

## SCIENTIFIC BRIEFING

# Tritium analysis shows apple trees may be transpiring water several decades old

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## Abstract

Recent work has shown evidence of ecohydrological separation whereby plants appear to use a less mobile soil water pool that does not mix with more mobile soil water, groundwater, and streamflow. Although many elements of this two water worlds hypothesis remain to be tested and challenged, one key question is “how old might the less mobile water used by plants be?” Such a question is methodologically difficult to answer: stable isotope tracing makes it difficult to resolve any water age older than a few years since the signal gets so damped. Tritium—a useful radiogenic isotope and age dating tool, is now difficult to use in natural systems because most bomb tritium has washed out of soil profiles. Here, we leverage new data from an unusually deep, homogenous soil profile that preserves the mid-1960s tritium bomb signal. We sample the Fuji apple trees (*Malus pumila* Mill) growing on this site that have root systems that penetrate over 15 m and utilize water from within the bomb peak soil water distribution (extracted via cryogenic extraction). Our data show that water used by these trees is on average 29 years old. Bayesian mixing analysis suggests that  $40 \pm 30\%$  of fruit tissue water came from depths between 4 and 9 m within the soil profile ( $36 \pm 9$  years old);  $60 \pm 29\%$  was equally divided between 0 and 4 m and 9–15 m ranges ( $13 \pm 5$  years old). These findings suggest that trees can use quite old less mobile water, highlighting the separation in ages between more mobile soil water and water in transit in sap flow.

## 1 | INTRODUCTION

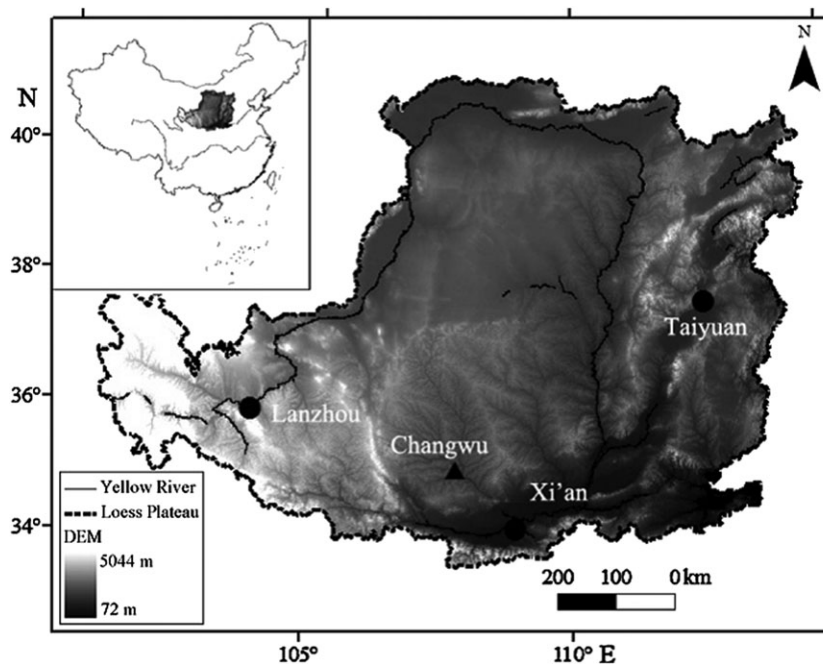
Field-based isotope sampling of plants and soil water has shown evidence of ecohydrological separation (Brooks, Barnard, Coulombe, & McDonnell, 2010; Evaristo, McDonnell, Scholl, Bruijnzeel, & Chun, 2016; Goldsmith et al., 2012). Here, we define ecohydrological separation as plants using water that is “less mobile” or in the range between field capacity and  $-15$  MPa, the range extracted via cryogenic extraction. Recent global synthesis has suggested that ecohydrological separation is widespread globally (Evaristo, Jasechko, & McDonnell, 2015). Such field-based empirical evidence has been supported recently by satellite-based global vapor measurements (Good, Noone, & Bowen, 2015). The separation between more mobile and less mobile water is also influencing biogeochemical conceptualization where recent work suggests “two nitrate worlds” (Hall, Weintraub, & Bowling, 2016)—denitrification inside soil aggregates (where anaerobic microsites dominate) and transport of nitrogen in soil lysimeter water (reflecting aerobic macropore water).

These studies notwithstanding, there are still many open questions surrounding the “two water worlds” hypothesis (Bowling,

Schulze, & Hall, 2016; Gaj et al., 2016; McDonnell, 2014). One question is key: How old is the less mobile water that is used by plants? To date, no one has been able to address this; no techniques are available to easily quantify it. Stable isotopes of water are unable to provide evidence a signal beyond about 5 years (Tetzlaff et al., 2009). Here, we use a unique record of soil water tritium concentration in the unsaturated zone together with fruit tissue water tritium sampling. We present these new data to answer the following questions (a) How old is the less mobile water used by plants?; and (b) What is the source distribution of less mobile water from the unsaturated zone. We leverage a unique experimental setting in the Loess Plateau of China where unusually deep unsaturated zone with a well-preserved bomb tritium profile is linked to fruit tissue water sampling for tritium.

### 1.1 | Study site

Our soil water sampling site is within the 1,504 km<sup>2</sup> Heihe watershed, a first order tributary of the Jing River in the table-gully region of southern Loess Plateau (Figure 1). The site is flat and unirrigated. The soil is predominantly silt loam with silt content greater than 50%. Mean



**FIGURE 1** The location map, lithography, and topography of the site

annual precipitation is 571 mm; mean annual temperature is 9.4 °C. Loess thickness is more than 100 m. The depth to water table ranges from 30 to 100 m (as reported by Li, Si, Zhang, & Jin, 2016). Fuji apple trees (*Malus pumila* Mill) located at the site are at a density of 1,000/hectare. They were planted in 1998, and the orchard has subsequently been fertilized and managed regularly each year since.

## 2 | METHODS

We extracted a 20-m soil core in a 17-year-old apple orchard at the end of September 2015. A hollow-stem auger was used to obtain soil samples at a sampling depth interval of 0.20 m. Each sample was split into two halves; one for gravimetric soil water content and the other cryogenic extraction for water sample recovery and later, tritium analysis of the liquid water. At the same site and on the same day in the field, five trees were randomly selected within a 50-m radius from the soil sampling location. Apple fruit was taken from each of the trees, peeled, pitted, and stored in sealed glass containers. Apple fruit tissue sampling is based on the following simplifying assumptions: (a) Transpiration tritium signal is conserved in the fruit tissue, albeit at lower fractions of total hydrogen incorporated than the component moving through the plant as tritiated water (Kramer, 1969; Murphy, 1990); (b) Majority of tritium (as tissue-free water tritium) in the fruit tissue comes from the soil (IAEA, 2007); and (c) There is negligible to nil water loss via the fruit tissue as any cuticular water loss (5–10%) is theoretically possible only via the plant leaf (Boyer et al., 2009).

### 2.1 | Cryogenic extraction method

We extracted 28 soil samples (note, not all samples were ultimately analyzed for tritium due to budget constraints) and five apple fruit samples using cryogenic extraction. Cryogenic extraction facilitated

the removal of liquid water from soil for tritium analysis of the liquid water. We followed methodology of Orłowski, Breuer, and McDonnell (2016), whereby the soil sample was heated at 95 °C for 2 hours, causing the water to evaporate from the soil (or plant) sample and then to become trapped via freezing in a (cryogenic) liquid nitrogen cold trap. After defrosting, the liquid water sample was then accessible for isotope analysis. Several recent papers describe the technique in detail, to which we refer the reader (Meissner, Koehler, Schwendenmann, Hoelscher, & Dyckmans, 2014; Orłowski et al., 2016).

### 2.2 | Tritium measurement

Each water sample was mixed with a fluorescent solution (Hisafe 3) at a ratio of 8:12 in a 20-ml container. A liquid scintillation counter (Quantulus 1220, Perkin Elmer, Singapore) at the Northwest A&F University, Xian, China, was used to measure the tritium activities in counts per minute, averaged over a 500 min counting period. The sample counts per minute was then converted to Tritium Unit (TU) based on the predetermined counting efficiency (E, obtained from a standard with known tritium activity). The standard deviation (SD) for the background was determined from the long-term reproducibility of background measurements of “dead water” with negligible tritium that were obtained between October 27, 2014, and April 15, 2016, in the laboratory. Water age was then derived by direct comparison of measured soil pore water values with a time series of tritium in precipitation (*sensu* Li et al., 2016). The detection limit, defined as 3.29SD according to Armbruster and Pry (2008) was 10.5 TU.

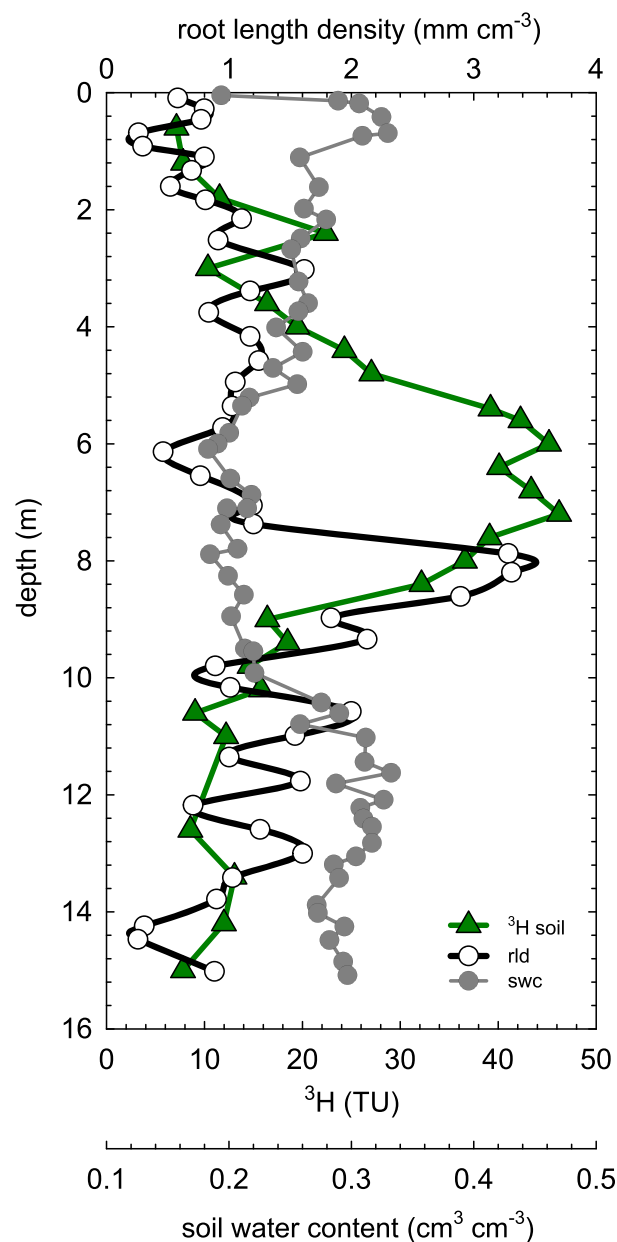
### 2.3 | Bayesian mixing model

We determined the source water proportions in fruit tissue water by using a simple linear mixing model implemented in a Bayesian framework. We employed the SIAR (stable-isotope analysis in R) Bayesian

mixing model statistical package (Parnell, Inger, Bearhop, & Jackson, 2010) to determine the most likely proportion of fruit tissue water from various depths in the soil profile using Markov Chain Monte Carlo methods. We discretized the soil tritium measurements into three groups: 0–4, 4–9, 9–15 m, on the basis of similarity of tritium values. That is, three depth regions where tritium values are similar to (statistically not different from) each other. The trophic enrichment factor and concentration dependence of the original model were set to zero. The model was run with 500,000 iterations (discarding the first 50,000), and a source water's most likely contribution (i.e., the mean of the posterior distribution of the Markov Chain Monte Carlo simulation) to fruit tissue water was obtained. We used known root length density information (Figure 2) per discretized depth range as priors in the Bayesian model: 0.78, 1.57, and 1.16 mm cm<sup>-3</sup>, respectively. We then presented this output together with a model run with little (or vague) prior information (i.e., uniform distribution). The SIAR method is an appropriate treatment of our data because our fruit tissue tritium values indicate contribution from multiple sources with considerable uncertainties along a 15-m soil profile. We note that this model is driven by the following simplifying assumptions: (a) The soil core sampled for tritium represents the entire soil depth within the soil rooting domain of the five apple trees sampled; (b) There is negligible difference with respect to rooting profile and water use between trees.

### 3 | RESULTS

Figure 2 shows the soil water tritium profile on the basis of cryogenic extraction. The peak tritium concentration was detected at 7.2 m. We reason that the peak at 7.2 m represents the bomb peak input—as such, this represents the distance that the 1963 tritium peak has moved downward during the past 53 years. Figure 2 also shows the root length density and soil water content profiles at the 18- and 17-year-old apple orchard sites, respectively. We then sampled the extracted water from five apple fruits growing on monitored trees on the plot. The age (mean  $\pm$  1SD,  $n = 5$ ) of fruit tissue water was  $29 \pm 9$  years. Figure 3 shows the source apportionment for each tree from the Bayesian mixing model. The model run whereby root length density information was used as model priors (Figure 3 box plots) shows that  $40 \pm 30\%$  of fruit tissue water came from depths between 4 and 9 m of the soil profile ( $36 \pm 9$  years old);  $60 \pm 29\%$  was equally divided between 0–4-m and 9–15-m ranges ( $13 \pm 5$  years old). The model run that assumed a uniform root distribution (i.e., not informed by any prior information, Figure 3 error bar plots) shows that  $51 \pm 20\%$  of fruit tissue water came from depths between 4 and 9 m of the soil profile;  $49 \pm 16\%$  was equally divided between 0–4-m and 9–15-m ranges. Finally, we note subtle differences in source proportion estimates between the two model runs (with and without use of prior information). Both models tend to agree in all source estimates, except in trees three and five, which showed the highest likelihood ( $\sim 42\%$ ) of contribution from depths 4 to 9 m ( $\sim 36$  years). These model run differences underline the value of prior information in Bayesian inference platforms such as SIAR, particularly in constraining source proportion estimates that may be overestimated in their absence.

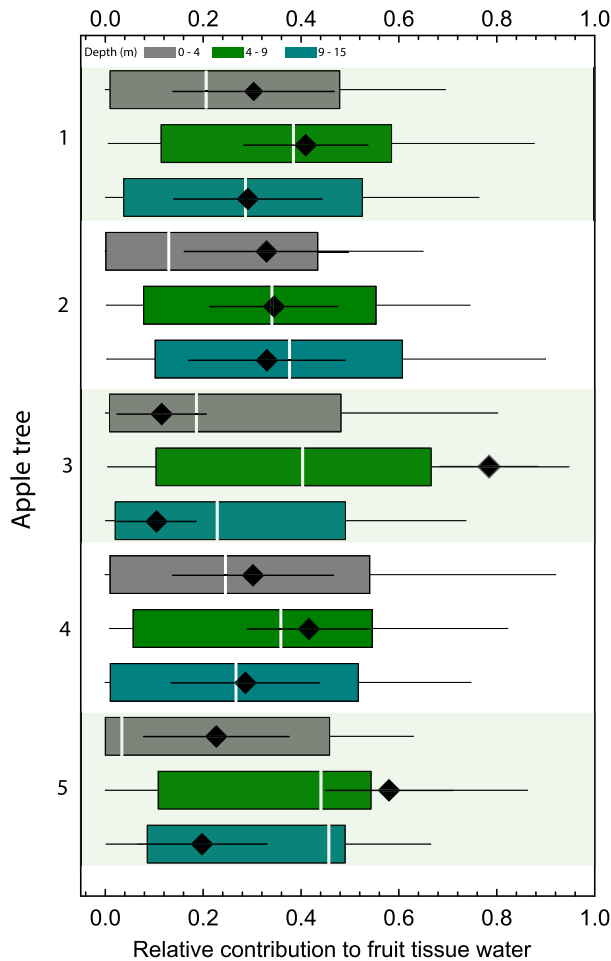


**FIGURE 2** Soil tritium, root length density (RLD), and soil water content (SWC) profile. TU = Tritium Unit

### 4 | DISCUSSION

#### 4.1 | On the surprisingly old less mobile water used by apple trees

Our soil water tritium profiles are not new. Others have reported similar profile distributions on the Loess Plateau (Lin & Wei, 2006; Zuo, Teng, Wang, Hu, & Guo, 2011; Li et al., in press). What is new is the linking of fruit tissue water to this age-based profile of less mobile water. With a half-life of 12.32 years, about 4 half-lives have passed since the bomb peak input with only 1/16 of peak concentrations (or 6.25%) remaining. Lin and Wei (2001, 2006) also found well-defined tritium profile in northern Chinese Loess Plateau; however, their peak location was at 10-m depth in their coarser soils; rather deeper than ours. Our studied apple trees have substantial roots at the depth of 4 to 10 m (Wang, Shao, Liu, & Zhang, 2015). Our root length density,



**FIGURE 3** Source water partitioning using Bayesian mixing model per depth interval and per tree. Model run informed by root length density information as priors is represented by box plots (the boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile; whiskers above and below the box indicate the 90th and 10th percentiles). Model run not informed by any prior information is represented by error bar plots (mean ± 1SD)

measured at a nearby 18-year-old apple orchard, suggests that while most roots are located in the upper 10 m, roots are rather uniformly

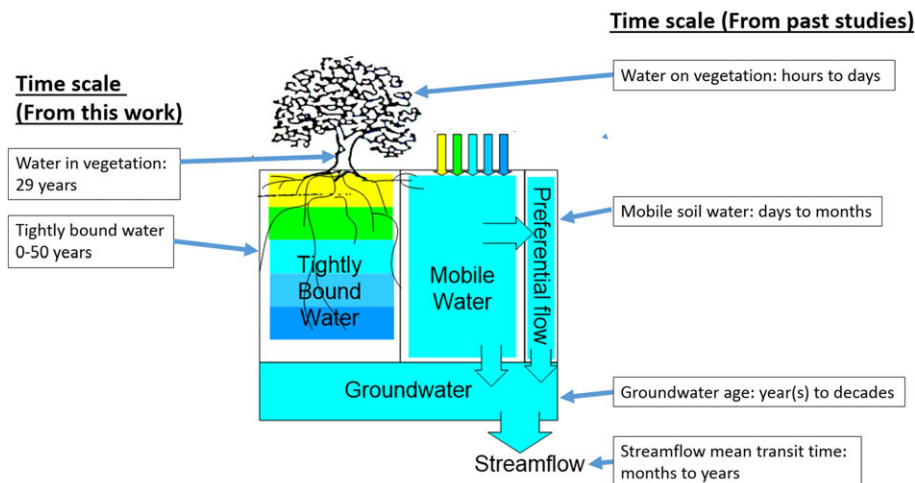
distributed between 0 and 7 m, and between 10 and 15 m. Soil water below a depth of 15 m was largely tritium-free. Although we do not have groundwater tritium data for our site, Lin and Wei (2006) reported that groundwater at 20- to 30-m deep has a tritium content of 34.4 to 43.4 TU.

**4.2 | Why the Bayesian mixing model?**

There are two main categories in the use of stable isotope methods in partitioning source contributions to plant available water: process-based mixing (PBM) models and simple linear mixing (SLM) models (Ogle, Tucker, & Cable, 2014). PBM models (e.g., RAPID by Ogle et al. (2014) and Ogle, Wolpert, and Reynolds (2004) integrate stable isotope data and a biophysical model into a Bayesian framework. We, however, lack the crucial biophysical parameters to be able to execute a PBM model. Where two or three water sources are identified, traditional SLM models may prove sufficient (e.g., Thorburn and Walker, 1994); Brunel, Walker, & Kennetsmith, 1995). These SLM models, however, are not appropriate when the putative sources are greater than the number of isotope tracers  $n$  plus one ( $n + 1$ ). In these underdetermined systems, iterative procedures (e.g., Phillips & Gregg, 2003) have been shown useful, providing the user with a range of feasible solutions. Notwithstanding the widespread use of IsoSource in systems with multiple sources, the method can only provide a range of feasible (not likely) solutions. The lack of a robust statistical foundation of an iterative approach, therefore, boosts the case for an alternative method that frames the mixing model atop a solid statistical (i.e., Bayesian) formulation. Moreover, we argue that our use of a Bayesian SLM (i.e., SIAR, Parnell et al., 2010) is an appropriate treatment of our data given the uncertainties that are inherent in the process dynamics of root water uptake (Ogle et al., 2004).

**4.3 | Water residence time in the soil-vegetation-atmospheric system**

Figure 4 shows a schematic diagram of how we conceptualize water residence time in the soil-vegetation-atmospheric system. A large body of work now exists for time scale of water in vegetation in the



**FIGURE 4** Conceptual model of water residence time in the soil-vegetation-atmospheric system (modified from Brooks et al., 2010)

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form of intercepted water (Muzyllo et al., 2009). These timescales are on the order of minutes to hours to days. For water transiting the soil profile and beyond to groundwater and streamflow, one must recognize the difference between celerity (of the hydraulic potentials) and the water velocity itself (McDonnell & Beven, 2014). Stable isotope tracing of the water velocity in the “more mobile” pool has shown soil water transit time on the order of weeks to months (Asano, Uchida, & Ohte, 2002) with upper limit on the order of 1 year (Munoz-Villers & McDonnell, 2013). Groundwater ages often extend from years to decades for shallow unconfined aquifers (Bethke & Johnson, 2008) and centuries and beyond for deeper or less permeable materials, with streamwater residence times often on the order 1–5 years (see Hale & McDonnell, 2016) but with substantial fractions less than a few months old (Jasechko, Kirchner, Welker, & McDonnell, 2016). These are shown schematically on the right-hand side of Figure 4. No studies that we know of have yet queried this less mobile water age.

#### 4.4 | Limitations of our study and future work

Our study has several limitations that we duly acknowledge. We lack any measure of mobile soil water in this dry site. We lack groundwater tritium data to be able to unequivocally exclude groundwater use by trees. However, we do know that the water table at our site is 58-m deep and that 20- to 30-year-old apple trees would very likely not have roots to anywhere near that depth. We also do not have fidelity in our tritium extractions from the apple trees themselves. This pilot work includes only pressed apples—with sufficient volume for liquid scintillation tritium analysis. We are actively working on new methods to express enough xylem water from trees for tritium analysis for comparison to our fruit-based extractions. Notwithstanding these limitations, we believe that these data are the first of their kind to show the age of transpiration-related less mobile water. Moreover, we underline that the “age” of plant available water described here represents the ages of various water parcels in the subsurface with their associated uncertainties. Although these uncertainties are accounted for in the Bayesian model with respect to source apportionment, we note that the same are likely to vary among ecosystems, and hence their associated “ages”. As more information about the site becomes available, future modeling work may invoke the use of a two-pool model (e.g., Trumbore, Davidson, Decamargo, Nepstad, & Martinelli, 1995), which may provide insights into the evolution of subsurface water parcels represented by tritium measurements.

## 5 | CONCLUSIONS

We have shown that tree water used by apple trees is 29 years old. Extraction distribution was somewhat uniform at the top 4 and bottom 6 m of the 15-m soil profile and slightly greater between 4 and 9 m. These data are the first that we are aware of to show the age of less mobile soil water vis-à-vis the ecohydrological separation hypothesis. Of course, this preliminary work poses many new questions regarding the effect of extraction procedure on resulting tritium concentrations and within-xylem transport time scales. Nevertheless, the work shows for the first time, the very old component of root water extracted.

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## AUTHOR CONTRIBUTIONS

JJM conceived the study and led the paper writing. ZQZ collected the field data, wrote the section on tritium measurement, drafted the earlier version of Figures 1 and 2 under the supervision of BS and ZL. JE performed the Bayesian mixing analysis, generated Figures 1 and 2, wrote and interpreted the Bayesian model results. BS, ZL and JE helped edit the manuscript. JE led the development of later revisions to the manuscript.

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