are the basis and not highlighted as results.

Reply: This section characterizes the hydrometeorological conditions during the two dry seasons (2014 and 2017) and the wet season (2017) studied. Since one of our objectives was to determine the sources of plant water under different soil water availability conditions (L137-144), we consider it important to present this information as part of the Results section.

[30] l. 335: Definition of normal vs. below-average dry season: In fact, both dry seasons sampled were below average, 2014 was about 20% lower (323 mm vs. 389 mm

C10

normal) and the 2016/17 one 40%....not sure if I would consider 20% below average a 'normal' year.

Reply: We will make this more clear.

[31] I.351-353: it is not surprising that the wet season is wetter the dry season, but it is notable that the wet season is drier than the 2014 dry season! Why is this information omitted?

Reply: Although the 2017 wet season showed slightly lower SWC values in the shallower soil layers in comparison to the 2014 dry season, the SWC values in the deeper layers were higher. We will add this information in the text.

[32] I.353: the API results don't tell the reader anything without proper explanation

Reply: Please see our reply to a previous comment.

[33] II.359-360: two digits after comma reported for 18O – more than precision – should be avoided; add 'for' delta 18O, 'for' delta 2H

Reply: We will make these changes.

[34] I.382-384: because of the effect of clay material on extraction? (see comment before)

Reply: The soil water was isotopically distinct from rainfall due to mixing and soil evaporation processes.

[35] – same for II. 387-388 I.417: the root biomass cannot be distinguished between species, right? (coffee vs. large trees?): : that means that the created informative prior would be quite biased: : .

Reply: Indeed, we were not able to distinguish between roots of coffee shrubs and shade trees, but we don't understand how this can have caused a bias in the prior information.

C11

[36] II.432-436: discussion Putting the rainfall amounts in the results section is debatable: : it sure is something that was done during the study, but it is not directly related to the objectives. As Hydrologist, I personally would've liked to read these numbers earlier to put the words 'dry season', 'less than average' etc. in perspective.

Reply: We would like to refer the reviewer to Section 3.1., in which we provide the rainfall amounts for the dry and wet seasons sampled and compare these with the long-term data from 1970-2000.

[37] Discussion II.522-525: So in the wet season both trees and coffee use shallow water, because it's abundant. In the dry season, the trees use deep water – because they have deeper roots and water in deeper soil is easier accessible (low matric potential of soils). The coffee uses shallower water in the dry season. What is the reason? – the fact that coffee plants cannot grow deep roots? – or is it because they don't need so much water compared to the trees and don't need deep roots? – or, because the coffee plant has another strategy and its roots can extract water from drier soil compared to tree roots? or: : :.... This is not a criticism; this question is out of interest. I wonder then, if this is really 'complementary' water use as such?

Reply: Many of these issues have been addressed in the Section 4.1 in the Discussion, and yes, there are open questions that need further research as mentioned in the lines 596-597 and 601-603.

[38] II.599- 600: Which recommendations based on their results would the authors give to coffee producers then? This would be a nice addition.

Reply: We would like to refer the reviewer to Section 4.3, in which we discuss the implications of our results and future research directions.

[39] II.606-612: this is a bit contradictory, because in the presented example using this additionally information did not affect the results much (both uncertainty and general outcomes). So which variables should be included in the future? Are there others that

C12

might be more suitable? Micronutrients? Soil moisture?....

Reply: As it is mentioned in the text, although our results did not change significantly by including or excluding the root and nutrient data (informative priors), exploring potential sources of water uptake using an informative and non-informative prior approach provided more confidence in our results. For other environments, the use of prior information may lead to different results and value to better understand water uptake patterns/processes (L604-610).

[40] Conclusions An experienced and well-known researcher a while ago gave me the advice: 'A good paper does not need a conclusion chapter – the reader draws them him/herself.' That stuck to me somehow. I think this is a good paper.

Reply: We believe that a conclusions section is essential for a paper, because it gives the reader a quick overview of the most important findings.

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C13

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C14