Matthias Beyer (Referee) matthias.beyer@bgr.de Received and published: 15 August 2019

Thank you for letting me review the manuscript hess-2019-329 'Coffee and shade trees show complementary use of soil water in a traditional agroforestry ecosystem' by Muñoz-Villers et al. I enjoyed reading. In their work, the authors investigate water uptake depths of large shade trees and coffee trees during two dry seasons and one wet season using water stable isotopes and a Bayesian mixing model. They find that coffee and shade trees show complementary water use patterns, i.e. preferential water use by coffee and deep-water use by the shade trees. During the wet season, both groups shift to shallower resources. Without doubt, this manuscript is well-prepared and written. The structure is clear, research questions are stated concisely, and the Introduction provides a thorough overview on the topic. The graphics are suitable. I like the study and the topic is interesting. Also – and this is the main scientific contribution of the paper – it is great to see that the authors integrated priors and used root information and macronutrient distributions for that. However, apart from that, the novelty and innovation of the soil water extraction and mixing model. The former might be answered, but the latter might require some more effort. I elaborate on those below. Minor comments are summarized further down. Good luck and all the best, Matthias Beyer

# 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.

**Major points:** I.249-250: was complete extraction somehow validated? Also note that clay-rich soils need higher extraction temperatures (see recent (Gaj et al., 2017; Orlowski 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 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:** <u>Validation of complete extraction</u>. 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, Orlowski 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 varies with 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)(Section 2.4, L250).

<u>Clay-rich soils need higher extraction temperatures</u>. 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 Orlowski et al. (2016) and Schoonheydt and Johston (2015) have discussed whether the extraction temperature should be increased. However, there has been no systematic investigation that clearly 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. We have incorporated this information in the revised manuscript now (Section 2.5, L278-283) to show that the contribution of this interlayer water bound in the clay mineral structure was small for our soils (Section 3.4, L446-447; Table 4), and therefore of little significance for the entire isotopic composition of the extracted soil water (Section 4.1, L519-535).

Importantly, we did state that the values of  $\delta$ 2H and  $\delta$ 18O in plant xylem water (-40.8 ± 15.0‰ and -4.6 ± 1.6‰, respectively) were on average more positive in comparison to bulk soil water (-46.7 ± 16.4‰ and -6.0 ± 2.3‰, respectively) (L386-388 in the original ms); however, comparing the isotopic composition range of xylem water and the soil water sources across sampling periods, we observed a good isotopic match between the tree xylem water and the soil water, while for the coffee plants, the xylem water had more enriched  $\delta$ 2H values in comparison to soil water. We have added this information in the revised version (Section 3.3, L425-433). To evaluate the effects of deuterium fractionation on coffee water sources, we compared the relative contribution of each water source obtained via the single isotope ( $\delta$ 2H) mixing model with those obtained via the informative prior distribution model. The results of these tests have been presented in Section 3.6. Finally, we have discussed this issue and its potential effect on the quantification of the plant water sources (Section 4.1, 519-558).

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  $100_{C}$ ) – waiting longer would not evaporate more water from the sample side unless the temperature is increased further.

**Reply:** Please see our reply to your previous comment.

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 mm), we acknowledge the difficulties with this approach. Hence, following the reviewer' suggestion, we decided to take the isotopic composition of the soil water at 5 cm depth as representative of near surface soil water. As a result, the discretization of the mixing model originally presented has changed in the revised version; Methods (Section 2.7, L303-315) and Results (Sections 3.2, 3.3 and 3.5; Table 2 and 3; Figure 3, 6 and 7) have changed accordingly. Please also see our reply to the next comment.

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 soil profile. In the original manuscript, we divided the soil water pool in two compartments: shallow (5-15 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 was used as the near surface water source following the reviewers' suggestion, we ran again the statistical tests to define the new classification of the soil water pool. Based on the results of these tests, the soil water pool was 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 (Section 2.7, L303-308).

**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. We have now added this information to the text (Section 2.6, L293-294).

- 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.

**Reply:** We have improved this in the discussion to stress the importance of using informative priors in the mixing models (Section 4.1, L509-518).

- (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.

Abstract I.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 have added this information in the Abstract (L23).

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

**Reply:** The percentages are mean values; we have added the +/- % standard deviation (L35-37).

I.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:** The sentence was rephrased for clarification (L37-38).

II.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 used the terms mentioned above (i.e., near surface for 5 cm depth and shallow for 15 cm depth).

II.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.

I.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 have changed this (L42). Also, we rephrased this sentence to represent our main conclusion (L41-43). The implications of our study are presented in Section 4.4 (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 deepest potential water source that we examined. It is unclear what the reviewer means with the question about water uptake vs. rooting depth with regard to line 44 (line 42 in the revised ms).

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 have provided more background information about Bayesian mixing models and highlight the novelty of including priors for the quantification of plant water sources (Section 1, L88-93).

I.55 and I.73: 'soil resources' sounds odd: : :can the authors specify please? **Reply:** We have been more clear.

l.87: However,

**Reply:** The suggestion has been followed.

I. 90-92: please note that mixing models are also frequently criticized, (Rothfuss 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).

1.92: '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 have added this reference to the text (Section 1, L95).

I.143: micrometeorological measurements (which)

**Reply:** We have changed this to "microclimatic measurements" (L143). The list of the microclimatic variables that were measured are provided in Section 2.2.

I. 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. We have included this reference in our Introduction (L91).

I.151/152: The answer to question no. 2 is not reflected in the abstract Materials/Methods **Reply:** We present the results of the two dry seasons investigated (the near normal and the more pronounced one), in the abstract (L26-27; L33), therefore we did answer the question #2. With regard to the Materials/Methods, in Section 2.3 we mentioned that the dry season of 2017 was warmer and drier offering the opportunity to examine the vegetation responses under more pronounced dry conditions.

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

I.214: 'carried out' rather than 'performed'? **Reply:** The change has been made.

I.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 (L224-228). For the trees, soil and rain samples, we have added this information in the text (L218-219; L232; L240).

I.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 have rephrased the sentence for clarification (L232-233).

I.247: refrigerated – was any mold developing on the samples? This can affect isotope ratiosReply: Some mold had developed on some of the samples of the trees and coffee shrubs, but this does not affect the xylem isotope ratios.

I.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 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.

I.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:** We have now added this information in the Supplementary Material.

#### Results

I.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 hydrometeorogical 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, we consider it important to present this information as part of the Results section.

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

**Reply:** Indeed, rainfall during the 2013-2014 dry season was about 20% lower than normal. Hence, following the suggestion of the reviewer we refer to this season as "near normal" in the revised manuscript.

1.351-353: 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 have added this information in the text (L366-368).

I.353: the API results don't tell the reader anything without proper explanation **Reply:** Please see our reply to a previous comment.

ll.359-360: two digits after comma reported for 180 – more than precision – should be avoided; add 'for' delta 180, 'for' delta 2H

**Reply:** We have made the changes.

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.Please also see our reply to one of your previous related questions.

– 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. As we mentioned earlier, we have included this information in the text (L293-294). However, we do not understand how this can have caused a bias in the prior information.

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 long-term data from 1970-2000.

Discussion LI.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 Section 4.2 in the Discussion, and yes, based on our findings, shade trees and coffee plants are complementary in their use of soil water.

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 4.4, in which we discuss the implications of our results and future research directions.

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 beincluded in the future? Are there others that 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 processes that lead to differences in the depth of plant water uptake (Section 4.1, L509-518).

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.

#### General comment

This study analyses plant water source partitioning in a coffee agroforestry system along seasons with contrasting soil moisture conditions. For that, the authors applied stable isotope techniques and Bayesian mixing models (MixSIAR) in order to test for the complementary use of soil water in space and time by coffee plants and shade trees. The importance of the study comes from the fact that ecohydrological relations in this type of traditional agroforestry systems are completely unknown, in contrast to those of intensive monospecific plantations. A novel aspect of the study is the inclusion of root and nutrient distributions within the framework of stable isotope mixing models, which is a usually underestimated capability of such models. That should improve their accuracy since plant water source partitioning is obviously constrained by root distribution and soil profiles of nutrient availability. Overall, this is a welldesigned, rigorous study, that is also clearly presented and well-written. Methods and results are concisely described and figures and tables are easy to interpret. Similar studies of plant water source partitioning are numerous, so it could be said that this study is not especially original. However, I find valuable to report this type of data from regions where they are scarce (i.e. Central and South America or Africa, see Barbeta & Peñuelas, 2017; Evaristo & Mcdonnell, 2017).

# We thank Adriá Barbeta for his positive and encouraging comments giving us the opportunity to further improve the article. Please find below our response to your comments.

Although my general assessment of the manuscript is highly positive, I miss some caution regarding stable isotope techniques. While this is a well-established approach, recent studies pointed to methodological issues linked to fractionation processes within the soil matrix (Orlowski et al., 2018; Gaj et al., 2019; Oerter & Bowen, 2019; Oerter et al., 2019), along the soil-plant continuum (Vargas et al., 2017; Barbeta et al., 2019) or within plant tissues (Zhao et al., 2016). Not all ecohydrological systems may be affected by those fractionation processes, and oxygen isotopes seem to still be highly reliable (Zhao et al., 2016; Vargas et al., 2017; Barbeta et al., 2017; Barbeta et al., 2019). Still, in Fig. 3, I observe that xylem water isotopes do not match very well with soil water isotopes from either depth. This is clearer for shade trees. A similar pattern arises in the deuterium excess boxplots. A thorough consideration of potential fractionation processes would require extensive additional analyses, which I think that it is not realistic to ask the authors to do. A more plausible solution is an explanation on why the authors think that fractionation processes are not relevant for their study. It might also be considered to run MixSIAR models separately for oxygen and hydrogen isotopes to check if there are significant discrepancies between them (as in Evaristo et al., 2017; Barbeta et al., 2019). As I said, it is known that fractionation processes do not affect in the same proportion oxygen and hydrogen isotopes. In any case, I believe that these emerging issues cannot longer be ignored by plant water source studies using stable isotopes.

**Reply:** We agree that fractionation processes may, and can no longer be omitted/discussed in the types of data our study presents. In fact, one of our co-authors has been an advocate and champion of doing the best possible research to discover when such affects might play a role (see Brantley et al. 2017; Oshun et al. 2016; Penna et al. 2018).

Calculating the isotopic composition range of xylem water and the considered sources across sampling periods and seasons, it is observed that all shade trees (-7.6 to -3.6 for  $\delta$ 180, and -65.5 to -32.2 for  $\delta$ 2H) and coffee plants (-6.3 to -0.6 for  $\delta$ 180 and -46.5 to -9.6 for  $\delta$ 2H) fell within the range of the soil water pool (-11.1 to -0.9 for  $\delta$ 180, and -83.4 to -11.9 for  $\delta$ 2H) during the 2014 dry season samplings (Fig. 3a)

In the 2017 dry season samplings, we again observed a good isotopic match between the tree xylem water (-6.0 to -3.2 for  $\delta$ 18O, and -56.7 to -34.5 for  $\delta$ 2H) and the soil pore (-7.5 to -1.6 for  $\delta$ 18O, and -54.8 to -19.0 for  $\delta$ 2H). However, for the coffee plants, the xylem water (-4.4 to -1.1 for  $\delta$ 18O and -39.6 to -7.9 for  $\delta$ 2H) had more enriched  $\delta$ 2H values in comparison to soil water (Fig. 3b).

In the 2017 wet season sampling, a very small mismatch was detected in  $\delta$ 2H between xylem water of coffee (-5.4 to -4.4 for  $\delta$ 18O and -42.2 to -34.5 for  $\delta$ 2H) and soil water (-8.5 to -4.1 for  $\delta$ 18O and -70.5 to -37.5 for  $\delta$ 2H), meanwhile the trees (-6.2 to -4.2 for  $\delta$ 18O and -60.6 to -45.6 for  $\delta$ 2H) showed again a good overlap with soil water (Fig. 3c). We have added this information in the Results (Section 3.3, L423-431), and based on these results, we carried out some tests to specifically evaluate the effects of deuterium fractionation on coffee water

sources by running a simple mass balance approach using hydrogen isotope ratios only in the MixSIAR model. The results of these tests have been presented in Section 3.6.

Finally, we have discussed this issue and its potential effect on the quantification of the plant water sources (Section 4.1, 519-558).

#### Minor comments

L38 It is not completely clear what does 'precipitation conditions' mean. **Reply:** We have rephrased the sentence for clarification (Abstract; L37-38).

L65 Species name (Cedrela odorata) should not be in capital letters. **Reply:** Agree. We have made the correction (L66).

L191 The high clay content is likely to produce soil water isotopic fractionation (Oerter et al., 2014).

**Reply:** 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 about the soil hydro-physical properties. For clayey soils, information about the cation exchange capacity (CEC) should be given, 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 have incorporated this information in the revised manuscript (Section 2.5 in the Methods: L278-283; Section 3.4 in the Results: L446-447 and Table 4) 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 (Section 4.1 in the Discussion: L519-535). See also our reply to a similar comment made by reviewer #1.

L218 The sampling of different plant parts in coffee plants and shade trees (cores VS branches) could have led to a different proportion of internal plant water pools in the xylem water samples of each group.

**Reply:** Agree. However, due to their much smaller size for the coffee, it was not possible to collect a xylem core from the main stem of the coffee plants without inflicting major damage. Therefore, to sample comparable plant xylem water pools between trees and coffee, segments (~6 cm) of mature branches were cut near the main stem of the coffee plants.

L223 I assume that bark was peeled off from coffee shrubs, too.

**Reply:** The bark (~ 1mm thick) from the branch segments of the coffee shrubs was not peeled off, because doing so would have taken considerable time and thus potentially expose the sample to evaporation; we have included this information in the Methods (L222-224) and also their potential effects on the enrichment observed in the deuterium composition of the coffee xylem water (Section 4.1 in the Discussion: L536-558).

L298 Recent precipitation, especially in periods with relatively wet soil conditions, could in fact percolate faster towards deeper layers. So, rainfall is not necessarily representative of near surface soil water.

**Reply:** We agree and we have revisited this assumption in response to your comment and a similar comment of Reviewer #1. Since each isotope sampling campaign was preceded by at least 6 days up to 22 days without or with minimum accumulated rainfall (< 5 mm), we acknowledge the difficulties with this approach. Therefore, we decided to take the isotopic composition of the soil water at 5 cm depth as representative of near surface soil water. As a result, the discretization of the mixing model originally presented has changed in the revised version; Methods (Section 2.7, L303-315) and Results (Sections 3.2, 3.3 and 3.5; Table 2 and 3; Figure 3, 6 and 7) have changed accordingly.

L304 The use of prior information is a very interesting point of the study. **Reply:** We appreciate your comment.

Daniele Penna (Referee) daniele.penna@unifi.it Received and published: 31 August 2019

Review of the manuscript 'Coffee and shade trees show complementary use of soil water in a traditional agroforestry ecosystem' by Muñoz-Villers et al. (hess-2019-329)

General comment

Reading this manuscript was pleasant. I particularly liked the idea of including the distribution of roots and nutrients in the mixing model approach, and I think that this point should be better stressed in the manuscript.

Overall, I fully agree with the comments provided by the reviews of Matthias Beyer and Adrià Barbeta, and I have only some minor comments to add. Good job! Daniele

# We thank Daniele Penna for his positive comments on our manuscript. Please find below our response to each comment.

Minor comments and technical corrections

42-43. This sentence is not immediate to understand without reading the paper. I suggest rephrasing. **Reply:** We have rephrased the sentence for clarification (L42-43)

45. Double negation (limitation: : :absent): I suggest simplifying the sentence. **Reply:** We have corrected the sentence (L44-45).

64. Complex sentence, rephrase. **Reply:** We have rephrased the sentence (L64-67).

149. I suggest to change into ": : :prevails over competition: : :" or, in any case, to include both the terms "complementary" and "competition" because the latter is logically linked to the second research question. **Reply:** We have followed the suggestion (L148-149).

268. Did you consider using the Normalized Antecedent Precipitation Index (NAPI, Heggen, 2001), instead of API? Heggen, R.J., 2001. Normalized antecedent precipitation index. J. Hydrol. Eng.

**Reply:** We did not consider it but reading about this method we learned that NAPI utilizes the same antecedent daily precipitation record as does API in determining antecedent moisture conditions. The difference is that NAPI treats precipitation earlier in the day of a specific event (i.e. storm runoff or in our case the isotope sampling) as antecedent to the event. Since our samplings were conducted in dry days, the use of NAPI would not make a difference.

385-405. I suggest to condense this part and let the figures talk for themselves. **Reply:** We have followed the suggestion. This part has been reduced in the revised version (L400-414).

Fig. 3. Caption: why panel (c) shows the GMWL whereas panels (a) and (b) the LMWL? **Reply:** We have corrected the caption of Figure 3.

Fig. 4. I suggest to replace "(a)" and "(b)" with "2014" and "2017" for more immediate understanding. **Reply:** We have made the change.

Fig. 5. What do error bars represent? Why are there only in panel (a) and not in panel (b)?

**Reply:** The bars represent the standard deviation; we have added this information in the figure caption. These bars are not showed in the panel (b) because the values in the *y* axis were normalized and expressed as ratio to their maximum values.

Fig. 8. I think that the result and discussion build around this figure should be taken with a bit of caution because based on few point only. I suggest to discuss this limitation in the manuscript. **Reply:** Agree. We have mentioned this limitation in the discussion (L634).

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1	Manuscript title
2	Coffee and shade trees show complementary use of soil water in a traditional agroforestry
3	ecosystem
4	
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18	
19	
20	Abstract

21 On a global scale Globally, coffee has become one of the most sensitive commercial crops that will 22 being affected by climate change. The majority of Arabica coffee (Coffea arabica) grows in 23 traditionally shaded agroforestry systems in tropical regions and accounts for ~70% of the coffee 24 production worldwide. Nevertheless, the interaction between plant and soil water sources in these 25 coffee plantations remains poorly understood. To investigate the functional response of dominant 26 shade trees species and coffee (C. arabica var. typica) plants to different soil water availability 27 conditions, we conducted a study during a near normal and a more pronounced dry season (2014 and 28 2017, respectively) and athe 2017 wet season (2017) in a traditional agroecosystem in central 29 Veracruz, Mexico. For the different periods, we specifically investigated the variations in water 30 sources and root water uptake via MIXSIAR mixing models using  $\delta^{18}$ O and  $\delta^{2}$ H stable isotopes of rainfall, plant xylem and soil water, along with micrometeorological and soil moisture measurements. 31 32 To further increase our mechanistic understanding about root activity, the distribution of belowground 33 biomass and soil macronutrients were also examined and considered in the model as prior 34 information. Results showed that, over the course of the two dry seasons investigated, all shade tree 35 species (Lonchocarpus guatemalensis, Inga vera and Trema micrantha) relied on average, on water 36 sources from intermediate (>15 to 30 cm depth:  $58 \pm 18\%$  (SD)) and deeper soil layers (> 3045 to 37 120 cm depth:;  $\frac{86-34 \pm 21\%}{100}$ , while coffee plants the used of much shallower water sources (< 5 cm 38 depth:  $42 \pm 37\%$  and <5-15 cm depth:  $5260 \pm 35\%$ ) was observed in coffee plants. In addition, in 39 these same periods, coffee water uptake was strongly influenced by antecedent precipitation 40 conditions, whereas trees showed little sensitiveness to short-termantecedent wetness-status. Our findings also showed that during the wet season coffee plants substantially increased the use of near 41 42 surface water ( $\pm 5648\%$  from  $\le 5$  cm depth), while shade trees extended the water acquisition to much 43 shallower soil layers (+3219% from < 15 cm depth) in comparison to drier periods. Despite the 44 plasticity in rootsoil water uptake observed betweenamong canopy trees and coffee plants, a spatial 45 segregation of the main complementary use of soil water source prevailed during the dry and wet 46 seasons studied. However, more variability in plant\_soil-water uptake patternssources was observed 47 among species in the rainy season when higher soil moisture conditions were present and water 48 stresslimitation was largely absent.

49

50 Key words: Shade trees, Coffea arabica; water stable isotopes, roots, nutrients, clay-rich soils,

51 MixSIAR, Mexico

#### 52 1. Introduction

53 Coffee agroforestry systems are highly valued because of their ecological, environmental, 54 economic and social benefits (Mas and Dietsch, 2004; Perfecto et al., 2007; Tscharntke et al., 2011). 55 Moreover, shade coffee of the species Arabica (*Coffea arabica*) accounts for ~ 70% of the total coffee 56 production (USDA, 2017). Although Arabica coffee is mainly grown in tropical montane regions, it 57 is cultivated under a wide range of climatic and soil conditions (Jha et al., 2014). Coffee Arabica 58 plantations can be broadly classified as traditional or modern coffee systems, according to vegetation 59 composition and structure and management practices (Moguel and Toledo, 1999). In the traditional 60 systems, coffee plants are cultivated under a diverse canopy of native and/or introduced shade tree 61 species. In contrast, monoculture coffee plantations exemplify the modern cultivation scheme, in 62 which the shade is provided by a single commercial tree species. The use of agrochemicals is also 63 typically required in this type of plantation (Moguel and Toledo, 1999).

64 Until recently, the vast majority of Arabica coffee was cultivated in traditionally managed 65 shaded coffee plantations, which have lower production costs and enhanced biodiversity, carbon 66 sequestration, soil fertility and biological pest control in comparison to modern systems (Greenberg 67 et al., 1997; Perfecto et al., 2002; Kellermann et al., 2008). However, coffee management practices 68 worldwide have increasingly become more intensive promoting the replacement of native trees with 69 fast-growing monospecific timber species (i.e. *Cedrela oodorata, Eucalyptus deplupta, Hevea* 70 *brasilensis*) (Nath et al., 2011).

71 Growing a crop in association with shade trees inevitably leads to some degree of competition 72 for the above-ground (light) and below-ground (water and nutrients) resources (Monteith et al., 1991). 73 In an agroforestry system, the outcome of competition for light is relatively predictable due to the 74 hierarchical structure of the canopy (i.e., shade trees intercept part of the sunlight, thereby reducing 75 the amount available for the understory crop). Conversely, competitive interactions for soil-below-76 ground resources can be much more diverse and complex. The central hypothesis of agroforestry 77 underscores that crops and trees are complementary in their use of soil resources-water (Cannell et 78 al., 1996), however the degree to which this occurs will be largely controlled by the spatial and 79 temporal patterns of resource availability, root distribution and root activity, which in turn depend on 80 factors such as climate, soil conditions, crop and tree species, and plantation age, density and 81 management practices (Beer et al., 1998; Lehmann, 2003; van Noordwijk et al., 2015). In addition, 82 below-ground competitive interactions for water and/or nutrients are much more difficult to elucidate 83 than above-ground relationships. So far, the most common approach is to measure the distribution of 84 root abundance of crops and trees, and examine to what extent they overlap or are separated (e.g., 85 Schaller et al., 2003; van Kanten et al., 2005). An important limitation of this method is, however, 86 that the spatial distribution of roots does not always mirror the actual resource capture along the soil 87 profile (Dawson et al., 2002; Lehmann, 2003). Another approach is to examine the vertical patterns 88 of soil water content (Cannavo et al., 2011; Padovan et al., 2015) or nutrient (Schroth et al., 2000, 89 cited in Lehmann, 2003) depletion. However, tThese methods are problematic because they cannot 90 provide information on whether resource depletion is caused by the crop, the trees, or both (Cannavo 91 et al., 2011; Padovan et al., 2015). HoweverRecently, the use of hydrogen ( $\delta^2$ H) and oxygen ( $\delta^{18}$ O) 92 water stable isotope techniques in combination with Bayesian mixing models based on Bayesian 93 theory has proved to be ides a powerful tool for quantifying the proportions and probability 94 distributions of different water sources to plant uptake across different ecosystems and regions 95 (Barbeta et al., 2015; Beyer<del>Muñoz Villers</del> et al., 2018; Penna et al., 2018), with the potential to which 96 and can largely overcome the above-mentioned limitations (Dawson et al., 2002; Lehmann, 2003; 97 van Noordwijk et al., 2015). Although rarely implemented, including nutrient and root distribution 98 data along the soil profile to inform these models could provide more comprehensive insights into 99 depth of plant water uptake (cf. Muñoz-Villers et al., 2018).

100 To date, research into plant-soil interactions and plant water source partitioning in coffee 101 agroforestry systems is extremely scarce. To our knowledge, only five studies have investigated the 102 water sources of shade trees and coffee shrubs using either information on the isotopic composition 103 of plant xylem and bulk soil water (Wu et al., 2016), soil water depletion (Cannavo et al., 2011; 104 Padovan et al., 2015) or root distribution (Schaller et al., 2003; van Kanten et al., 2005). Moreover, 105 all of these studies have been carried out in intensive monospecific plantations characterized by high 106 coffee planting densities (4000-5000 shrubs ha-1), low density ( $\sim 150-280$  trees ha-1) and very low 107 diversity (1-2 species) of shade trees. While recognizing the limitations of some of the methods used 108 in these previous studies, the available information suggests that competition for water between coffee 109 and trees can be strong at sites with a pronounced seasonal dry period (Wu et al., 2016; Padovan et 110 al., 2015), while it seems to be virtually absent at sites with no or a relatively short dry season 111 (Schaller et al., 2003; Cannavo et al., 2011). Further, although most coffee roots are usually located 112 in the upper soil layers (< 30 cm depth; van Kanten et al., 2005, and references therein), the plant and 113 soil interactions for water during the dry season seem to occur below the main crop rooting zone (> 114 30 cm depth) (Wu et al., 2016). The latter reflects the ability of coffee to develop an extensive root 115 system, and to increase the root water uptake at greater soil depths once the available water has been 116 depleted in shallower layers (Huxley et al., 1974, cited in Lehmann, 2003).

117 Currently, we lack of information on plant water sources in traditional shade coffee 118 plantations. In these agroforestry systems, the higher density and diversity of shade trees could 119 potentially lead to stronger and more diverse tree-crop interactions (van Noordwijk et al., 2015). On the other hand, the dense tree canopy reduces light availability <u>and hence</u> limit<u>sing</u> coffee water use.
This could lead to a lower soil water demand and thus increased plant water availability during the
dry season.

123 Further, ecohydrological research in these shade coffee systems is becoming increasingly 124 important since trees have been promoted as a strategy for mitigating and adapting to future climate 125 (Schroth et al., 2009; Vaast et al., 2016; Rice, 2018). Shaded coffee plantations store more carbon 126 than sun-grown coffee systems, thereby contributing to the reduction of greenhouse gases (Vaast et 127 al., 2016; Rice, 2018, and references therein). In addition, the tree canopy provides some level of 128 protection against the rising mean and maximum air temperatures (Baker and Haggar, 2007; Schroth 129 et al., 2009; Vaast et al., 2016), which in recent modeling studies have been pointed out as the key 130 climatic changes affecting coffee growth, yield and quality (Schroth et al., 2009; Baca et al., 2014; 131 Bunn et al., 2015). Although there are important differences across sites, rainfall is also predicted to 132 decrease and become more variable in many of the world's coffee-growing regions. For example, 133 Giorgi (2006) estimated that rainfall will decrease by about 17% (per 100 years) during the dry season 134 and by about 9% during the wet season in Mexico and Central America. Similarly, predictions by 135 Karmalkar et al. (2011) for the same regions pointed out changes in rainfall of -24% to +8% (per 100 136 years) during the dry season and of -39% to -1% during the wet season (with the range reflecting 137 variability among regions). As such, if warming is accompanied by decreases in rainfall, this could 138 lead to, or exacerbate, competition for water sources between coffee shrubs and shade trees (Baker 139 and Haggar, 2007), which in turn could affect the long-term sustainability of these agroecosystems.

140 Mexico is among the largest shade coffee producers in the world, and the central region of 141 Veracruz constitutes the second most important coffee zone in the country. In this area, we selected 142 a representative traditional shade coffee plantation to investigate plant water sources of dominant 143 shade trees species and coffee (C. arabica var. typica) shrubs under different conditions of soil water 144 availability. DHence, during a near normal and a more pronounced dry season (2014 and 2017, 145 respectively) and athe 2017-wet season (2017), variations in depth of plant water uptake were 146 examined using the stable isotopic composition ( $\delta^{18}$ O and  $\delta^{2}$ H) of rainfall, plant xylem and soil water 147 in combination with a Bayesian mixing model (MixSIAR), along with microclimaticmeteorological 148 and soil moisture measurements. To further increase our understanding about root activity and water 149 uptake, the distribution of roots and macronutrients along the soil profile were also examined and 150 considered in the mixing model as prior information. Specifically, we addressed the following 151 questions:

- Does a complementary water use strategy between shade trees and coffee shrubs prevail
   <u>over competition</u> in a traditional shaded agroforestry system?
- 1552. Does competition exist for water sources among tree and coffee species during morepronounced dry periods?
- 157
- 3. What are the seasonal patterns in plant-water source partitioning?
- 158
- 159 2. Materials and methods
- 160 *2.1 Study site*

The research was carried out in the "La Orduña" coffee plantation (~100 ha) located on a flat
plateau at an elevation of 1210 m a.s.l. on the eastern slopes of the Cofre de Perote mountain (19°28'
N, 96°56' W) in central Veracruz State, Mexico (Fig. 1). The coffee plantations in this region occur
between elevations of 1000 and 1350 m a.s.l. (Hernández-Martínez et al., 2013; Marchal and Palma,
1985).

166 The climate is classified as temperate humid with abundant rains during the summer (Garcia, 167 1988). Two distinct seasons can be distinguished: (1) a wet season (May-October), during which 168 rainfall is associated primarily with cumulus and cumulonimbus clouds formed during convective 169 and orographic uplift of the moist maritime air masses brought in by the easterly trade winds; and (2) 170 a (relatively) dry season (November-April), during which most rainfall falls from stratus clouds 171 associated with the passage of cold fronts (Báez et al., 1997). Mean annual rainfall measured nearby 172 the study site during the period 1971–2000 was 1765 mm, with on average monthly rainfall of 389 173 mm falling during the dry season and 1376 mm falling during the wet season (SMN, 2018). Mean 174 annual temperature over this period was 19.5 °C, with a minimum and maximum monthly average 175 value of 15.5 and 22.5°C observed in January and May, respectively (SMN, 2018). Annual potential 176 evapotranspiration (ET<sub>0</sub>) is about 1120 mm (Holwerda et al., 2013).

177 The investigated shade coffee plantation is a so-called traditional commercial polyculture 178 system (sensu Moguel and Toledo, 1999), which was established more than 80 years ago. The tree 179 canopy wasis diverse and consisteds predominantly of the species Inga spp., Citrus spp., 180 Lonchocarpus guatemalensis, Trema micrantha and Enterolobium cyclocarpum (Holwerda et al., 181 2016). The shade trees were planted at a density of ca. 500 ha-1, and currently form a canopy of 182 about 14 m high. The Arabica coffee plants were of the variety typica. Typica – a tall cultivar of Coffea 183 arabica- was the first coffee variety that arrived from Ethiopia to Mexico (Renard, 2010); it has 184 bronze-tipped young leaves and the berries are large. Plants of typica variety are tolerant to conditions 185 of low soil fertility and drought, but vulnerable to most pests and diseases (Escamilla et al., 2005). In 186 the study site, this cultivar was planted approximately 20 years ago at a density of about 1700 shrubs ha-1, currently having an average height of ~ 2 m. In this region, the coffee flowering occurs in
March or April, fruit development between May and October, and ripening and harvest between
October and February (Villers et al., 2009). The management of the plantation involves weed control
practices and selective pruning of mature coffee plants and shade trees at irregular times once every
7 years (cf. Hernández-Martínez et al., 2009). No pruning activities occurred during or in between
our study periods. A photograph of the coffee plantation is providedshown in the Supplementary
Material.

194 The soil type is an Andic Acrisol derived from volcanic ashes. Soil profiles (~150 cm) are 195 multilayered (A, B1/BT and BC) and have clay (~ 65%) as the dominant texture across all layers. A 196 general description of the soil profile showed a dark brown to dark yellowish brown, clay silty organic 197 A horizon (0–20 cm) overlying a dark yellowish brown, clay silty sand B1/BT horizon (20–135 cm), 198 followed by a dark yellowish brown, clay sandy BC horizon (>135 cm). Average soil bulk densities 199 and porosities were 1.2 gr cm-3 and 63%, respectively, along the A and B horizons (Holwerda et al., 200 2013). The underlying material consists of deeply weathered old lava and sandy-gravelly pyroclastic 201 flow deposits (Rodríguez et al., 2010). Soils are mostly covered by a thin (1-2 cm) but continuous 202 layer of litter.

203

#### 204 2.2 Hydrometeorological measurements

During the study period, rainfall and microclimate conditions were continuously monitored above the canopy in an 18 m high tower, located in the southwestern part of the coffee plantation. Rainfall (P, mm) was measured using a TR–525 M tipping bucket rain gauge (Texas Electronics, USA). Temperature (T, °C) and relative humidity (RH, %) were measured using a HC2-S3 probe (Rotronic, USA). Data were recorded every 30 s, accumulated (P) or averaged values (all other parameters) were stored at 5<sub>-</sub>-min intervals using a CR1000 datalogger (Campbell Scientific Ltd., USA).

212

# 213 2.3 Isotope sampling

To examine the water sources of overstory shade trees and understory coffee shrubs, plant tissue and soil samples were collected for isotope analysis at the middle (Jan. 23) and end (Apr. 11 and 26) of the 2014 dry season. In 2017, the dry season was warmer and drier offering the opportunity to examine the vegetation responses to more pronounced dry conditions. Therefore, a second sampling campaign was <u>carried outperformed</u> to collect plant and bulk soil samples at the middle (Feb. 27), end (Apr. 5) and late end (May. 20) of the 2017 dry season. Another sampling was carried out in the middle of the 2017 wet season (Aug. 4) to evaluate plant-soil water uptake patterns at highersoil water availability conditions.

222 In all seven samplings, xylem samples were obtained from three individuals of each of the 223 three dominant shade tree species (Lonchocarpus guatemalensis, Inga vera and Trema micrantha) by 224 extracting ~5-6 cm cores using a Pressler increment borer inserted at 1.2 m above ground (n = 60) 225 samples of trees in total). On each occasion, xylem samples were taken from the same individuals but 226 from various aspects of the trunk. The bark was immediately removed after core extraction to avoid 227 contamination of phloem water. For the coffee plants, samples were obtained from ~6 cm segments 228 of mature suberized branches that were cut near the main stem of several shrubs each time. The bark 229 (~1mm thick) and cambium were not stripped from the coffee branches, to avoid exposure of the 230 samples to evaporation. All coffee plants were sampled randomly (n = 40 samples of coffee shrubs)231 in total). During the 2014 and 2017 dry seasons, sampling of coffee shrubs involved 5-6 individuals 232 each time. Since only one sampling occasion was performed during the 2017 wet season, a larger 233 number of individuals (10) was sampled to reduce the uncertainties associated with different sampling 234 sizes between wet and dry seasons respectively. For each tree, we measured diameter at breast height 235 (DBH) and height, and for the coffee plants the diameter of the main stem was measured below its 236 bifurcation in small branches (Table 1).

Bulk soil samples were collected at three locations and at <u>depth of</u> 5, 15, 30, 60, 90 and 120 cm-<u>depth each</u>, using a hand auger (n = 126 samples of soil in total). Auger sampling points were located so that each of the sampled shade trees and coffee plants had <u>a total of threeone</u> soil sampling points within <u>atheir</u> 3 m radius.

Samples of xylem and bulk soil were collected during the morning and early afternoon (between 8:30 to 13:30 hrs), and each sampling campaign was preceded by at least 6 days up to 22 days without or with minimum accumulated rainfall (< 5 mm). All xylem and soil samples were collected quickly and carefully and <u>stored</u> in water-tight vials to avoid any evaporation (see section below).

To establish the local meteoric water line and compare soil water sources with recent rainfall, bulk samples of rainfall (n = 80 in total) were collected weekly at a nearby (~ 5 km) meteorological station over the course of the twofour years studied (Nov. 2013 – Oct. 2014 and Nov. 2016 – Oct. <u>2017 December 2013 to December 2017</u>) as part of a long-term isotope sampling of precipitation (cf. Muñoz-Villers et al., 2018).

251

#### 252 **2.4 Isotope collection and analysis**

253 Samples of precipitation, plant xylem and bulk soil for isotope analysis were collected in 30-254 ml borosilicate glass vials sealed with polycone caps to prevent evaporation. All samples were 255 refrigerated until extraction and analysis at the Center of Stable Isotope Biogeochemistry (CSIB) at 256 the University of California-Berkeley, USA.

257 Xylem and soil samples were extracted using cryogenic vacuum distillation (temperature: 258  $100 \pm 1.1^{\circ}$ C, vacuum:  $3 \pm 1.5$  Pa and time: 60-70 min) following the method of West et al. (2006). 259 The  $\delta^2$ H and  $\delta^{18}$ O isotopic compositions of extracted water samples were then determined using an 260 isotope-ratio mass spectrometer (Thermo Delta Plus XL, Thermo Fisher Scientific, USA). The 261 analytical precision of the instrument was  $\pm 0.60\%$  (1 SD) for  $\delta^2 H$  and  $\pm 0.12\%$  (1 SD) for  $\delta^{18} O$ . 262 Samples of precipitation were analyzed for  $\delta^{2}$ H and  $\delta^{18}$ O using a laser water isotope analyzer (L2140-263 i) from Picarro Inc. (Santa Clara, CA, USA) in high precision and without Micro-Combustion Module mode. The analytical precision was  $\pm$  0.65% (1 SD) and  $\pm$  0.20% (1 SD) for  $\delta^2$ H and  $\delta^{18}$ O, 264 265 respectively.

The isotope values are expressed in delta notation (‰) relative to Vienna Standard Mean 266 267 Ocean Water (VSMOW). To evaluate evaporative enrichment in the soil and xylem water isotopes relative to rainfall, we calculated the deuterium-excess parameter ( $d = \delta^2 H - 8 * \delta^{18} O$ ; Dansgaard, 268 269 1964).

270

271

#### 2.5 Soil sampling and laboratory determinations

272 To determine volumetric soil water content (SWC), samples were collected at 5, 15, 30, 60, 273 90 and 120 cm depth from each of the three boreholes excavated during the soil isotope samplings. 274 Soil moisture content was determined gravimetrically and converted to volumetric values by using 275 bulk density of the soil sample. In addition, to determine the antecedent moisture conditions for the 276 15 days prior to each sampling date, an antecedent precipitation index (API) was calculated following 277 Viessman et al. (1989).

278 To examine pH and N, P and K macronutrient concentrations along the soil profile, soil 279 samples were collected at 5, 15, 30, 60, 90 and 120 cm depth from each borehole (n = 3 samples per 280 soil depth) during three isotope sampling campaigns: Apr. 11, 2014 (dry season), Feb. 27, 2017 (dry 281 season) and Aug. 4, 2017 (wet season). Samples (n = 18) for determining other chemical properties 282 were collected at the same depths in soil profiles. All ssamples were first air-dried and then sieved 283 using 2 mm screens. Soil pH was determined using a glass electrode pH meter in a 1:2 soil: water 284 ratio. Organic matter content was determined by the Walkley-Black method. Total carbon (C) and 285 total nitrogen (N) were measured using a TruSpec dry combustion CN analyzer (LECO, USA). 286 Extractable phosphorus (P) was determined by the Bray I method (Bray and Kurtz, 1945). 287 Exchangeable cations (Ca+, Mg+, K+, Na+) were determined by extracting soil with 1 MNH4OAc

288 (pH 7.0). Ca+ and Mg+ were analyzed using atomic absorption spectrometry and K+ and Na+ were

analyzed using flame photometry. Soil cation exchange capacity (CEC) was determined by the

ammonium acetate 1N (pH 7.0) method (Van Reeuwijk, 2002) and base saturation (BS) was

- calculated as the portion of CEC that is occupied by exchangeable bases: (Ca+, Mg+, K+, Na+)/CEC.
- 292

#### 293 **2.6 Root biomass**

294 To examine the root biomass distribution along the soil profile in the study plot, 33 soil cores 295 were obtained using 5 cm diameter and 10 cm long samplers. Soil cores were extracted at 5, 20, 40, 296 60 and 90 cm depth (from 5 to 40 cm: n = 9 for each depth, and from 60 to 90 cm: n = 3 for each 297 depth). All cores were processed immediately in the laboratory. Soil samples were first sieved using 298 2 mm screens to separate the bigger roots. Next, the samples were washed using a fine nylon mesh 299 sieve, and then separated into diameter classes (< 1 mm, 1-2 mm and > 2 mm) and dried at 70 °C for 300 48 hours. Root biomass (g m-3) was calculated from the dry weight of the roots and the volume of 301 the core sampler for each class and soil depth. No differentiation between roots of coffee shrubs and 302 shade trees was made.

303

# 304

## 4 2.7 Plant water uptake sources and temporal patterns

305 The MixSIAR Bayesian mixing model framework (Moore and Semmens, 2008; Stock et al., 306 2018) was used to determine the most likely contributions of water sources for the shade tree species 307 and coffee shrubs sampled over the course of the 2014 (Jan. 23, Apr. 11 and 26) and 2017 (Feb. 27, 308 Apr. 5, May. 20) dry seasons and the 2017 wet season (Aug. 4). To assess temporal changes of the 309 different plant water sources, the seven sampling occasions were modeled separately. The mixture 310 data for the model was the mean xylem water isotopic ( $\delta^{2}$ H and  $\delta^{18}$ O) composition of the shade tree 311 species and coffee shrubs, changing accordingly with the sampling date. Based on statistical tests, 312 tThe relative contributions of fourthree potential plant xylem end-member water sources was-were 313 evaluated. These included rainfall as surrogate for and restricted to the following soil groups: near 314 surface water (< 5 cm  $\frac{\text{depth}}{\text{depth}}$ ), shallow-soil water (average of 5 to -15 cm  $\frac{\text{depth}}{\text{depth}}$ ), intermediate (> 15 315 to 30 cm) and deep soil water (average of > 30 to- 120 cm-depth). For each sampling date, the mean 316 and standard deviation of the soil water isotopeie ( $\delta^2 H$  and  $\delta^{18}O$ ) signatures from the four different 317 grouped soil depths of the water sources were introduced into the model, as follows: rain water isotope 318 data from a month prior to the xylem sampling and soil water isotope data from the two different 319 grouped soil depths, all corresponding to the date of xylem tissue collection.

320 Further, we also considered the use of additional data such as soil macronutrients (N, P, K) 321 and root biomass information to constrain model estimates by specifying an 'informative' prior 322 distribution of the soil source proportions (Stock et al., 2018). These data were also grouped into 323 fourtwo classes based on the depth of the soil samplings and corresponding largely with the grouping 324 for soil water: <u>near surface (< 5 cm)</u> shallow (0-5 to 20 cm), <u>intermediate (> 20</u> to 40 cm) and deep 325 (> 40 to 120 cm). In addition, the nearest corresponding dry or wet season dataset of soil 326 macronutrients were used according to the date of sampling. More details on the informative prior 327 parametrization are provided in the Supplementary Materials. The effect of using these priors (i.e. a 328 weight proportion before considering the isotope data) on the water sources distribution was then 329 examined by comparing these with the results of 'non-informative' (i.e. all the combinations of 330 proportions of water sources were equally likely) simulations. The results of each of these model runs 331 were accepted based on the examination of Markov Chain Monte Carlo convergence using the 332 Gelman-Rubin and Geweke diagnostic tests (Gelman et al., 2014).

Furthermore, the effect of isotope fractionation on the quantification of plant water sources was specifically explored by comparing the results of the informed two-isotope mixing model with those from a mixing model using only one water stable isotope ratio in the MixSIAR Bayesian framework. This approach has been used elsewhere (Evaristo et al., 2017; Barbeta et al., 2019) to provide some initial insights. Nevertheless, we are aware that the use of a single isotope ratio approach in a multiple water source model could lead to erroneous results due to the overlap of feasible solutions with poor constrained of uncertainties (see Parnell et al., 2010).

Lastly, the relative contributions of the water sources were compared among shade trees and coffee shrubs across all sampling dates using factorial ANOVA and Tukey's HSD post-hoc tests. The analyses were carried out in R Statistical Software version 3.2.4 (R Core Development Team, 2016).

343

344 3. Results

# 345 3.1 Hydrometeorological conditions

346 Precipitation (P) was 1650 mm in the first study year (Nov. 2013 – Oct. 2014) and 1423 mm 347 in the second study year (Nov. 2016 - Oct. 2017). During the 2013-2014 dry season (Nov - Apr.), 348 rainfall was 323 mm, and mean daily values of temperature (T) and vapor pressure deficit (VPD) 349 were  $17.6 \pm 3.0^{\circ}$ C and  $0.65 \pm 0.39$  kPa, respectively. The lowest monthly amounts of P and the highest 350 values of T and VPD were observed in April at the end of the dry season (Fig. 2a,b). During the 2016-351 2017 dry season, rainfall amounted towas 235 mm, with lowest monthly values registered in January 352 and February at the middle of the season (Fig. 2b). Mean daily T was  $18.3 \pm 2.6^{\circ}$ C, with the highest 353 values observed at the end of the dry period. Generally Overall, high VPD was high during the entire 354 values prevailed over the course of this dry season (0.78 ± 0.46 kPa on average), and reached although
 355 maximum values were particularly observed in February and May.

Compared to long-term (1971–2000) climatic records of the region, rainfall in the first <del>year</del> of-study <u>year</u> was very close to the mean annual precipitation of 1765 mm (SMN, 2018). In contrast, the second year was drier (~ 300 mm less); <u>especially the difference was particularly observed</u> during the dry season, which had about 40% lower precipitation than the average value of 389 mm. Also, higher mean monthly temperatures (+ 0.54°C) prevailed across the 2017 dry season in comparison with the 1971–2000 period. <u>Although rainfall during the 2013-2014 dry season was also about 20%</u> lower than normal, this season was considered as near average.

Rainfall during the 2017 wet season (May – Oct.) was lower in comparison to 2014 (1188 mm vs. 1326 mm, respectively) (Fig. 2b). Further, the mean air temperature and vapor pressure deficit were slightly higher in the 2017 wet season than in the 2014 wet season ( $20.7 \pm 1.6$  °C and  $0.67 \pm$ 0.25 kPa vs. 20.1 ± 1.5 °C and  $0.60 \pm 0.21$  kPa, respectively) (Fig. 2a).

367

# 368 3.2 Soil moisture and antecedent precipitation during sampling campaigns

369 During the 2014 dry season campaign (Jan. – Apr.), mean soil water content (SWC) was on 370 average  $339.8 \pm 16.7\%$  at 5 cm depth,  $40.2 \pm 14.5\%$  at 15 cm depth,  $38.9 \pm 6.4\%$  at 30 cm depth in 371 the shallower layers (5–15 cm depth) and  $48.36.0 \pm 1.45.2\%$  in the deeper layers (at 630-to -120 cm 372 depth) (Fig. 2b). In comparison, SWC in the 2017 dry season campaign (Feb. – May.) was lower in 373 the shallower layers first 30 cm ( $32.5 \pm 3.95.8\%$ ), meanwhile water content in the deeper layers was 374 similar (49.08.9  $\pm$  2.9%) with respect to the 2014 dry period. In 2014, lowest SWC values were 375 observed at the end of the dry season (April), whereas the greatest soil moisture depletion in 2017 376 was registered at the middle of the dry season (February) (Fig. 2b).

377 During the wet season sampling in August 2017, higher SWC values in the shallower at 5 cm 378  $(28.235.0 \pm 23.67\%)$ , 15 cm  $(30.9 \pm 4.3\%)$ , 30 cm  $(38.4 \pm 4.8\%)$ -and 60 to -120 cm at deeper (498.09) 379  $\pm 2.97.2\%$ ) soil depthslayers were generally observed in comparison to the 2017 dry period (Fig. 2b). 380 Although the 2017 wet season sampling showed slightly lower SWC values in the shallower soil 381 layers in comparison to the 2014 dry season, the SWC values in the deeper layers were higher. For 382 the different samplings, antecedent precipitation wetness conditions (API) were, respectively, 4, 30 383 and 13 mm for Jan. 23, Apr. 11 and 26, 2014 and 1, 12, 9 and 43 mm for Feb. 27, Apr. 5, May. 20 384 and Aug. 4, 2017.

385

#### 386 **3.3** Stable isotope composition of waters

387 Over the study periods, a greater range of variation was found in the rainfall isotope 388 composition of the 2013-2014-year (from -126.7 to 14.4% for  $\delta^2$ H; from -17.7 to 0.04% for  $\delta^{18}$ O) 389 in comparison to the 2016-2017-year (from -113.3 to 15.5% for  $\delta^2$ H; from -15.9 to 0.04% for  $\delta^{18}$ O) 390 (p > 0.05) (Fig. 3). Overall, mean dry season rainfall was significantly more enriched than the mean 391 wet season rainfall in  $\delta^{2}$ H and  $\delta^{18}$ O ( $p \le 0.001$ ) (Table 2 and 3). On average, In the second study year, 392 the isotopic compositions of the dry and wet season rainfall were both on average more depleted 393 during the second study year than during the first study year; thus, the local meteoric water line of 394 2016-2017 had a slightly steeper slope in comparison to the one for 2013-2014 (Fig. 3). Nevertheless, 395 the range of variation of deuterium excess values was similar between years (9–29‰ for the first year 396 vs. 9–31‰ for the second year; Fig. 3), and deuterium excess values of rainfall withinbetween the 397 dry and wet seasons were not statistically different ( $p \ge 0.05$ ).

For all sampling dates, hydrogen and oxygen isotope composition of bulk soil water showed a consistent pattern of increasing isotope depletion with <u>soil</u> depth (Supplementary Materials), in which shallower (5-15 cm) soil water was significantly more enriched than <u>intermediate (15-30 cm)</u> and deeper (<del>6</del>30-120 cm) soil water ( $p \le 0.001$ ) (Table 2 and 3; Fig. 3). In correspondence, lowe<u>str</u> values of deuterium excess generally characterized the <u>near surface-shallower</u> soil water pool.

For the 2014 dry season samplings, bulk soil ranged from -83.3 to -11.9% for  $\delta^2$ H and from -11.1 to -0.9% for  $\delta^{18}$ O (Fig. 3a). For the 2017 dry season samplings, bulk soil water showed a narrower range of variation and more enriched isotope values (from -54.8 to -19.1%  $\delta^2$ H and from -7.5 to -1.5%  $\delta^{18}$ O) in comparison to 2014 (Fig. 3b). However, statistical differences were only suggested for the intermediate and the deeper soil layers in both water isotopes between the two dry seasons investigated ( $p \le 0.001$ ).

In the 2017 wet season sampling, bulk soil isotope composition ranged from -70.5 to -37.5%for  $\delta^2$ H and from -8.4 to -4.1% for  $\delta^{18}$ O (Fig. 3c), showing significant differences in the shallow, <u>intermediate</u> and deep soil water pools in comparison to 2017 dry season ( $p \le 0.001$ ). In all sampling periods, bulk soil water across <u>the different</u> depth\_groupss was isotopically distinct from rainfall during the 2014 and the 2017 dry seasons ( $p \le 0.001$  for both water isotopes).

Across all sampling periods, xylem water of coffee shrubs was more enriched than that of shade trees ( $p \le 0.001$ ) (Table 2 and 3; Figure 3). Further, the isotopic composition of plant xylem water (7.64 to 0.56 for 180, and 65.47 to 9.64 for 2H) fell within the bulk soil water isotope range (11.10 to 0.87 for 180, and 83.35 to 11.86 for 2H), and no statistically differences were found, and values of  $\delta^2$ H and  $\delta^{18}$ O plant xylem (40.8 ± 15.0‰ and 4.6 ± 1.6‰, respectively) were on average more positive in comparison to bulk soil water (46.7 ± 16.4‰ and 6.0 ± 2.3‰, respectively)\_(p > 0.05)(Fig. 3).

421 In the 2014 dry season, xylem water isotope values of shade trees ranged from -65.5 to -422 32.1‰ for  $\delta^2$ H and from -7.6 to -3.6‰ for  $\delta^{18}$ O, meanwhile a larger variation was observed in the 423 xylem water of coffee shrubs (from -46.5 to -9.6  $\% \delta^2$ H and from -6.3 to -0.6  $\% \delta^{18}$ O) ( $p \le 0.001$ ) 424 (Fig. 3a). Among tree species, Lonchocarpus guatemalensis showed the most depleted xylem water 425 isotope signature (-58.1 ± 4.8‰  $\delta^2$ H and -6.8 ± 0.5‰  $\delta^{18}$ O), whereas *Inga vera* reported the most 426 enriched values with a greater range of variation (-51.0  $\pm$  10.2‰  $\delta^{2}$ H and -5.3  $\pm$  1.1‰  $\delta^{18}$ O). 427 Intermediate  $\delta^2$ H and  $\delta^{18}$ O values were observed in *Trema micrantha* (57.1 ± 5.4% and 6.6 ± 0.6%), 428 respectively) (Fig. 4a). Statistical tests showed that *Inga vera* was significantly different from the 429 other tree species *L. guatemalensis* and *T. micrantha* in  $\delta^{18}$ O (p < 0.05).

430 In the 2017 dry season, the isotopic composition of shade trees varied from -56.7 to -34.5%431 for  $\delta^2$ H and from -6.0 to -3.2‰ for  $\delta^{18}$ O; corresponding values for coffee shrubs varied from -39.6 432 to -7.8 % for  $\delta^2$ H and from -4.4 to -1.1% for  $\delta^{18}$ O ( $p \le 0.001$ ) (Fig. 3b). Contrary to 2014, L. 433 guatemalensis showed the most enriched isotope value (-41.3  $\pm$  5.7% for  $\delta^2$ H and -4.6  $\pm$  0.5% for 434  $\delta^{18}$ O), and *I. vera* reported the most depleted values (-48.5 ± 5.1% for  $\delta^{2}$ H and -4.8 ± 0.8% for  $\delta^{18}$ O), 435 with differences being statistically significant suggested for  $\delta^2 H$  (p < 0.05). Intermediate  $\delta^2 H$  and  $\delta^{18} \Theta$ 436 values were observed in the xylem water of T. micrantha ( $45.9 \pm 3.6\%$  and  $3.9 \pm 0.6\%$ . 437 respectively), showing differences in  $\delta^{18}$ O with the other two species (p < 0.05).

438 Overall, more enriched-isotope values of plant xylem water were <u>more enriched observed</u> 439 induring the 2017 dry season <u>than during in comparison to those inthe</u> 2014 <u>dry season ( $p \le 0.001$ )</u> 440 (Fig. 3a,b; Fig. 4). <u>DAlso, lower deuterium excess values were also lower obtained in shade trees and</u> 441 coffee shrubs <u>during in</u> 2017, <u>indicating as sign of a more evaporative signature</u> (Table 2 and 3; Fig. 442 3). Plots of  $\delta^2$ H xylem water against height for the individual shade trees and coffee shrubs sampled 443 in both dry seasons are shown in Figure 4, in which a similar  $\delta^2$ H pattern was displayed between trees 444 and coffee shrubs in the 2014 and 2017 years.

445 During the 2017 wet season sampling, more depleted  $\delta^2$ H and  $\delta^{18}$ O values in xylem water of 446 trees and coffee shrubs were more depleted observed in comparison to the 2017 dry season (p < 0.05) 447 (Fig. 3c). The range of variation was from -60.6 to -45.6 ‰  $\delta^2$ H and -6.2 to -5.4‰  $\delta^{18}$ O for trees, 448 and from -42.2 to -34.4 ‰  $\delta^2$ H and -5.4 to -4.4‰  $\delta^{18}$ O for coffee shrubs ( $p \le 0.001$ ).

It was observed that the xylem isotopic composition of all shade trees and coffee plants fell within the range of the soil water sources during the 2014 dry season samplings (Fig. 3a). For the 2017 dry season, we again observed a good isotopic match between the shade tree xylem water and soil water. However, for the coffee plants, the xylem water was more enriched in  $\delta^2$ H in comparison to soil water (Fig. 3b). During the 2017 wet season sampling, a slight enrichment in  $\delta^2$ H was again observed in the xylem water of coffee, while trees showed a good overlap with soil water (Fig. 3c). Based on these results, tests were carried out to specifically evaluate the effects of deuterium
 fractionation on coffee water sources by running a simple mixing model using only hydrogen isotope
 ratios in the MixSIAR framework.

458

# 459 3.4 Root biomass and macronutrients along soils profile

460 Overall, most roots were concentrated in the first 5 cm of soil with a sharp decline in biomass 461 at 20 cm depth (Fig. 5a). Fine roots (< 1mm) followed by bigger roots (> 2 mm) dominated the 462 shallower soil layers (< 20 cm), meanwhile roots in general were scarce at deeper depths (> 60 cm). 463 Soil acidity was highestr at the near the surface layers and decreaseding gradually withas soil depth 464 increased (Table 4). Organic matter (OM) and total carbon wereas also greatest between 5 and 15 cm 465 depth, whilehowever values decreased rapidly below ~30 to 60 cm depth. Although hHighest concentrations of nitrogen were found in the first 15 cm<u>of soil</u>, although values remained relatively 466 467 high and constant at deeper layers (Fig. 5b). Phosphorus showed its highest concentration at the 468 topsoil with values decreasing sharply below 30 cm depth. In contrast, lowest concentrations of 469 potassium, -sodium and magnesium were lowestfound at the near surface layers (<in the first 15 cm, 470 of soil depth) while maximum values were observed below > at 90120 cm depth. Base saturation (BS) 471 was very low along the soil profile, indicating poor availability of soil macronutrients. Soil cation 472 exchange capacity (CEC) was generally low across depths, indicating little potential for interaction 473 between clay particles and cations.

474

### 475 3.5 Plant water sources

476 In general, We found there was a good agreement between the MixSIAR Bayesian mixing 477 model results using a non-informative and an informative prior distribution (on average 5% difference 478 across all xylem water contributing sources; p > 0.05). This indicates that the independent distribution 479 (soil macronutrients and root data) set a *priori* to optimize model source proportion estimates 480 (informative approach) in the model was not influential enough to significantly modify the results 481 obtained using the isotope signatures of the xylem end-member water sources alone (non-informative 482 approach). Having this agreement between models, we present the results of the water source 483 contribution based on the informative prior distribution. Results of the non-informative approach have 484 beenare provided in the Supplementary Materials.

The model results showed that the <u>intermediate and</u> deep soil water pools (> 15 to 120 cm soil depth) wereas the main sources for the shade trees over the course of the 2014 dry season ( $91 \pm$ 37% on average; Fig. 6 and Supplementary Materials). Across this period, *L. guatemalensis* and *T*. *micrantha*-showed on average the highestr proportion of deep soil-water uptake between 3060 and 489 120 cm soil depth (4991  $\pm$  263712% and 88  $\pm$ 10%, respectively), while. In contrast, T. micrantha 490 and in comparison with I. vera depended strongly on soil water sources between at 15 and 30 cm 491 depth (5474  $\pm$  1842% 14 and 67  $\pm$  638%) (p < 0.001 > 0.05). In contrast, For the coffee plants, the 492 analysis showed that the water uptake of coffee plants was mainly sustained by sources from the first 493 15 cm of soil ( $94 \pm 27\%$  on average; Fig. 6 and Supplementary Materials), shallow soil water sources 494  $(65 \pm 23\%)$  (Fig. 6), having significant differences with all shade tree species *L. guatemalensis* ( $p < 10^{-10}$ 495 0.001)., *T. micrantha* (p < 0.001) and *I. vera* (p < 0.05) tree species across the 2014 dry period studied. 496 During the 2017 dry season, the same trend with most water extracted from intermediate and 497 deep soil layers was observed in the shade trees (91  $\pm$  39% on average; Fig. 7a,b,c and Supplementary 498 Materials). L. guatemalensis showed almost equal proportions of intermediate and all shade tree 499 species were tapping high proportions of deep soil water ( $4686 \pm 4113\%$  and for *L. guatemalensis*; 500 85.42 ± 1935%, respectively for T. micrantha and 92 ± 12% for I. vera; Fig. 7a,b,c and Supplementary 501 Materials), while. T. micrantha followed by I. vera were both tapping high proportions of soil water 502 at 30 cm depth  $(72 \pm 18 \text{ and } 55 \pm 39\%)$  (p > 0.05). Among samplings dates, differences between tree 503 species only suggested appeared to occur between L. guatemalensis and I. vera at the end of the dry 504 period (Apr. 5) (p < 0.05). Coffee water sources were again restricted to mainly obtained from much 505 shallower soil layers (0-5cm: 53  $\pm$  4426% and 5-15 cm: 5442  $\pm$  413229%; )-(Fig. 7a,b,c and 506 Supplementary Materials) compared to shade trees.

507 <u>Overall, we did not find any although in 2017 the contribution of this water source was</u> 508 slightly smaller (9%) in comparison to 2014 (Supplementary Materials), <u>Ss</u>tatistically significantly 509 differences between dry periods among main plant water sources between the dry periods investigated 510 (p > 0.05).

511 Across the individual samplings throughout the two dry seasons, we observed that antecedent 512 precipitation had a stronger effect oin the water uptake sources of coffee plants than trees (Fig. 8). 513 For example, when dry antecedent wetness prevailed (API<sub>15</sub> < 5 mm; Fig. 2b) coffee water sources 514 were mainly composed of  $\frac{1}{9} \frac{1}{23\%}$  and  $\frac{1}{3} \frac{1}{3} \frac{1}$ 515 depth (91  $\pm$  317%). Alternatively, meanwhile when wetter antecedent conditions were present (API<sub>15</sub> 516 > 10 mm), the shallower-near surface soil water layer ( $5863 \pm 31322\%$ ) was the main contributing 517 source. On the contrary, tree water uptake was essentially sustained by deeper soil water sources 518 between 30 and 120 cm (91 $\pm$  13% and 80  $\pm$  15%, respectively) at low and relatively high antecedent 519 wetness conditions (94  $\pm$  23% and 87  $\pm$  23%, respectively) (Fig. 8). Nevertheless, For for all species 520 investigated, the relationships between API and the contribution of near surface deep-soil water 521 sources were not found statistically significant (p > 0.05).

522 DuringIn the 2017 wet season, water source partitioningsources of tree water uptake- differed 523 significantly among shade tree species (Fig. 7d and Supplementary Materials). During this period, L. 524 guatemalensis and I. vera still showed the greatest use of deep soil water at 60-120 cm depth (764  $\pm$ 525 37% and  $6970 \pm 4136\%$ , respectively) (*p* > 0.05), mean while *T. micrantha* relied on much shallower 526 soil water was the main source for T. micrantha ( $9172 \pm 2339\%$ ), having significant differences with 527 the other two tree species (p < 0.001). Coffee consistently showed the use of near surface water 528 sources  $(9869 \pm 522\%)$ ; )-(Fig. 7d and Supplementary Materials), which wasbeing significantly 529 different from in relation to all shade tree species (p < 0.001).

530

## 531 <u>3.6 Fractionation effects on coffee water sources</u>

532 To evaluate the effects of xylem deuterium fractionation on our results for coffee water source 533 uptake, we compared the relative contribution of each soil water source obtained via the single-534 isotope ( $\delta^2$ H) mixing model with those obtained via the informative two-isotope mixing model. In 535 general, we observed that the  $\delta^2$ H model consistently estimated a lower contribution of the shallow 536 soil water source and a higher contribution of the near surface soil water source (Supplementary 537 Materials). On average, the reduction in the shallow soil water source  $(-25.7 \pm 29.0\%)$  coincided very 538 well with the increase in the near surface soil water source ( $+28.1 \pm 30.6\%$ ). These differences were 539 most pronounced for the 2017 dry season samplings (p > 0.05; Supplementary Materials), during 540 which the differences in  $\delta^2$ H between coffee xylem and soil water were greatest. However, there were 541 no significant differences between the relative contributions of the intermediate and deep soil water 542 sources estimated by the two models (p > 0.05). In summary, the results of the  $\delta^2 H$  mixing model 543 suggested an even more pronounced soil water partitioning between coffee and shade tree species 544 than those obtained with the informative two-isotope mixing model.

545

## 546 4. Discussion

# 547 <u>4.1 Methodological aspects</u>

548 To our knowledge, the ecohydrological study presented here is one of the first that 549 incorporates biophysical properties as prior information alongside plant water source information 550 from stable isotopes ( $\delta^{18}$ O and  $\delta^{2}$ H) data into a MixSIAR Bayesian mixing model framework, as a 551 way to improve our understanding of the processes that lead to differences in the depth of plant water 552 uptake. Even though our findings did not change significantly by including or excluding the prior 553 information such as soil macronutrients and root data, exploring plant water source partitioning using 554 these two model approaches provided more confidence in our results. Therefore, we call for more 555 studies that combine soil nutrient and root biomass distribution with plant water source information 556 <u>from  $\delta^{18}$ O and  $\delta^{2}$ H data, to explore the additional value of these biophysical parameters elucidating</u> 557 plant-soil interactions in different regions and environments.

558 In recent years, some plant, soil and/or deep subsurface water source studies that have used 559 stable isotopes have identified isotope variation that could be the result of isotope fractionation 560 processes caused by water molecules interacting with clay surfaces, partially filled pore spaces or 561 even salts (Chen et al., 2016; Gaj and McDonnell, 2019; Lin et al., 2017Oerter et al., 2014; Oshun et 562 al., 2015). Our soils were rich in clay content and according to some studies this type of soil structure can impart isotope fractionation (Lin et al., 2017; Meißner et al., 2014; Oerter et al., 2014; Orlowski 563 564 et al., 2016a). Thus far, however, these isotope effects have been more evident in clay-rich soils 565 having high cation exchange capacities (CEC ~ 30 to 70 cmolc kg-1; Oerter et al., 2014; Orlowski et 566 al., 2016b) in combination with low soil water contents (SWC < 20% Meißner et al., 2014; Orlowski et al., 2016b). In this respect, the soils in our study area are characterized by low CEC (< 21 cmolc 567 568 kg-1; Table 4). This reflects relatively little interaction between cations adsorbed and clay mineral 569 particles, which indirectly suggests minimal impacts of interlayer water bound in the soil structure 570 (cf. Vidal and Dubacq, 2009). In addition, our soil samples were collected at relatively high SWC 571 across the different sampling periods ( $\sim 30\%$  to 60%; Figure 1). As such, we have assumed that the 572 probability of fractionation due to soil properties that may impact water extraction efficiency, was 573 very small or completely absent and therefore, the extracted soil water was the same the plants had 574 access to.

575 With regard to our plant samples, we specifically observed enrichment in the deuterium 576 composition of the xylem water in the coffee plants in comparison to bulk soil water. It is not 577 surprising that fractionation was evident for  $\delta^2 H$  and not  $\delta^{18}O$ , given the higher fractionation factor 578 of <sup>2</sup>H relative to <sup>18</sup>O (Rundel et al., 2012). Some possible explanations for this xylem water 579 enrichment could be related to bark evaporation (Ellsworth and Sternberg, 2015) and/or xylem-580 phloem water exchange (Cernusak et al., 2005), since we did not remove the bark and cambium from 581 our coffee branch samples. On the other hand, like many other crops, coffee plants associate 582 symbiotically with arbuscular mycorrhizal fungi (López-Andrade et al., 2009; Perea-Rojas et al. 583 2019). Studies in our coffee growing region of Veracruz have documented the presence of 584 mycorrhizal structures in coffee roots (Arias et al., 2012; Muleta et al., 2008), which can promote 585 increases in plant water and nutrient uptake (Augé, 2004; Scheneiger and Jakobsen, 2000). Although no research has been carried out yet to test the influence of mycorrhizal fungi on isotope fractionation 586 587 during coffee root water uptake, this effect could have been present and being also responsible for the 588 isotopic mismatch between xylem water and soil water sources, as it has been reported elsewhere 589 (Poca et al., 2019).

590 We did evaluate the effects of these isotope enrichments in the coffee xylem water on the 591 relative contributions of the coffee water sources using a single-isotope ( $\delta^2$ H) mixing model. Consistently, the model results estimated a higher near surface water and a lower shallow soil water 592 593 source contribution in comparison to the dual isotope informative prior mixing model. In contrast, 594 the estimated proportions of the intermediate and deep soil water sources were similar between 595 models. Thus, the effect of fractionation was translated into a more pronounced spatial separation 596 between the main soil water sources of the coffee plants and shade trees, but our overall results were 597 not different.

598

# 599 4.24 Complementary water use strategy between shade trees and coffee shrubs

600 Our <u>findings\_ecohydrological\_research\_consistently</u> showed that all shade tree species (*L.* 601 *guatemalensis, I. vera* and *T. micrantha*) relied mainly on water sources from deep soil layers (> 15 602 to 120 cm depth), while the use of much shallower water sources (< 15 cm) was observed in the coffee 603 (*C. arabica* var. *typica*) over the course of the <u>near</u> normal and <u>the</u> more pronounced dry seasons 604 studied. These findings suggest a spatial and temporal partitioning of <u>soil\_belowground</u>-water 605 <u>sourcesresources</u> between <u>shade</u> trees and coffee plants during drier periods and water-resource 606 complementary in th<u>is coexistence e mixed</u> species <u>environmentplantings</u>.

607 Although comparisons of our findings with other traditional shade Arabica coffee plantations 608 are difficult because studies are essentially lacking in this type of agroecosystems, there are a handful 609 of other investigations carried out in shade coffee monospecific plantations in the humid tropics in 610 which complementary rather than competitive water use strategies prevailed. For example, Cannavo 611 et al. (2011) compared the water use and soil water availability of an unshaded coffee vs. a shaded 612 monoculture (Inga densiflora) coffee plantation in Costa Rica, both of 7-8 years old, using soil 613 moisture measurements and water balance calculations. Their results showed that soil water content 614 in the deeper soil layers (> 120 cm depth) was lower in the shaded coffee than in the full-sun-grown 615 coffee system, while water content in the shallower layers was similar. This suggested that associated 616 shade trees preferentially used water from deeper soil horizons providing some evidence of 617 complementarity water use between coffee plants and native Inga trees during the dry season. 618 However, the authors acknowledged that they were unable to separate roots from coffee than those 619 of trees in the soil profiles, so they could not be certain whether trees were the only individuals 620 extracting water from deeper sources. In this respect, our study showed that there was always a 621 mixture in water uptake from different sources (soil depths), but a separation between the main 622 sources of water for shade trees and coffee shrubs clearly prevailed.

623 Other investigations in Costa Rica have examined the belowground resource competition of 624 Arabica coffee in association with fast-growing timber species using data of plant growth, root 625 distribution and density, and soil moisture and nutrients patterns. For example, the study of Schaller 626 et al. (2003) carried out in a commercial (*Eucalyptus deplupta*) shade coffee plantation where soils 627 are highly fertilized, showed that coffee had a relatively even root distribution along the first 40 cm 628 of soil depth with a higher root density in the proximity of the coffee rows. Conversely, the root 629 system of *E. deplupta* was much shallower having most roots concentrated in the upper 10 cm of soil. 630 In this case, the tree root density was found highest in the alleys between the coffee rows. The authors 631 explained that the apparent complementary resource exploitation of this tree-crop system was mainly 632 attributed to high soil resources availability and the high competitiveness of the coffee limiting the 633 expansion of tree roots (cf. Lehmann, 2003). Although in our study we did not determine the depth 634 distribution of coffee and tree roots, our findings showed that all shade tree species were tapping 635 water from deeper soil layers than coffee, suggesting that trees are deep rooted and being able to 636 explore larger soil volumes causing little competition with coffee.

637 In Nicaragua, Padovan et al. (2015) compared the root distribution, soil moisture, 638 transpiration and leaf water potential patterns in a sun-grown-unshaded coffee systemplantation and 639 an agroforestry system of coffee planted with two timber trees (deciduous Tabebuia rosea and 640 evergreen Simarouba glauca). Their findings showed that coffee roots were more abundant than tree 641 roots and mainly concentrated in the shallower soil layers (0–80 cm depth). Most roots of both tree 642 species were observed in deeper layers (>100 cm) suggesting a clear niche differentiation with coffee. 643 During the 3-year study period, volumetric water content along a 2 m soil profile was higher in the 644 full-sun\_-grown coffee than in the shaded coffee, which was explained by greater soil water uptake 645 from trees below the crop rooting zone (Padovan et al., 2015). Moreover, coffee shrubs in the shaded 646 plantation were more water stressed (i.e. lowest midday leaf water potentials) during the pronounced 647 dry season studied (Padovan et al., 2018). Theirse results suggest that despite the clear hydrological 648 niche segregation, competition between coffee and shade trees may occur if the dry season is long 649 and severe enough.

650 Our findings also showed that during the wet season coffee plants substantially increased the 651 use of near surface water ( $\pm$ -<u>5670</u>%) in comparison to the dry season, while all shade trees also 652 extended their water acquisition to much shallower soil water pools (+19%). This is largely explained 653 by the increases in soil moisture in the first 30 cm depth due to frequent rainfall inputs that 654 characterize the wet season in our study area. This <u>also</u> suggests that coffee had a higher root activity 655 in <u>the topsurface</u> soil layers during the wet season in comparison to the dry season, as has been 656 documented in other studies (Huxley et al., 1974). Regarding the shade tree<u>s</u>-species, we observed

657 that T. micrantha showed the greatest response to the wet season conditions by drawing most water 658 from the first 15 cm of soil (972%), whereas this was much less evident in L. guatemalensis (2130%) 659 and I. vera (279%). Although we did not determine the vertical distribution of roots for each of the 660 shade tree species studied, these findings suggest that T. micrantha has a shallower rooting system 661 than the other tree species. The fact that the *T. micrantha* trees were more recently planted (i.e. 662 younger with less developed root system) than the L. guatemalensis and I. vera trees supports this 663 idea. On other hand, the high temperature and rainfall that characterize the wet season at our study 664 site may favor rapid mineralization of nutrients and their subsequent leaching to deeper soil layers 665 (i.e. potassium, calcium and magnesium; Table 4). Hence, for the larger trees studied (L. 666 guatemalensis), the water and nutrients available at deeper depths could have been an important 667 resource for plant growth in this period, partly explaining the lower activity of their shallower roots. 668 Despite the changes and the higher variability in depth of water uptake observed among canopy trees 669 and coffee shrubs, a complementary use of soil water prevailed during the wet season. Future work 670 should be focused on the distribution and dynamics of tree and crop roots and their seasonal variation 671 in relation to the availability of nutrients and water in the soil. Also, it would be desirable to relate 672 these dynamics to crop and shade tree phenology to elucidate temporal synergistic or competitive 673 water requirements.

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# 675

#### 4.32 The role of antecedent wetness in coffee water uptake

676 Despite the relatively small sample size, oOur study showed that antecedent wetness strongly 677 influenced the water uptake patterns of coffee plants (cf. Huxley et al., 1974). We found that under 678 relatively wet antecedent conditions prevailing after small rainfall events during the dry season, coffee 679 substantially increased the use of shallower near surface soil water sources, possibly as an 680 opportunistic strategy to overcome the soil water deficits in this period and taking advantage of their 681 much shallower rooting system compared to trees. Conversely, tree water uptake was mainly sourced 682 by deeper soil water layers showing less sensitiveness to higher antecedent wetness. In this respect 683 there are no comparative studies in shade coffee agroecosystems evaluating short term dynamics the 684 functional response -of plant water sources-uptake over a range of at different antecedent wetness 685 conditions. Nevertheless, plant and soil water interactions under dry and relatively wet conditions 686 have been examined in other types of agroforestry systems. For example, in the study of Gao et al. (2018) carried out in a semiarid region in China, the authors evaluated the seasonal variations in water 687 688 use of jujube (Ziziphus jujuba) trees planted with annual (Brassica napus) and perennial 689 (Hemerocallis fulva) crops under various soil wetness status. Using stable isotope techniques and 690 Bayesian mixing modelling, their results showed that jujube trees generally tapped water (> 58%) 691 from deep soil layers (60-200 cm depth) at low antecedent wetness, while B. napus and H. fulva crops 692 primarily extracted water (> 65%) from intermediate (20-60 cm) and shallow (0-20 cm) soil layers. 693 This exhibits a complementary water use strategy between trees and crops. However, at higher 694 antecedent precipitation conditions both the jujube trees and the inter-row crops extracted most water 695 from the first 0-60 cm of soil depth (> 65%). This indicated that both species exhibited an 696 opportunistic strategy for accessing resources at shallower soil depths. In this case, contrary to our 697 findings, tree roots rather than crop roots showed the stronger capacity to switch rapidly from deep 698 to shallow sources in response to increased soil water availability.

699

#### 700 4.43 Implications and future directions

701 The consistent complementarity in plant water use strategies observed under different 702 hydrometeorological conditions in the coffee plantation studied provides support to the central tenet 703 of agroforestry systems (Cannel et al., 1996). Based on our findings, the L. guatemalensis, I. vera and 704 T. micrantha provide good choices for coffee shade trees due to their complementarity in soil water 705 use. Since these tree species obtained their water from deeper soil layers than the coffee, this could 706 mean that they utilize nutrients leaching beyond the reach of the coffee plants, and so contribute to 707 improved nutrient cycling and increased overall productivity of the system (van Noordwijk et al., 708 2015).

709 Nevertheless, the current outcome may change given the new coffee management practices 710 that consist on replacing traditional coffee varieties (e.g. C. arabica var. typica) with others (C. 711 arabica var. costa rica; C. canephora) that may exhibit deeper roots systems and perhaps different 712 water (and nutrient) uptake strategies, in response to prevalent diseases such as leaf rust or root 713 nematodes. Therefore, future research should be focused on evaluating the water source partitioning 714 of traditional vs. new coffee disease-resistant varieties and their relation to shade tree water use. In 715 this respect, there are further questions with regard to strategic use of shade tree species, whereby 716 fast-growing species might be more (commercially) productive but also more competitive. Some 717 evidence from elsewhere has shown that such management practices do not necessarily increase 718 competition and may even enhance the water use efficiency as part of drought-avoidance 719 mechanisms. For example, in southeast China, Wu et al. (2016) used  $\delta^2 H$  and  $\delta^{18}O$  stable isotope 720 methods to examine the seasonal water use of a fast-growing rubber tree species (*Hevea brasilensis*) 721 planted with Arabica coffee. Their findings showed that rubber trees were mostly accessing water 722 from intermediate (15-50 cm depth) and deep soil layers (50-110 cm), meanwhile coffee was mostly 723 tapping water from the topsoil (< 15 cm). Additionally, rubber trees showed strong root plasticity in 724 soil water uptake avoiding competition with coffee during the rainy and relatively dry seasons. However, more research is needed since these results depend largely on tree-crop specie combinationsand local climatic and soil conditions.

727 In addition to effects of changing management practices, climate warming may induce 728 changes in plant transpiration throughout the year (e.g. Karmalkar et al., 2011). In our study, we used 729 a water stable isotope approach along with root and macronutrients data to estimate the relatively 730 contribution of the plant water sources. However, for a more complete assessment of the plant and 731 soil interactions, seasonal plant water fluxes need to be quantified. Our results so far have made the 732 first steps towards serving coffee producers to make better decisions on sustainable coffee and water 733 management, as well as providing new insights into water resources in general, which are urgently 734 required for implementing efficient and equitable management programs in humid tropical 735 environments (Hamel et al., 2018). However, future work should be focused on water use of 736 individual trees and coffee shrubs using ecophysiological and hydrological techniques in order to 737 better understandknow how much water is used from the different soil water pools.and where from.

Finally, in our methodology we used prior information alongside the stable water isotope approach to better understand plant water uptake dynamics. Even though our results did not change significantly by including or excluding the root and nutrient data, exploring plant water source partitioning using these two approaches provided more confidence in our results. We would recommend that other authors also consider using nutrient and root data in combination with plant xylem water end members to better understand water uptake patterns, especially to explore the additional value of this information in different environments.

745

#### 746 5. Conclusions

747 This study provides the first baseline information on plant water sources for a traditional 748 shade coffee plantation in the humid tropics. Our results showed that coffee water uptake was mainly 749 sustained from shallow soil sources (< 15 cm depth) while all shade trees relied on water sources 750 from deeper soil layers (>15 to 120 cm depth). This complementary strategy in belowground resource 751 use between crops and trees was consistent over the course of the near normal and the more 752 pronounced dry seasons investigated. Across these same periods, we observed that antecedent 753 precipitation had a strong influence in coffee plants increasing their water uptake to shallower near 754 surface soil water sources as an opportunistic strategy to overcome the reduced water availability. In 755 the wet season, coffee plants substantially increased the use of near surface water (<\_5 cm depth), 756 whereas shade trees expanded their water acquisition to the first 15 cm soil depth. Overall, a greater 757 soil water partitioning prevailed among tree and coffee species when higher soil moisture conditions 758 were present. Nevertheless, despite such variability in plant-soil water interactions across seasons, a clear spatial segregation of the main water source prevailed between trees and crops during the rainyand dry periods investigated.

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Author contributions. LEMV designed the experiment. LEMV, MSAB and FH collected the field
data. MSAB performed all the Bayesian mixing model analysis. JG contributed in the data analysis.
LEMV prepared the first draft of the manuscript. FH, MSAB and JG edited and commented on the
manuscript several times, and- TED carried out the final revision. Later, all the co-authors
contributed with revisions.

767

768 **Competing interests.** The authors declare that they have no conflict of interest.

769

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**Table 1.** Characteristics of the shade trees and coffee plants sampled for water isotope analysis during 2014 and 2017. Numbers between parentheses are the standard deviation.

Escuito	Species	Canopy layer	2014		2017		п
Family			DBH cm	Height m	DBH cm	Height m	
Fabaceae	Lonchocarpus guatemalensis	Overstory	101.5 (12.6)	20.3 (1.3)	119.8 (12.1)	21.0 (1.2)	3
Fabaceae	Inga vera	Overstory	39.3 (15.7)	10.7 (4.8)	48.1 (13.3)	9.6 (1.2)	3
Cannabaceae	Trema micrantha	Overstory	13.16 (6.8)	8.15 (3.1)	23.3 (7.2)	15.2 (2.2)	3
Rubiaceae	Coffea arabica var. typica	Understory	12.7 (2.1)	2.83 (0.7)	n.a.	n.a.	5* 6** 10***

 $\ast$  Number of individuals sampled each time in the 2014 dry season

\*\* Number of individuals sampled each time in the 2017 dry season

\*\*\* Number of individuals sampled in the 2017 wet season

**Table 2.** Mean  $\pm$  (SD) H and O stable isotope composition of 2013-2014 precipitation, tree xylem water and bulk soil water of the 2014 dry season sampling, and corresponding *d*-excess values (‰)

		Precip	oitation	<u>1</u>							Bulk so	il wate	<u>er</u>								9		
		<u>n =</u>	= 41								<u>n =</u>	54						<u>S</u> XJ	hade ti /lem w	<u>rees</u> rater	<u>Co</u> <u>X</u>	ffee sh vlem w	<u>irubs</u> vater
	Dry sea	<u>ason</u>	V	Vet sea	<u>son</u>	<u>0-:</u>	<u>5 cm d</u>	<u>epth</u>	<u>&gt;5-</u>	<u>15 cm</u>	<u>depth</u>	<u>&gt;15-</u>	- <u>30 cm</u>	<u>ı depth</u>	<u>&gt;:</u>	<u>30-120</u> <u>depth</u>	<u>cm</u> <u>1</u>		<u>n = 2</u>	7		<u>n = 1</u> 4	<u>4</u>
<u>δ</u> 2 <u>Η</u>	$\frac{\underline{\delta}^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>SS</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	$\frac{d}{exce}$ $\frac{ss}{ss}$	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	$\frac{d}{exce}$ $\frac{ss}{ss}$	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	$\frac{d}{\text{exce}}$ $\frac{\text{ss}}{\text{ss}}$
<u>1.</u> <u>6</u> ± <u>8.</u> <u>5</u>	= <u>1.9</u> <u>±</u> <u>1.4</u>	$\frac{17.0}{\pm 5.1}$	$ \begin{array}{r} \underline{-} \\ \underline{42.} \\ \underline{4 \pm} \\ \underline{36.} \\ \underline{1} \end{array} $	$\begin{array}{c} \pm \\ \underline{7.2} \\ \pm \\ \underline{4.3} \end{array}$	$\frac{14.9}{\pm 2.8}$	$\frac{1}{20.}$ $\frac{5 \pm}{7.8}$	$ \begin{array}{c} \pm \\ \underline{2.4} \\ \pm \\ \underline{1.0} \end{array} $	$\frac{-1.5}{\pm 4.1}$	$\frac{\underline{30.}}{\underline{8\pm}}$ $\underline{9.4}$		$\frac{-1.2}{\pm 6.3}$	$ \begin{array}{r} = \\ \underline{54.} \\ \underline{7 \pm} \\ \underline{10.} \\ \underline{3} \end{array} $	$\begin{array}{c} \pm \\ \underline{7.0} \\ \pm \\ \underline{0.9} \end{array}$	<u>1.2 ±</u> <u>6.6</u>	$\frac{\underline{66.}}{\underline{8\pm}}$		<u>3.0 ±</u> <u>4.7</u>	$\frac{55.}{4 \pm 7.6}$		$\frac{-5.8}{\pm 4.1}$	$\frac{=}{25.}$ $\frac{5 \pm}{10.}$ $\frac{8}{8}$	$ \begin{array}{c} \pm \\ \underline{3.4} \\ \pm \\ \underline{1.8} \end{array} $	<u>1.7 ±</u> <u>5.0</u>

		Precip	itation	L							Bulk so	oil wate	er										
		<u>n =</u>	<u>    39</u>								<u>n =</u>	<u>= 54</u>						<u>S</u> xy	<u>hade t</u> ylem w	<u>rees</u> vater	<u>Co</u> <u>X</u>	<u>ffee sh</u> /lem w	<u>rubs</u> ater
ļ	Dry sea	<u>son</u>	V	Vet sea	ison	<u>0-</u> .	<u>5 cm d</u>	lepth	<u>&gt;5-</u>	<u>15 cm</u>	<u>depth</u>	<u>&gt;15</u>	<u>-30 cm</u>	<u>ı depth</u>	<u>&gt;</u> .	<u>30-120</u> deptl	<u>) cm</u> <u>1</u>		<u>n = 2</u>	<u>4</u>		<u>n = 1</u>	<u>8</u>
$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	$\frac{d}{\underline{exce}}$	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	$\frac{\underline{d}}{\underline{exce}}$	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	$\frac{d}{exce}$ $\frac{ss}{ss}$	$\frac{\delta^2}{\mathbf{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>SS</u>	$\frac{\delta^2}{\underline{H}}$	$\frac{\delta^{18}}{\underline{O}}$	<u>d-</u> <u>exce</u> <u>ss</u>
$ \begin{array}{r} = \\ \underline{2.9} \\ \pm \\ \underline{16.} \\ \underline{0} \end{array} $	$\begin{array}{c} \pm \\ \underline{3.0} \\ \pm \\ \underline{1.8} \end{array}$	$\frac{21.5}{\pm 4.3}$	$\frac{=}{47.}$ $\frac{8 \pm}{34.}$ $\frac{4}{4}$	$\begin{array}{c} \pm \\ \underline{7.9} \\ \pm \\ \underline{4.1} \end{array}$	$\frac{15.2}{\pm 3.3}$	$\frac{\underline{-}}{\underline{24.}}$ $\underline{3\pm}$ $\underline{3.9}$	$\begin{array}{c} -\\ \underline{2.2}\\ \pm\\ \underline{0.5} \end{array}$	$\frac{-6.9}{\pm 6.6}$	$\frac{=}{32.}$ $\frac{1 \pm}{5.3}$	$\begin{array}{c} \pm \\ \underline{3.6} \\ \pm \\ \underline{0.5} \end{array}$	$\frac{-3.4}{\pm 4.1}$	$\frac{-}{41.}$ $\frac{9 \pm}{5.7}$	$\begin{array}{c} \pm \\ \underline{5.7} \\ \pm \\ \underline{0.6} \end{array}$	$\frac{3.4}{\pm 4.8}$	$\frac{\underline{-}}{\underline{47.}}$ $\underline{3 \pm}{\underline{3.8}}$	$\begin{array}{c} \pm \\ \underline{6.5} \\ \pm \\ \underline{0.5} \end{array}$	$\underline{5.0} \\ \pm 3.2$	$\frac{\pm}{9\pm}$ $5.6$	$\begin{array}{c} \pm \\ \underline{4.4} \\ \pm \\ \underline{0.7} \end{array}$	-9.7 $\pm 5.4$	$\frac{\underline{-}}{\underline{21.}}$ $\underline{3 \pm}{\underline{7.2}}$	$\begin{array}{c} = \\ \underline{2.8} \\ \pm \\ \underline{1.0} \end{array}$	<u>1.3 ±</u> <u>6.2</u>

**Table 3.** Mean  $\pm$  (SD) H and O stable isotope composition of 2016-2017 precipitation, tree xylem water and bulk soil water of 2017 dry season sampling, and corresponding *d*-excess values (‰)

<u>Soil</u> depth	<u>pH</u> ( <u>H</u> 2O)	<u>P</u>	<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	<u>CEC</u>	<u>BS</u>	<u>OM</u>	<u>C</u>	<u>Organic</u> <u>carbon</u>	<u>N</u>	<u>Clay</u>
<u>(cm)</u>		<u>(mg kg<sup>-1</sup>)</u>			(cmol <sub>c</sub> kg	<sup>-1</sup> )					<u>(%</u>	)	
<u>5</u>	<u>4.07</u>	<u>33.33</u>	<u>1.47</u>	<u>0.60</u>	<u>3.86</u>	<u>0.87</u>	<u>16.10</u>	<u>0.42</u>	<u>5.18</u>	<u>3.01</u>	<u>2.54</u>	<u>0.38</u>	<u>60.8</u>
<u>15</u>	<u>4.12</u>	<u>4.60</u>	<u>1.08</u>	<u>0.47</u>	<u>0.95</u>	<u>0.12</u>	<u>13.27</u>	<u>0.20</u>	<u>2.89</u>	<u>1.90</u>	<u>1.67</u>	<u>0.30</u>	<u>63.8</u>
<u>30</u>	<u>4.34</u>	<u>n.d.</u>	<u>2.22</u>	<u>0.77</u>	<u>1.92</u>	<u>0.54</u>	<u>14.65</u>	<u>0.37</u>	<u>1.55</u>	<u>1.31</u>	<u>0.90</u>	<u>0.23</u>	<u>70.9</u>
<u>60</u>	<u>4.95</u>	<u>n.d.</u>	<u>2.36</u>	<u>0.93</u>	<u>3.81</u>	<u>1.21</u>	<u>20.35</u>	<u>0.41</u>	<u>1.02</u>	<u>0.69</u>	<u>0.59</u>	<u>0.22</u>	<u>66.9</u>
<u>90</u>	<u>5.10</u>	<u>n.d.</u>	<u>2.75</u>	<u>1.11</u>	<u>3.78</u>	<u>1.27</u>	18.85	<u>0.47</u>	0.48	<u>0.50</u>	0.28	0.20	<u>66.1</u>
<u>120</u>	5.16	<u>n.d.</u>	<u>3.00</u>	<u>1.45</u>	<u>3.76</u>	<u>1.20</u>	17.60	<u>0.53</u>	<u>0.41</u>	<u>0.51</u>	0.24	0.20	<u>65.1</u>

 Table 4. Soil characteristics (average values) determined at the different depths



**Figure 1.** Study site location in the municipality of Coatepec, Veracruz, Mexico. Source: QuickBird Satellite Image (2010). Copyright DigitalGlobe, Inc.



**Figure 2.** (a) Daily mean air temperature and vapor pressure deficit (VPD) and (b) and daily total rainfall (*P*), as measured from November 2013 to October 2014 and from November 2016 to October 2017, and volumetric soil water content (SWC) measured at different depths during the sampling campaigns in the study area; different depths are indicated by the unique symbols shown in the lower panels (the key to the symbols is at top). The blue-colored areas indicate the 6- to 22-day period of minimum rainfall (< 5 mm) preceding the dates of isotope sampling in January (mid dry season) and April (late dry season) of 2014, and in February (mid dry season), April and May (late and end of dry season), and August (mid wet season) of 2017.



**Figure 3.** (a) Isotope composition of xylem water for shade trees and coffee shrubs, bulk soil at different depths as observed during the three sampling dates (Jan. 23, Apr. 11 and Apr. 26, 2014), and isotope values of rainfall during the period December 2013 to November 2014. The dashed line represents the 2013–2014 local meteoric water line (LMWL;  $\delta^2 H = 17.82 + 8.26^* \delta^{18}O$ ), (b) Isotope composition of xylem water for shade trees and coffee shrubs, bulk soil at different depths during the three sampling dates (Feb. 27, Apr. 5 and May. 20, 2017) and isotope values of rainfall during the period December 2016 to November 2017. The dashed line represents the 2016–2017 local meteoric water line (LMWL;  $\delta^2 H = 21.0 + 8.36^* \delta^{18}O$ ), and (c) Isotope composition of xylem water for shade trees and coffee shrubs, bulk soil at different depths during the trees and coffee shrubs, bulk soil at different depths during the period December 2016 to November 2017. The dashed line represents the 2017 local meteoric water line (LMWL;  $\delta^2 H = 21.0 + 8.36^* \delta^{18}O$ ), and (c) Isotope composition of xylem water for shade trees and coffee shrubs, bulk soil at different depths during the middle of the 2017 wet season (Aug. 4) and isotope values of rainfall during the period December 2016 to November 2017. The dashed lines in panels (b) and (c) represent the 2016–2017 local meteoric water line (LMWL;  $\delta^2 H = 21.0 + 8.36^* \delta^{18}O$ ). The solid line represents the global meteoric water line (GMWL;  $\delta^2 H = 10 + 8^* \delta^{18}O$ ). The panels on the right show the deuterium excess values for the plants and soil water sources and rainfall preceding the sampling campaigns. The dashed blue line represents the deuterium excess value of the GMWL.



**Figure 4.** Plant height vs  $\delta^2$ H xylem water for coffee plants and shade tree species corresponding to (a) the 2014 and (b) 2017 dry season samplings.



**Figure 5.** (a) Distribution of root biomass for three size classes of roots (different color bars), <u>the</u> <u>error bars in (a)</u>-represent one standard deviation of uncertainty and (b) macronutrients distribution along the soil profile, here normalized and expressed as in ratio to their maximum values (absolute values in Table 4).



**Figure 6.** MixSIAR Bayesian mixing model results showing the mean likely contribution of each water source to the xylem water of shade canopy trees and coffee shrubs. (a), (b) and (c) show results for the sampling dates of Jan. 23, Apr. 12 and Apr. 26, 2014 respectively, using the informative prior distribution. *Lg: L. guatemalensis; Tm: T. micrantha; In: I. vera and Ca: Coffea Arabica*. Error bars represent one standard deviation of uncertainty.



**Figure 7.** MixSIAR Bayesian mixing model results showing the mean likely contribution of each water source to the xylem water of shade canopy trees and coffee shrubs. (a), (b), (c) and (d) show results for the sampling dates of Feb. 27, Apr. 5, May. 20 and Aug. 4, 2017 respectively, using the informative prior distribution. *Lg: L. guatemalensis; Tm: T. micrantha; In: I. vera and Ca: Coffea aArabica*. Error bars represent one standard deviation of uncertainty.



**Figure 8.** Contribution of deep soil water to plant uptake at different antecedent precipitation conditions across the 2014 and 2017 dry seasons.



Figure S1. Photo of the "La Orduña" shade coffee plantation.



-O- Soil profile 1 -O- Soil profile 2 -O- Soil profile 3

**Figure S2.** Hydrogen stable isotope ratios of the bulk soil water collected at different depths along the soil profile 1 (grey filled circles), soil profile 2 (open circles) and soil profile 3 (black filled circles) on January 23, April 12 and April 26, 2014 (dry season), February 27, April 5 and May 20, 2017 (dry season) and August 4, 2017 (wet season)

#### Construction of the informative prior in the MixSIAR model: parameters and information

Macronutrients (N, P, K) and root biomass data collected at the different depths were first grouped (averaged) in order to match the soil depth groups that represent the four potential plant water sources: Near Surface (5 cm), Shallow (> 5 to 20 cm), Intermediate (> 20 to 40 cm) and Deep (> 40 to 120 cm). Importantly, no significant difference was found between the dry and wet season nutrient profiles for 2017. Next, the nutrient and root biomass distributions were normalized such that the sum of the values for all the depths was 100. Then, for each sampling year, a composite depth distribution was constructed by averaging the N, P, K and root biomass profiles. The composite distribution was the prior probability for each source used in the Bayesian mixing model ("informative" approach). The resulting prior proportions used for the 2014 sampling dates were: Near Surface = 58, Shallow Soil = 15, Intermediate = 17 and Deep Soil = 10. For 2017 samplings, the following proportions were used: Near Surface = 53, Shallow Soil = 14, Intermediate = 17 and Deep Soil = 16. This configuration produced sharp proportions for each source, contrasting with those prescribed in the "non-informative" prior distribution (i.e. all the combinations of proportions of water sources were equally likely) as observed in Figure S3 for the 2014 dry season simulations.





**Table S1.** Relative contributions of the different water sources to plant xylem water (mean  $\pm$  SD) per species and for the three sampling dates performed in 2014 dry season. Contributions were derived with the MixSIAR Bayesian mixing model framework, using the 'informative' prior approach

		<u>Samplin</u> (Jan. 23, 2	<u>ig 1</u> 2014)			<u>Sampling</u> (Apr. 11, 2	<u>g 2</u> 014)			<u>Samplir</u> (Apr. 26,	n <u>g 3</u> 2014)	
	<u>S</u>	hade trees		<u>Coffee</u> <u>shrubs</u>	<u>S</u>	hade trees		<u>Coffee</u> <u>shrubs</u>	<u>S</u>	Shade trees		<u>Coffee</u> <u>shrubs</u>
	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> <u>vera</u>	<u>C.</u> arabica	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> <u>vera</u>	<u>C.</u> arabica	<u>L.</u> guatemalensis	<u>T.</u> <u>micrantha</u>	<u>I.</u> <u>vera</u>	<u>C. arabica</u>
<u>Near</u> surface water	<u>0.01±0.03</u>	<u>0.01±0.02</u>	<u>0.03±0.07</u>	<u>0.03±0.10</u>	<u>0.03±0.06</u>	<u>0.05±0.08</u>	<u>0.10±0.15</u>	<u>0.23±0.3</u> <u>3</u>	<u>0.04±0.08</u>	<u>0.05±0.08</u>	<u>0.07±0.11</u>	<u>0.68±0.37</u>
<u>Shallow</u> soil water	<u>0.02±0.06</u>	<u>0.01±0.03</u>	<u>0.07±0.14</u>	<u>0.93±0.06</u>	<u>0.03±0.06</u>	<u>0.04±0.08</u>	<u>0.09±0.16</u>	<u>0.68±0.4</u> <u>0</u>	<u>0.04±0.08</u>	<u>0.04±0.09</u>	<u>0.08±0.14</u>	<u>0.26±0.38</u>
<u>Intermedia</u> <u>te soil</u> <u>water</u>	<u>0.48±0.47</u>	<u>0.36±0.46</u>	<u>0.62±0.43</u>	<u>0.02±0.09</u>	<u>0.19±0.32</u>	<u>0.55±0.43</u>	<u>0.66±0.38</u>	<u>0.06±0.1</u> <u>0</u>	<u>0.68±0.39</u>	<u>0.72±0.37</u>	<u>0.74±0.34</u>	<u>0.03±0.06</u>
<u>Deep soil</u> water	<u>0.49±0.47</u>	<u>0.63±0.45</u>	<u>0.28±0.39</u>	<u>0.02±0.09</u>	<u>0.75±0.31</u>	<u>0.36±0.36</u>	<u>0.15±0.22</u>	<u>0.04±0.0</u> <u>6</u>	<u>0.24±0.32</u>	<u>0.19±0.29</u>	<u>0.12±0.21</u>	<u>0.02±0.04</u>

**Table S2.** Relative contributions of the different water sources to plant xylem water (mean  $\pm$  SD) per species and for the three sampling dates performed in 2014 dry season. Contributions were derived with the MixSIAR Bayesian mixing model framework, using the 'non-informative' prior approach

		<u>Samplin</u> (Jan. 23, 2	1 <u>g 1</u> 2014)			<u>Samplin</u> (Apr. 11, 2	<u>g 2</u> (014)			<u>Sampli</u> (Apr. 26,	<u>ng 3</u> 2014)	
	<u>S</u>	hade trees		<u>Coffee</u> <u>shrubs</u>	<u>:</u>	Shade trees		<u>Coffee</u> <u>shrubs</u>		Shade trees		<u>Coffee</u> <u>shrubs</u>
	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> vera	<u>C.</u> arabica	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> <u>vera</u>	<u>C.</u> arabica	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> vera	<u>C. arabica</u>
<u>Near</u> surface water	<u>0.01±0.03</u>	<u>0.01±0.02</u>	<u>0.03±0.07</u>	<u>0.03±0.11</u>	<u>0.03±0.05</u>	<u>0.04±0.08</u>	<u>0.09±0.14</u>	<u>0.18±0.3</u> <u>0</u>	<u>0.03±0.07</u>	<u>0.04±0.08</u>	<u>0.05±0.10</u>	<u>0.64±0.40</u>
<u>Shallow</u> soil water	<u>0.02±0.06</u>	<u>0.01±0.04</u>	<u>0.08±0.15</u>	<u>0.90±0.23</u>	<u>0.03±0.06</u>	0.05±0.09	<u>0.09±0.17</u>	<u>0.73±0.3</u> <u>7</u>	0.03±0.08	<u>0.04±0.09</u>	<u>0.07±0.14</u>	<u>0.32±0.41</u>
<u>Intermedia</u> <u>te soil</u> <u>water</u>	<u>0.46±0.47</u>	<u>0.34±0.44</u>	<u>0.57±0.43</u>	<u>0.03±0.10</u>	<u>0.19±0.31</u>	<u>0.54±0.43</u>	<u>0.67±0.39</u>	<u>0.05±0.1</u> <u>0</u>	<u>0.70±0.39</u>	<u>0.74±0.37</u>	<u>0.78±0.32</u>	<u>0.03±0.06</u>
<u>Deep soil</u> water	<u>0.51±0.46</u>	<u>0.65±0.44</u>	<u>0.32±0.39</u>	<u>0.03±0.10</u>	<u>0.76±0.31</u>	<u>0.38±0.36</u>	<u>0.15±0.23</u>	<u>0.04±0.0</u> <u>7</u>	<u>0.23±0.33</u>	<u>0.19±0.29</u>	<u>0.10±0.19</u>	<u>0.02±0.04</u>

**Table S3.** Relative contributions of the different water sources to plant xylem water (mean  $\pm$  SD) per species and for the three sampling dates performed in the 2017 dry season. Contributions were derived with the MixSIAR Bayesian mixing model framework, using the 'informative' prior approach

		<u>Samplin</u> (Feb. 27, 2	<u>g 1</u> 2017)				<u>Samplin</u> (Apr. 5, 2	u <u>g 2</u> (017)			<u>Samplir</u> (May 20,	n <u>g 3</u> 2017)	
	<u>S</u>	hade trees		<u>Coffee</u> <u>shrubs</u>			Shade trees		<u>Coffee</u> <u>shrubs</u>	5	Shade trees		<u>Coffee</u> <u>shrubs</u>
	<u>L.</u> guatemalensis	<u>T.</u> <u>micrantha</u>	<u>I.</u> <u>vera</u>	<u>C.</u> arabica		<u>L.</u> guatemalensis	<u>T.</u> <u>micrantha</u>	<u>I.</u> <u>vera</u>	<u>C.</u> arabica	<u>L.</u> guatemalensis	<u>T.</u> <u>micrantha</u>	<u>I.</u> <u>vera</u>	<u>C. arabica</u>
<u>Near</u> surface water	<u>0.01±0.03</u>	<u>0.02±0.06</u>	<u>0.03±0.08</u>	<u>0.03±0.09</u>	-	<u>0.11±0.16</u>	<u>0.07±0.12</u>	<u>0.03±0.06</u>	<u>0.82±0.3</u> <u>0</u>	0.03±0.07	<u>0.02±0.04</u>	<u>0.01±0.02</u>	<u>0.73±0.40</u>
<u>Shallow</u> soil water	0.02±0.07	<u>0.07±0.21</u>	<u>0.07±0.23</u>	<u>0.89±0.28</u>		<u>0.16±0.26</u>	<u>0.11±0.21</u>	<u>0.04±0.08</u>	<u>0.13±0.2</u> <u>9</u>	0.04±0.10	<u>0.02±0.09</u>	<u>0.01±0.03</u>	<u>0.25±0.40</u>
<u>Intermdiat</u> <u>e soil</u> <u>water</u>	<u>0.22±0.38</u>	<u>0.76±0.39</u>	<u>0.73±0.41</u>	<u>0.07±0.23</u>		<u>0.53±0.41</u>	<u>0.61±0.41</u>	<u>0.44±0.44</u>	<u>0.03±0.0</u> <u>7</u>	<u>0.63±0.43</u>	<u>0.79±0.37</u>	<u>0.47±0.48</u>	<u>0.01±0.03</u>
<u>Deep soil</u> water	<u>0.76±0.39</u>	<u>0.15±0.32</u>	<u>0.17±0.34</u>	<u>0.02±0.07</u>		<u>0.19±0.25</u>	<u>0.22±0.31</u>	<u>0.49±0.42</u>	<u>0.02±0.0</u> <u>5</u>	<u>0.31±0.42</u>	<u>0.17±0.35</u>	<u>0.52±0.48</u>	<u>0.01±0.03</u>

**Table S4.** Relative contributions of the different water sources to plant xylem water (mean  $\pm$  SD) per species and for the three sampling dates performed in the 2017 dry season. Contributions were derived with the MixSIAR Bayesian mixing model framework, using the 'non-informative' prior approach

		<u>Samplir</u> (Feb. 27,	n <u>g 1</u> 2017)			<u>Samplin</u> (Apr. 5, 2	<u>g 2</u> 017)			<u>Samplin</u> (May 20,	<u>ng 3</u> 2017)	
	4 2	Shade trees		<u>Coffee</u> <u>shrubs</u>	<u>S</u>	Shade trees		<u>Coffee</u> <u>shrubs</u>	<u>S</u>	hade trees		<u>Coffee</u> <u>shrubs</u>
	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> <u>vera</u>	<u>C.</u> arabica	<u>L.</u> guatemalensis	<u>T.</u> <u>micrantha</u>	<u>I.</u> <u>vera</u>	<u>C.</u> arabica	<u>L.</u> guatemalensis	<u>T.</u> <u>micrantha</u>	<u>I.</u> <u>vera</u>	<u>C. arabica</u>
<u>Near</u> surface water	<u>0.01±0.03</u>	<u>0.02±0.06</u>	<u>0.02±0.07</u>	<u>0.02±0.08</u>	<u>0.10±0.15</u>	<u>0.05±0.11</u>	<u>0.02±0.06</u>	<u>0.80±0.34</u>	<u>0.02±0.06</u>	<u>0.01±0.04</u>	<u>0.01±0.02</u>	<u>0.71±0.41</u>
<u>Shallow</u> soil water	<u>0.02±0.08</u>	<u>0.07±0.21</u>	<u>0.09±0.25</u>	<u>0.88±0.29</u>	<u>0.16±0.27</u>	<u>0.11±0.22</u>	<u>0.03±0.08</u>	<u>0.16±0.33</u>	<u>0.04±0.10</u>	<u>0.02±0.09</u>	<u>0.01±0.02</u>	<u>0.27±0.42</u>
<u>Intermedia</u> <u>te soil</u> <u>water</u>	<u>0.23±0.39</u>	<u>0.76±0.38</u>	<u>0.72±0.41</u>	<u>0.08±0.24</u>	<u>0.58±0.41</u>	<u>0.66±0.41</u>	<u>0.45±0.44</u>	<u>0.03±0.07</u>	<u>0.63±0.43</u>	<u>0.80±0.36</u>	<u>0.46±0.48</u>	<u>0.01±0.04</u>
<u>Deep soil</u> water	<u>0.75±0.39</u>	<u>0.15±0.32</u>	<u>0.17±0.33</u>	<u>0.02±0.07</u>	<u>0.17±0.25</u>	<u>0.19±0.30</u>	<u>0.49±0.43</u>	<u>0.02±0.05</u>	<u>0.31±0.42</u>	<u>0.16±0.34</u>	<u>0.53±0.48</u>	<u>0.01±0.03</u>

	<u>Sai</u> (Aug	<u>mpling</u> .4, 2017)		
	5	Shade trees		Coffee shrubs
	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> <u>vera</u>	<u>C.</u> arabica
Near surface water	<u>0.12±0.19</u>	<u>0.01±0.04</u>	<u>0.03±0.09</u>	<u>0.98±0.05</u>
Shallow soil water	<u>0.09±0.24</u>	<u>0.91±0.23</u>	<u>0.24±0.37</u>	<u>0.01±0.03</u>
Intermediate soil water	<u>0.06±0.13</u>	<u>0.01±0.05</u>	<u>0.03±0.10</u>	<u>0.01±0.02</u>
Deep soil water	0.74±0.37	<u>0.07±0.21</u>	<u>0.69±0.41</u>	0.01±0.03

**Table S5.** Relative contributions of the different water sources to plant xylem water (mean  $\pm$  SD) per species and for the sampling performed in the 2017 wet season. Contributions were derived with the MixSIAR Bayesian mixing model framework, using the 'informative' prior approach

	<u>(A</u>	<u>Sampling</u> ug.4, 2017)		
		Shade trees		<u>Coffee</u> <u>shrubs</u>
	<u>L.</u> guatemalensis	<u>T.</u> micrantha	<u>I.</u> vera	<u>C.</u> <u>arabica</u>
Near surface water	<u>0.11±0.18</u>	<u>0.01±0.03</u>	<u>0.03±0.08</u>	<u>0.98±0.05</u>
Shallow soil water	<u>0.08±0.23</u>	<u>0.93±0.21</u>	<u>0.23±0.37</u>	0.01±0.02
Intermediate soil water	<u>0.05±0.13</u>	<u>0.01±0.05</u>	<u>0.03±0.10</u>	<u>0.01±0.02</u>
Deep soil water	<u>0.76±0.37</u>	<u>0.05±0.20</u>	<u>0.71±0.40</u>	<u>0.01±0.04</u>

**Table S6.** Relative contributions of the different water sources to plant xylem water (mean  $\pm$  SD) per species and for the sampling performed in the 2017 wet season. Contributions were derived with the MixSIAR Bayesian mixing model framework, using the 'non-informative' prior approach

			<u>C. arabica</u>				
		<u>2014</u>			<u>20</u>	<u>17</u>	
-	<u>Sampling 1</u> (Jan. 23)	<u>Sampling 2</u> (Apr. 11)	<u>Sampling 3</u> (Apr. 26)	<u>Sampling 1</u> (Feb. 27)	<u>Sampling 2</u> (Apr. 5)	<u>Sampling 3</u> (May. 20)	<u>Sampling 4</u> (Aug.4)
Near surface water	<u>0.20±0.29</u>	<u>0.41±0.44</u>	<u>0.75±0.36</u>	<u>0.92±0.21</u>	<u>0.99±0.04</u>	<u>0.94±0.20</u>	<u>0.98±0.04</u>
Shallow soil water	<u>0.73±0.38</u>	<u>0.54±0.46</u>	<u>0.22±0.37</u>	<u>0.05±0.18</u>	<u>0.01±0.04</u>	<u>0.05±0.20</u>	<u>0.01±0.03</u>
Intermediate soil water	<u>0.04±0.09</u>	<u>0.03±0.07</u>	<u>0.02±0.05</u>	<u>0.02±0.10</u>	<u>0.00±0.01</u>	<u>0.01±0.02</u>	<u>0.00±0.02</u>
Deep soil water	<u>0.04±0.08</u>	<u>0.02±0.05</u>	<u>0.02±0.03</u>	<u>0.01±0.03</u>	0.00±0.01	<u>0.01±0.02</u>	<u>0.01±0.03</u>

**Table S7.** Relative contributions of the different water sources to coffee xylem water (mean  $\pm$  SD) for the samplings performed in 2014 and 2017 dry seasons and 2017 wet season. Contributions were derived with the MixSIAR using the single-isotope ( $\delta^2$ H) mixing model