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Interactive Comment

Interactive comment on "A method to determine plant water source using transpired water" *by* L. B. Menchaca et al.

L. B. Menchaca et al.

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Reviewer #1 raises the following concerns:

1. The method is based on the wrong assumption that leaf water isotope ratios are simply the result of evaporation. 2. The method cannot be applied generally because leaf evaporation lines do not always extrapolate back to source water. 3. The study makes no comparison with xylem water extraction methods. 4. There is a pointless mention of the isotopic ratio of tap water at the site. 5. The isotopic ratio of soil water does not seem credible given the climate of the site. 6. The paper does not review the most recent work on leaf water isotope research and the experimental results were obtained a long time ago.

Response to reviewer #1



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Points 1 and 2:

We agree that the isotopic composition of leaf water and transpired water does not necessarily result from evaporative processes alone. However, the reliability of the proposed method does not depend on assumptions about the processes (including evaporation) affecting leaf water isotopic ratios. We will change the text to make it clear that this assumption is not the foundation for the paper, and to emphasize that the paper presents an empirical method to determine the isotopic ratio of plant source water, using transpired water.

It is true that not all "leaf evaporation lines" extrapolate back to the source water isotopic ratio. For example, Allison et al., (1985) reported that isotope ratios of leaf tissue water from excised leaves in a greenhouse experiment did not extrapolate back to source water, but data from another experiment did, when the source water was in equilibrium with the atmosphere.

Excised leaves may contain waters from different origins and waters that have gone through different degrees of fractionation. If the water in the leaves comes from both, root uptake and atmospheric input, it is unlikely that leaf water isotopic ratios will plot on a line extrapolating to the subsurface source water.

In our method, however, the complicating effects of atmospheric input or evaporation are largely avoided. The stable isotope regression lines in this paper are not comparable to leaf evaporation lines. The method uses changes in the isotopic ratios of transpired water generated from the same leaves during the entire length of the experiment. The leaves are not excised from the plant and are protected from the effects of ambient vapor by a plastic bag. Inside the bag, the output (transpired) water collected, comes from two isotopically distinct sources, the water being conducted to the leaf and the water already present in the leaf before the plastic bag was put in place. The accumulated water in the bag is periodically emptied (and sampled) through a small hole and sealed back immediately. Every time the bag is emptied, the proportion of leaf wa-

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ter (as it was before it was covered with the plastic bag) in the sample will be smaller and the proportion of source water will increase. Therefore, the isotope ratios of these samples will plot on a line that extrapolates in the direction of the isotopic ratio of the source water. Contrary to the reviewer's observation that this method cannot be taken as "a generalization that can be depended upon," it seems reasonable to expect that the method will work in most circumstances, and that there will be exceptions, such as in extreme climates and for particular plant physiognomies.

Point 3:

We disagree with the reviewer's concern that the paper does not compare the results with other methods. In the paper, results generated by the method proposed are compared with results from direct sampling of soil waters and ground waters (located a few feet away from the trees used in the study) using standard methods and equipment such as vacuum lysimeters and wells. The paper does not include data generated from xylem water extraction methods. If we can agree with the current consensus, that xylem water is not fractionated, for the case of our study one should expect no difference between xylem water and either soil or groundwater. Since these waters were sampled using direct methods, there should be no need to sample xylem water in order to demonstrate the validity of the method proposed. Again, we should make sure that this is clearly indicated in the text, and we will modify the text accordingly.

Point 4:

Isotopic ratios of waters known at the site (including tap water) are reported in the paper. The plants sampled were not irrigated. Tap water may, or may not have been contaminating the soil water. It is possible, but not known, that the tap water from irrigation or leaking underground water pipes from nearby areas infiltrated the soil at the sampling site. LBNL lies in the Berkeley Hills, within a mixture of natural and urbanized areas with complex surface and groundwater systems. For this reason, it was necessary to know the isotopic ratios of all possible water sources at the site where

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the plants were growing. For the plants sampled, the isotope regression lines produced by the method presented, extrapolated back to the isotopic ratios of soil (lysimeter) and groundwater (wells), indicating that these plants were using these sources and not others, such as tap water or rainfall.

Point 5:

We agree that normally one would expect soil water to be isotopically heavier than groundwater. However, in this particular case the opposite is true. Although we are not exactly sure of why, there are several possible explanations. For example, the near surface soil water may be in part tap water lost from broken underground pipes. This facility is located on a steep hill in a tectonically active area. Another possibility is that the groundwater dates to a time period when weather conditions were somewhat different. Storm systems affecting the Bay Area, can have very different geographical origins and contrasting isotopic signatures. In a similar way, the frequency of summer fog affecting the site may also have changed. Summer fog is an important characteristic of coastal California and the LBNL property is located directly within the fog belt.

Point 6:

The purpose of this paper is to propose a new and practical methodology for identifying the isotopic ratio of plant source water. The experimental results were obtained in 1994 but we have no reason to doubt their reliability. We have included a brief discussion of recent work on the modeling of stable isotopes of leaf water and transpiration, making the text more informative on this subject. However, the method proposed in this paper does not depend on the use of predictive models, instead, this method is of practical and diagnostic nature, and treats the problem as the progressive mixing of two isotopically distinct waters within a closed system where the volume of the first component is fixed and relatively small, while the volume of the second component is that of the source water and is allowed to flow freely into the bag. In contrast, the isotopic ratio of

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the first component may be determined by a number of factors, such as, vapor initially trapped within the collecting bag or chamber, leaf exudates, bulk leaf water, transpired fractionated water, and other components that may have been in the leaves prior to the sealing of the bag.

Reviewer #3 raises the following concerns:

1. The lack of model predictions greatly reduces the value of this study.

2. Capturing transpired water vapor in a leaf chamber under isotopic steady-state (ISS) conditions should be a straightforward solution to the problem, rather than the method proposed.

3. The method seems to be a theoretically complicated way to assess plant source water.

4. Leaf water at the first collection period is somewhat 'contaminated' by atmospheric vapor.

5. A more serious concern is that the condensate drained from the collection chamber is easily fractionated to a degree dependent on temperature of the collection system. How much back-diffusion of bulk leaf water into the stem and thus removed from the enclosed leaf and chamber is taking place, and would this affect the isotopic composition over time?

6. The paper does not describe the theoretical framework needed to understand the results, nor does it sufficiently describe details of the field collection methods (temperature, etc.) to adequately reproduce the work or assess the results.

Response to reviewer #3

Point 1:

We disagree with the reviewer's opinion that the paper is of little value because it does not use predictive models. The purpose of the paper was not to develop or use pre4, S786–S793, 2007

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dictive models but rather to present a practical and inexpensive field method that can be used to find out the isotopic ratio of the water being used by plants. Because of the method's simplicity, practicality and portability, we expect it to be of value in environmental monitoring and surveillance, and in field hydrological and ecological investigations.

Point 2:

We agree completely with the reviewer's comment that in the laboratory transpired water vapor in a leaf chamber under isotopic steady state conditions should match the isotopic ratio of the plant's water source. In the method described in our paper, the plastic bag is used in a manner that is analogous to a leaf chamber. We must strongly emphasize here that the method described in this paper does not constitute a replacement or a substitute for the leaf chamber. The leaf chamber is used to monitor and control conditions at the leaf/atmosphere interface, and to sample water vapor and other gases. Leaf chambers are used in experiments where careful recording of parameters is required, such as, for the construction of predictive models that aim to understand how plant isotopic ratios relate to physiological and environmental parameters. The method presented here is used to determine the isotopic ratio of plant source water regardless of how the isotopic ratios of leaf water are achieved and whether the plant- soil- atmosphere system is or is not in an isotopic steady state. This method is not concerned with the complicated processes and relationships that determine the isotopic ratios in the leaf water but rather with circumventing those complications.

The reviewer's observation of the differences in cost is also accurate. The plastic bags used in our method can be replaced by gas chambers. However, leaf gas chambers in the US can cost about 700 US dollars each and are not as light and portable as simple plastic bags. Simple plastic bags do not record humidity, temperature, gas flow, etc. but this is not necessary if one only needs to find out the isotopic characteristics of the plant water source. According to the reviewer, using a leaf gas chamber one can wait long enough to determine when isotopic steady state has been reached (90-120 min)

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and then take only one sample. A question here is how does one know that an isotopic steady state has been reached after 90 to 120 min? Also, does one need to know the isotopic ratio of the source water to be able to determine whether or not the vapor in the chamber has reached isotopic steady state?

The reviewer's concerns that gas chambers should be used instead of using the method we propose here are unfounded. The two methods are different, and are appropriate for different experimental situations. Obviously, the gas chamber method is more appropriate in the laboratory or in a detailed field investigation using one or a few plants. In contrast, the method we propose can be used on a large scale investigation involving hundreds of individual plants.

Point 3:

The reviewer's comment that the method seems to be a theoretically complicated way to assess plant source water is not true. We start by assessing the isotopic ratio of a water sample from a bag enclosing actively transpiring leaves on the plant. The isotopic ratio of this first sample may differ from the source water isotopic ratio depending on the conditions in which the plant has been growing. As xylem water is allowed to re-fill the bag through transpiration from the leaves, the isotopic ratio of the water samples will change in the direction of the isotopic ratio of the source water. Every subsequent water sample contains proportionally more and more xylem water and less and less of the fractionated or mixed "first sample" water. Therefore, a plot of these isotopic ratios will extrapolate toward the "pure" source water isotopic ratio.

Point 4: The reviewer is concerned that the leaf water at the first collection period may be somewhat 'contaminated' by atmospheric vapor. Indeed, the leaf water and the first accumulated water sample may be 'contaminated' by atmospheric vapor. This however will be reflected in the differences between the isotopic ratio of this sample and subsequent samples. It is the demonstration of these differences that make the proposed method powerful.

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Point 5: Back diffusion of bulk leaf water into the stem and condensation inside the chamber does not affect the effectiveness of the method. At every sampling interval the isotopic ratio of the mixture of waters within the bag will change toward the source water isotopic ratio to a greater or lesser extent depending on how important the proportion of stem, leaf, and bulk leaf water is in the mixture sampled. Perhaps it will help here to point out that in our paper the volumes of transpired water accumulated in the bags at every time interval were very large (200 - 700ml). With these volumes, the relative importance of air trapped in the bag and leaf water or bulk water contributions is almost certainly small.

Point 6: The proposed method is described in sufficient detail to allow anyone to replicate it and the relevant field conditions existing during the study presented. The proposed method does not require measurements of temperature within the plastic bags.

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