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The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward

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Abstract

Recent studies using water isotopes have shown that trees and streams appear to return distinct water pools to the hydrosphere. Cryogenically extracted plant and soil water isotopic signatures diverge from the meteoric water lines, suggesting that plants would preferentially use bound soil water, while mobile soil water that infiltrates the soil recharges groundwater and feeds streamflow all plots on meteoric water lines. These findings have been described under the “two water worlds” (TWW) hypothesis. In spite of growing evidence for the TWW hypothesis, several questions remain unsolved within the scope of this framework. Here, we address the TWW as a null hypothesis and further assess the following: (a) the theoretical biophysical feasibility for two distinct water pools to exist, (b) plant and soil processes that could explain the different isotopic composition between the two water pools, and (c) methodological issues that could explain the divergent isotopic signatures. Moreover, we propose a way forward under the framework of the TWW hypothesis, proposing alternative perspectives and explanations, experiments to further test them, and methodological advances that could help illuminate this quest. We further highlight the need to improve our sampling resolution of plants and soils across time and space. We ultimately propose a set of key priorities for future research to improve our understanding of the ecohydrological processes controlling water flows through the soil–plant–atmosphere continuum.

KEYWORDS

bulk soil water, ecohydrological separation, preferential flow, stable isotopes, two-domain flow

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1 | INTRODUCTION

Understanding the complex linkages and interactions among precipitation inputs, water reservoirs in the soil and groundwater, and catchment output water fluxes (e.g., evaporation, transpiration and streamflow) is still a major challenge in ecohydrology (Vose et al., 2011). Stable isotopes have become a useful tool for tracking water sources and improving our understanding of the linkages between rain, soil, groundwater, streams, and trees (Kendall & McDonnell, 1998). Recent work using a dual water isotope approach ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) has proposed a so-called “two water worlds” hypothesis (henceforth TWW), under the idea that trees and streams return distinct water pools to the hydrosphere (Bowling, Schulze, & Hall, 2016; Brooks, Barnard, Coulombe, & McDonnell, 2010; Evaristo, Jasechko, & McDonnell, 2015; Evaristo, McDonnell, Scholl, Bruijnzeel, & Chun, 2016; Goldsmith et al., 2012; Hervé-Fernández et al., 2016; McDonnell, 2014). The TWW stems from distinct fractionation of water isotopologues.

The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ measured in meteoric waters (i.e., ice, snow, rain and fog) shows an empirical linear relationship, described by a meteoric water line (MWL; Rozanski, Araguás-Araguás, & Gonfiantini, 1993). This relationship can be affected by meteorological conditions (i.e., temperature and relative humidity [RH]) and other occurring processes (e.g., evaporation, condensation, and mixing). A well-known fractionation process is evaporation. This process can be energy- or diffusion-driven process (equilibrium or nonequilibrium, respectively). Under equilibrium conditions, lighter isotopologues (i.e., $^1\text{H}_2^{16}\text{O}$, mass 18) are preferentially evaporated, while the heavier ones (i.e., $^1\text{H}^2\text{H}^{16}\text{O}$ and $^1\text{H}_2^{18}\text{O}$, mass 19 and 20, respectively) are preferentially condensed (Gat, 1996). This type of fractionation is dependent on temperature and occurs in saturated environmental conditions (RH = 100%). In contrast, under nonequilibrium conditions, heavier water isotopologues (i.e., $^1\text{H}_2^{18}\text{O}$, mass 20) in the liquid phase evaporates and diffuses at slower speeds to the gaseous phase, opposite to lighter water isotopologues (i.e., $^1\text{H}_2^{16}\text{O}$ and $^2\text{H}^1\text{H}^{16}\text{O}$, mass 18 and 19, respectively). This generates an enhanced loss of the lighter isotopologues, resulting in higher accumulation of ^{18}O compared to that of ^2H in the residual water (Gat, 1996). This mass-dependent effect causes evaporating water in soils, in nonsaturated environments (RH < 100%), to follow a characteristic evaporation line (EL). In nature, streams, groundwater, and meteoric water (i.e., fog, rain, hail, and snow) will usually plot along the MWL, while water that has undergone evaporation in soils often will plot along the EL (Gat, 1996). The MWL and EL therefore represent two forms of evaporation processes, which

enrich water isotopes differently. These enrichment processes are used to demonstrate the TWW hypothesis (McDonnell, 2014).

The notion is that nonmobile, bound soil water is used preferentially by plants, while a second pool of mobile soil water is associated with infiltration and groundwater recharge contributing to streamflow (McDonnell, 2014). While early studies focused mainly on tropical and Mediterranean environments, a recent global synthesis suggests that this ecohydrological separation between mobile and nonmobile water may be widespread across most biomes (Evaristo et al., 2015).

Of course, in some sense, “two-domain flow” in soil physics and hillslope hydrological studies is common (Beven & Germann, 2013). Many studies have also shown for different ecosystems that large pores may facilitate rapid lateral subsurface flow that is not in chemical or hydrological equilibrium with the soil matrix (Luxmoore, Jardine, Wilson, Jones, & Zelazny, 1990; Newman, Campbell, & Wilcox, 1998). But the TWW hypothesis challenges the translatory flow paradigm (Hewlett & Hibbert, 1967), which states that water in soils are completely mixed. Therefore, infiltrating precipitation displaces water held in the soil prior to the precipitation event, pushing water deeper into the soil profile and ultimately into the stream. The translatory flow concept has been widely supported through paired catchment studies, indicating that plants utilize water that would otherwise form streamflow, thus linking transpiration and streamflow (reviewed by Brown, Zhang, McMahon, Western, & Vertessy, 2005 and Farley, Jobbágy, & Jackson, 2005). However, studies supporting the TWW hypothesis suggest that there are two distinct pools of water on the basis of their respective isotopic composition: mobile water sampled from precipitation, soil lysimeters, groundwater, and streamflow that generally fall on a meteoric water line (MWL), while plant xylem water and surface bulk soil water (e.g., less than 1 m depth) plots below the MWL, and follows the characteristic EL. However, deeper bulk soil water plots along the MWL. This could be related, but not strictly, to mixing of rainfall with water already in shallow soil as well as the rainfall amount, groundwater depth and ^2H and ^{18}O non-equilibrium fractionation parameters (Gat, 1996; Sprenger, Leistert, Gimbel, & Weiler, 2016). The TWW hypothesis postulates that plants utilize preferentially bound water even when more mobile water is present, while groundwater and streamflow are generated primarily from a mobile water pool that is poorly mixed with the surface soil matrix (Brooks et al., 2010; Evaristo et al., 2015, 2016; Goldsmith et al., 2012; Hervé-Fernández et al., 2016; McDonnell, 2014).

The TWW hypothesis also contradicts basic observations of plant biology where plant water use increases significantly immediately following rain events with increased hydrological connectivity during

rainy seasons (e.g., De Swaef, Steppe, & Lemeur, 2009; Hervé-Fernández et al., 2016). If the TWW is a plausible hypothesis, it remains to be tested why plants would preferentially use more tightly bound water (particularly when soil water is above field capacity) that requires more energy to obtain. If these different concepts cannot be reconciled, can the divergence of isotopic ratios be explained through other processes? This will require well-designed studies that consider plant or soil processes that could explain divergent isotopic ratios.

Here, we examine the TWW as a null hypothesis (some soil and plant water diverge significantly from the MWL) and consider various testable alternative explanations. In doing so, we address the following questions: (a) Is it theoretically possible for two water pools to exist and when might this occur?; (b) What soil and plant processes could explain the different isotopic signature of plant tissues?; (c) What methodological issues could explain the divergent isotopic signatures? Finally, based on our observations we propose a set of experiments

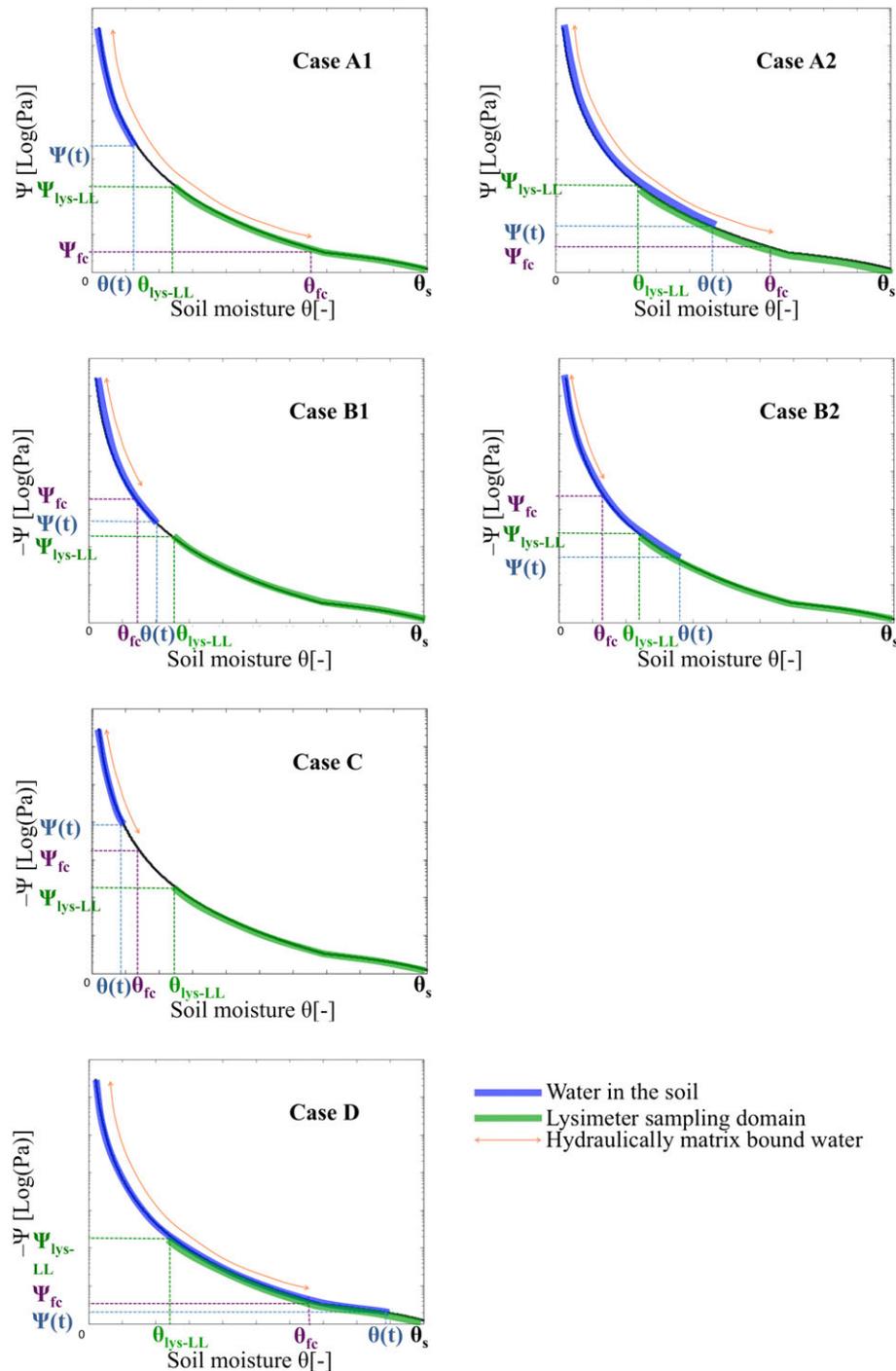


FIGURE 1 Moisture release curve schematic diagram of the six main cases defining the relative values of the field capacity (θ_{fc}), the soil water content at sampling time ($\theta(t)$), and the lower limit of sampling of the lysimeter (θ_{lys-LL}) with their associated matric potential values (Ψ [log(Pa)]). The blue shade denotes the part of the theoretical retention curve that describes the water in the soil, the green shade denotes the lysimeter sampling domain, and the red line denotes the matrix bound water. Subcases where plant water uptakes are analyzed are not represented on this figure for clarity. The cases included are the following: Case A1. $\theta(t) < \theta_{lys-LL} \leq \theta_{fc}$; Case A2. $\theta_{lys-LL} \leq \theta(t) < \theta_{fc}$; Case B1. $\theta_{fc} \leq \theta(t) < \theta_{lys-LL}$; Case B1. $\theta_{fc} < \theta_{lys-LL} < \theta(t)$; Case C. $\theta(t) \leq \theta_{fc} < \theta_{lys-LL}$; Case D. $\theta_{lys-LL} \leq \theta_{fc} < \theta(t)$

that will test the TWW and alternative hypotheses. It is our hope that this framework will stimulate interest in testing all plausible hypotheses to explain plant and soil water divergence from the MWL and thus advance our understanding of water reservoirs and fluxes through ecosystems.

2 | THE HYDRAULIC AND PHYSIOLOGICAL BASIS OF THE TWW HYPOTHESIS (FIGURE 1)

To examine when a TWW could exist, we considered different cases that account for the lower limit (LL) of water extracted cryogenically ($\theta_{\text{cryo-LL}}$), the LL of water sampled using suction lysimeters ($\theta_{\text{lys-LL}}$), and the amount of matrix-bound water (θ_{MB}), defined as the water retained below field capacity (θ_{fc} ; Geris et al., 2015).

For a TWW to be “true,” both of the following conditions would have to be met:

Condition 1: Hydraulically, water that is not freely draining is bound to the soil matrix (θ_{MB}) at a certain matric potential, i.e., θ_{MB} is the water held below θ_{fc} (Veihmeyer & Hendrickson, 1931).

$$\theta_{\text{MB}} \text{ as } \theta \in [\theta_{\text{cryo-LL}}, \theta_{\text{lys-LL}}]. \quad (1)$$

Condition 2: Physiologically, contradiction with the assumption that a plant will take up water at the highest available water potential (i.e., easiest to withdraw; Gardner, 1960). It follows that despite freely draining water above θ_{fc} being available for plant uptake until drainage is complete, plants still withdraw more tightly bound water.

$$\theta_{\text{MB}} \text{ as } \theta \in [0, \theta_{\text{fc}}]. \quad (2)$$

Given that there are two unmixed fractions of water (hence TWW), we can consider the threshold of $\theta_{\text{lys-LL}}$ relative to $\theta_{\text{cryo-LL}}$ because lysimeters sample water only at very low water potentials (typically up to ~ 85 kPa). Below we consider hypothetical cases (Figure 1) that can arise and consider whether a TWW would be plausible in each case. These are divided into four cases (A-D) on the basis of where the LL of lysimeter sampled water and the soil water content at the time of sampling are relative to field capacity. By considering the relationships between each variable and the conditions for a TWW to exist above, we consider the possibility of TWW in each case.

CASE A : $\theta_{\text{lys-LL}} \leq \theta_{\text{fc}}$ and $\theta(t) < \theta_{\text{fc}}$

$$\text{A1. } \theta(t) < \theta_{\text{lys-LL}}$$

$$\text{A2. } \theta(t) \geq \theta_{\text{lys-LL}}$$

$$\text{A2a. } V_p(t) > V_s [\theta(t) - \theta_{\text{lys-LL}}]$$

$$\text{A2b. } V_p(t) \leq V_s [\theta(t) - \theta_{\text{lys-LL}}]$$

where $\theta(t)$ is the soil water content at the root interface at the time of sampling, V_s (in liters, L) is the volume of the soil in the root zone (assumed constant), $V_p(t)$ is the volume of water (in L) taken by the plant roots at time t , and θ_{fc} is the soil water content at field capacity.

Observing a divergence in the isotopic signatures in case A1 would not contradict any known plant physiological process, as all water in the soil is matrix bound according to the hydraulic definition (i.e., Condition 2), and compared to what the suction lysimeters can extract as $\theta(t) < \theta_{\text{lys-LL}} < \theta_{\text{fc}}$, plants are indeed limited to matrix-bound water that cannot be sampled by a suction lysimeter (Figure 1).

Finding a divergence in the isotopic signatures in case A2 needs to be analyzed more closely. In case A2a, the plants need a volume of water that is greater than the volume of water that the lysimeter can sample. This could happen in a water-limited situation (dry season, drought, etc.) but would vary with local soil and plant interactions. Therefore, the hypothesis that the plant takes easy-to-access water “first” cannot be discarded (the volume of water that the plant takes up would therefore be a “mixture” of water held at $(\theta_{\text{lys-LL}}, \theta(t))$ and water held below $\theta_{\text{lys-LL}}$).

In case A2b, however, plants do not need more water than what the lysimeter can sample. If a divergence in the isotopic signatures is found, the plants take up at least some water that is more tightly matrix-bound (i.e., below $\theta_{\text{lys-LL}}$) than what is available and which would suffice to fulfill their needs. This contradicts the common assumption about plant physiology and would therefore be a novel finding and support of the TWW with ecohydrological separation. The hydraulic definition (Condition 2) is respected as the water taken by plants would all be in $(0, \theta_{\text{fc}})$.

CASE B : $\theta_{\text{lys-LL}} > \theta_{\text{fc}}$ and $\theta(t) > \theta_{\text{fc}}$

$$\text{B1. } \theta(t) < \theta_{\text{lys-LL}}$$

$$\text{B2. } \theta(t) \geq \theta_{\text{lys-LL}}$$

$$\text{B2a. } V_p(t) > V_s [\theta(t) - \theta_{\text{lys-LL}}]$$

$$\text{B2b. } V_p(t) \leq V_s [\theta(t) - \theta_{\text{lys-LL}}]$$

In case B1, there is no available water above the lysimeter sampling capability. This should be expected in soils with high clay content, suction lysimeters with low suction applied, or no water to be sampled by the suction lysimeters (e.g., during dry season). Finding a difference in the isotopic signatures is therefore to be expected. Regarding case B2, however, the volume needed by the plant is important. In fact, if this volume is greater than what the lysimeter can sample (similar to A2a), it cannot be discarded that the plant takes up water that is the easiest to access (in $(\theta_{\text{lys-LL}}, \theta(t))$), yet the isotopic signatures would be different as the plant would probably also reach the water that is comparatively harder to access (below $\theta_{\text{lys-LL}}$) to fulfill its needs.

Where the plant water needs $V_p(t)$ is smaller than the volume of water possibly sampled by the lysimeter (B2b), observing a divergence in the isotopic signatures would contradict our current knowledge of plant physiology. However, because it is not possible to discard the hypothesis that the plant only takes up water that is above θ_{fc} (in $(\theta_{\text{fc}}, \theta_{\text{lys-LL}})$), Condition 1 is not fulfilled (i.e., the water taken up by the plant is not necessarily “matrix bound”).

CASE C : $\theta_{\text{lys-LL}} > \theta_{\text{fc}}$ and $\theta(t) \leq \theta_{\text{fc}}$

These results would be consistent with traditional plant physiology. In this case, plants would not have access to freely draining water, and thus would only take matrix-bound water to the soil according to the hydraulic definition (Condition 2). This scenario is also most

plausible in soils with high clay content, using suction lysimeters with low suction, or during dry season where there is no water to be sampled with the suction lysimeter.

$$\begin{aligned} \text{CASE D : } & \theta_{\text{lys-LL}} \leq \theta_{\text{fc}} \text{ and } \theta(t) > \theta_{\text{fc}} \\ \text{D1. } & V_p(t) > V_s \cdot [\theta(t) - \theta_{\text{lys-LL}}] \\ \text{D2. } & V_p(t) \leq V_s \cdot [\theta(t) - \theta_{\text{lys-LL}}] \end{aligned}$$

Case D1 is similar to Cases A2a and B2a: observing a divergence in the isotopic signatures could be explained by the needs of the plant to take up more water than what can be sampled by the lysimeter, and would therefore not be surprising.

In case D2, the plant would theoretically have enough water from what is relatively easy to access (in $[\theta_{\text{lys-LL}}, \theta(t)]$), however at least part of the water they take up is matrix-bound (below $\theta_{\text{lys-LL}}$). This would contradict current knowledge of plant physiology and would be a novel finding. In that case, the use of the term “matrix bound water” does not violate the hydraulic definition (Condition 2).

Overall, we conclude from this analysis that a divergence in the isotopic signatures cannot always be interpreted as a confirmation of the TWW hypothesis (Figure 1). We can distinguish four types of outcomes from this list of possible experimental cases: (a) Outcomes do not contradict our current understanding of plant physiology (cases A1, B1, and C; see Figure 1): they do not fulfill Condition 2 and therefore cannot confirm the TWW hypothesis; (b) Outcomes may present a contradiction to known plant physiology (cases A2a, B2a, and D1): they do not necessarily fulfill Condition 2 and more investigations would be needed to confirm the TWW hypothesis (cases A1, B1, and C; see Figure 1); (c) Outcomes are in contradiction to known plant physiology (Condition 2) and would constitute a novel finding in that aspect. However, in the latter case, outcomes do not fulfill Condition 1 (B2b) as water withdrawn by the plant is not necessarily hydraulically matrix bound. Therefore, the term “bound water” would probably be misused and should rather be used in relative terms (i.e., plants take up water that is more tightly bound than what is available); and (4) Outcomes contradict the known plant physiology (fulfill Condition 2), and water withdrawn by the plant is necessarily hydraulically tightly bound (fulfill Condition 1)—A2b and D2. This would constitute a novel finding (i.e., TWW), and the use of the expression “tightly bound” in absolute terms would be justified.

This thought experiment highlights some potential conditions and the assessment of when a TWW is plausible by more precisely measuring soil water content at the root interface ($\theta[t]$), site specific field capacity (θ_{fc}), the volume of water taken up by roots in the sample period ($V_p(t)$), and the lower limits of lysimeter samples ($\theta_{\text{lys-LL}}$).

3 | FACTORS AFFECTING DIVERGENCE IN ISOTOPIC SIGNATURES BETWEEN XYLEM FROM PLANTS, SOIL (BULK AND MOBILE), AND STREAM WATER

3.1 | Soil processes

The TWW hypothesis challenges soil process paradigms of translatory flow in soils and complete mixing of subsurface water. The extreme

example of translatory flow has been described as piston displacement, in which all preexisting water is displaced by incoming water (Kutilek & Nielsen, 1994). The other extreme is macropore flow or bypassing, whereby incoming water flows down through large continuous pores or structural features with little or no contact with the preexisting water, and hence, no mixing (Beven & Germann, 2013). Recently, Sprenger, Seeger, Blume, and Weiler (2016), described a slower mobile water compartment in clay and silty soils.

Most models simulate a process with an intermediate level of interaction between preexisting and incoming water, termed convective–dispersive flow (Vereecken et al., 2016), in which preexisting soil water is displaced imperfectly. Following a rain event, some new incoming water travels rapidly through soil while, simultaneously, some new water “mixes” with and displaces the preexisting water. A common variation of this is known as the mobile–immobile water model, in which water stored in small pores or aggregates is not directly connected to primary flow paths and is only transported by diffusion (e.g., Gerke & Genuchten, 1993). In natural systems one expects a combination of bypassing and convective–dispersive flow depending on variables such as the matrix conductivity, media structure, rate of input, and preevent conditions (De Vries & Simmers, 2002; Seyfried & Rao, 1987). These well-documented processes lead to the expectation that slowly draining, matrix, or bound water, which may be exposed to evaporation for multiple events, will have a different isotopic signature than stream or groundwater that is fed by rapidly draining water that is not. Additionally, as the isotopic signature of rain events varies seasonally, the input of quickly draining rain water at discrete times of year needs to be accounted for. Since soil conductivities near saturation are often one or two orders of magnitude greater than transpiration rates, this rapidly draining water is not available for plant uptake regardless of the plant’s “preference.”

Even if all new event water were exclusively transported via macropores with minimal mixing in the soil matrix, and static matrix water were preferentially absorbed by trees, there must be a source to replace soil water lost by plant uptake, maintaining the process in time, and returning a significant proportion of soil water to the atmosphere (Hervé-Fernández et al., 2016; Jasechko et al., 2013). As plants extract water from moist soil, soil and plant water potentials progressively decline, and water moves from areas of the soil with higher water potentials to areas with lower water potentials near roots. Inevitably, tightly bound matrix water must be replaced by water from larger pores held at lower tension. This suggests that some additional mixing process must occur between the soil water and plant xylem.

At hillslope scales, streamflow and groundwater recharge are generated by nonuniform infiltration processes across the landscape. These processes are influenced by variables such as soil thickness, depth to water table, and rainfall characteristics. Consequently, experiments testing the TWW hypothesis need to be site specific with precise monitoring of the nonbounded and bounded water in the soil matrix across space and time.

3.2 | Plant processes

There are several key points where the TWW hypothesis is inconsistent with currently accepted plant ecophysiological theory and thus

should be an important focus of future experiments. We know that the majority of water enters the plant at the root interface primarily as bulk flow and passes through the endodermis where it encounters the Casparian strip (Weatherley, 1976). From there, water passes from the apoplastic to the symplastic pathway into the xylem and travels through the vasculature to the leaves. The common assumption is that isotope fractionation does not occur at each of these transitions. Therefore, the isotopic signature of plant xylem water and water in the soil at the site of root uptake are similar (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002). However, growing evidence hints toward several physiological processes that can enrich plant water and lead to a divergence of the isotopic signal from the original isotopic composition of the source soil water (e.g., Eller, Lima, & Oliveira, 2013; Ellsworth & Sternberg, 2015; Ellsworth & Williams, 2007).

First, there is evidence that fractionation could occur throughout the pathway. Hydrogen isotope fractionation from 3 to 9‰ has been measured at the soil-root interface in halophytic and xerophytic plant species, with a significant positive correlation between salinity tolerance and magnitude of fractionation (Ellsworth & Williams, 2007). While additional studies have also found fractionation at the root interface (Sternberg, Ish-Shalom-Gordon, Ross, & O'Brien, 1991), it should also be noted that several have not (e.g., Dawson & Ehleringer, 1991) leaving a need for understanding transport through these pathways. Further, it is plausible that water exchange across the Casparian strip and mycorrhiza could preferentially change isotopic composition. Within the vascular tissue, regular embolisms cause a phase shift to water vapor inside the xylem conduits. Such a phase shift has the potential to fractionate water in the bubble, but whether this can affect the isotope ratio of the bulk water taken from plant tissues has not been explored. Moreover, recent evidence has demonstrated that water may evaporate from stems with suberized bark in deciduous trees during leafless periods (Ellsworth & Sternberg, 2015; Phillips & Ehleringer, 1995). Additionally, preferential flow through aquaporins (Mamonov, Coalson, Zeidel, & Mathai, 2007) and during cell metabolism (Yakir, 1992) may further alter isotopic composition.

Second, growing evidence that dew, fog, and atmospheric water is directly absorbed by plants through leaf and bark tissues (Berry, White, & Smith, 2014; Earles et al., 2016; Eller et al., 2013) provides another possible source of isotopic variation within plants. Eller et al. (2013) reported that fog water diffusing directly through leaves contributed up to 26% of maximum transpiration, while Gotsch et al. (2013) found that fog uptake resulted in the recovery of 9% of all dry-season water transpired from individual branches. Since fog tends to be more enriched compared to rain (see Scholl & Murphy, 2014 for review), the internal cycling of enriched water from leaves is likely to affect the isotopic signature of water sampled in the xylem. There is evidence that fog water taken up by leaves even reaches belowground water pools (Eller et al., 2013), further supporting the contribution of isotopically-enriched fog water to divergent isotopic signatures. While this cannot entirely explain the TWW hypothesis as it is not a universal occurrence, quantifying recirculated water and its subsequent effect on isotopic signature of plant water is possible and should be considered in future studies.

Third, significant spatial variation in isotopic signatures within trees may also affect isotopic composition of stem water in ways that

challenge the TWW. For instance, a recent experiment using in situ sap flux sensors located at different positions around the stem showed that uptake of labeled water was not homogeneous (Volkman, Haberer, Gessler, & Weiler, 2016) due to limited lateral mixing (Orians, van Vuuren, Harris, Babst, & Ellmore, 2004; Sperry, 1995) and differences in connectivity of stem xylem with different root sectors (Nadezhkina, 2010). Twisting plant vessels within the stem found in many tree species (Orians et al., 2004; Zimmerman & Brown, 1971) also suggests that xylem water measured along the trunk will not necessarily be linked to the same root section and thus water source.

Furthermore, because we now understand water movement in plants to be 3-dimensional and not unidirectional, cycling and storage is likely to play a role in altering sampled plant water isotopic signatures. Since phloem water is likely to be sourced from various origins (i.e., soil depths and landscape heterogeneity) across time, the signature could consistently skew plant water isotopic composition. Indeed, Cernusak, Farquhar, and Pate (2005) showed phloem water to be significantly enriched in ^{18}O by 0.5 to 0.8‰ compared with xylem water. Transfer of phloem water to the xylem may be especially pronounced during periods of drought stress when such transfers are speculated to occur to support embolism repair (Nardini, Gullo, & Salleo, 2011; Pfautsch, Holta, & Mencuccini, 2015). There is also evidence suggesting diel patterns of phloem-xylem water exchange, with water moving from bark to xylem in the morning, and a reversal of the flow direction occurring during the evening (De Schepper, van Dusschoten, Copini, Jahnke, & Steppe, 2012; Pfautsch, Renard, Tjoelker, & Salih, 2015; Steppe, De Pauw, Lemeur, & Vanrolleghem, 2006). Evaporation of stem water through bark and/or exchange with isotopically-enriched phloem water has been proposed as underlying mechanisms explaining the enrichment in xylem water along stem and branches and between younger and more mature tissue material (Cernusak et al., 2005; Dawson & Ehleringer, 1993; Thorburn, Walker, & Brunel, 1993).

3.3 | Plant-soil dynamics

Having separately addressed complexities in soil and plant processes, it is also necessary to consider temporal dynamics of these processes that, together, could explain divergent isotopic signatures, in addition to seasonal variability of precipitation isotopic signatures. Seasonal variability in precipitation can effect bulk soil water and is typically caused by temperature, atmospheric relative humidity and rainout processes which all influence isotopic signatures along the MWL (Dansgaard, 1964; Gat, 2005; Sprenger, Leistert, et al., 2016).

Even if it is possible to sample soil water precisely at the point of soil water uptake and within xylem, there is an inherent time lag between water entering the root and reaching xylem at the point of sampling. Water taken up by roots can reach xylem in the trunk on the order of hours to days and subsequently reside for significant amounts of time (up to 44 days in conifers, Meinzer et al., 2006) depending on tree-specific anatomy (soil uptake rates, xylem vessel size, and storage cells) and environmental conditions (temperature, humidity, rainfall, etc.). The decoupling of these water pools can also vary seasonally. A recent study has shown that xylem water in larch (*Larix decidua* Mill.) was enriched in ^{18}O (and decoupled from the

strongly isotopically depleted soil water) before the growing season and steadily decreased to a low before needle maturation (Treydte et al., 2014). Similar seasonal effects have also been found in *Acer grandidentatum* Nutt and *Quercus gambelii* Nutt (Phillips & Ehleringer, 1995). Assuming that transpiration is negligible during winter in temperate climates, water storage in plants from the previous autumn (Brandes et al., 2007; Waring, Whitehead, & Jarvis, 1979) coupled with evaporation effects in the twigs and stems (Dawson & Ehleringer, 1993) may explain ^{18}O and ^2H enrichment in xylem water in early spring (Brandes et al., 2007; Treydte et al., 2014). Also, water remaining in stems over dormant periods or during periods with low transpiration rates allows for gradual transfer of isotopically enriched phloem water (Cernusak et al., 2005) into xylem water, which has been suggested to explain this pattern (Brandes et al., 2007).

Hydraulic redistribution is also a common occurrence (Domec et al., 2010; Prieto, Armas, & Pugnaire, 2012; Sardans & Peñuelas, 2014) that will likely mix water volumes between the soil and plant that originated at different points in time. Likewise, soil water isotopic composition may be affected if plant water with a different signature (sourced across plant organs, e.g., leaf and bark uptake) is released into the soil, as previously mentioned.

Furthermore, in many environments, recharge of groundwater occurs during periods when the incoming precipitation overwhelms evaporative demands (Jasechko et al., 2013). This happens locally, for example, in shallow soils over fractured bedrock, as well as in seasonally cool Mediterranean climates. In either case, the recharge water can be expected to have a very strong precipitation signature that is relatively unaffected by fractionation processes. Therefore, it is actually quite expected that soil water would develop a different signature from xylem water over time with progressive precipitation, displacement, and fractionation sequences occurring out-of-sync with plant uptake.

3.4 | Limitations of water extraction techniques

Isotopic composition of water is inherently prone to fractionation. This serves as a scientific advantage by providing discrete signatures to various pools but also a challenge to successfully maintain consistent water signature from collection to isotopic analysis. Recent evidence suggests that extracted water (i.e., plant and some soil water) could be prone to fractionation through the extraction process. Below, we highlight the potential for fractionation and explain how the TWW hypothesis could be partially explained as an artifact of methodology.

In ecohydrological studies, soil pore water is sampled through wells, piezometers or low-tension lysimeters representing mobile and matrix-bound water. In contrast, soil and plant water is often extracted using cryogenic vacuum distillation representing bulk soil or plant water, i.e., a mixture of tightly-held matrix bound and loosely-held mobile water. During cryogenic vacuum extraction, the sample is heated under a vacuum whereby the water is extracted by evaporation and caught in a liquid nitrogen cold trap. The isotopic signature of the condensed water follows a Rayleigh distillation curve and an unfractionated water sample is obtained when all water is extracted (Ingraham & Shadel, 1992). Samples (both soil and plant) that are not fully extracted are more depleted in ^2H and ^{18}O (West,

Patrickson, & Ehleringer, 2006). The time necessary to yield unfractionated samples strongly depends on tissue type (wood, branch, root, or leaf) and plant species (Araguás-Araguás, Rozanski, Gonfiantini, & Louvat, 1995; Koeniger, Marshall, Link, & Mulch, 2011; West et al., 2006).

Several studies have demonstrated fractionation of soil water through cryogenic vacuum extraction, particularly in clay-rich soils and soils with low water content (Orlowski, Breuer, & McDonnell, 2016 and references therein). In clay soils, there is an interlayer of adsorbed water that allows for some water fraction to be partly bound to minerals, such that it is not completely released during the cryogenic extraction technique (Orlowski, Frede, Brüggemann, & Breuer, 2013; Orlowski et al., 2016). Oerter et al. (2014) stated that soils with high cation exchange capacity can retain large amounts of structured water around the ion (the so-called hydrated radii due to the high ionic potential of, e.g., Ca^{2+} and Mg^{2+}) and are capable of causing fractionation between bound and bulk soil water (O'Neil & Truesdell, 1991; Orlowski et al., 2016). Although these studies have demonstrated issues with extraction methods, more work is needed to discern the best methodology for each experimental design.

4 | A PATH FORWARD: RESEARCH ADVANCES AND FUTURE EXPERIMENTS

To determine comprehensively if a TWW exists, we need to address experimentally the limitations and alternative explanations for diverging isotopic signatures presented in this paper. To do so, we have compiled three areas that should be considered in future experiments: (a) internal flow dynamics within trees and how they contribute to sampled isotopic ratios; (b) improved sampling resolution of soils and plants for greater precision across time and space; and (c) a determination of potential sampling errors when soils and plants are sampled and water is extracted.

4.1 | Internal cycling of water in trees

There is a clear need to test whether internal stem cycling of phloem and xylem water within plant, known as Münch counterflow (Münch, 1930), and water exchange between xylem, bark, and other sources can be responsible for alterations in xylem isotopic composition. For this, a manipulative experiment of the water status of the tree could be used to determine whether (a) more internally-stored water is used from the bark, or (b) more counterflow occurs. Technologies now exist for quantifying Münch's counterflow and the amount of water exchanged between xylem and bark (De Schepper & Steppe, 2010; Steppe et al., 2006). Then, to address if this exchange significantly alters xylem composition, xylem and phloem sap can be sampled in situ across days, hours, and thus transpiration rates. Xylem water can be sampled with a hydraulic pump as described by Lambs and Berthelot (2002) and phloem sap can be sampled by using aphids (Steppe, Sterck, & Deslauriers, 2015). Sampling could be done across controlled or induced (through heat) transpiration rates to link the isotopic signatures of xylem and phloem waters to transpiration rates.

4.2 | Improved spatial and temporal resolution of sampling soil and xylem water

In order to break new ground and effectively address the TWW hypothesis, we need to abandon our existing crude, “shotgun” and snapshot techniques in stable isotope ecohydrology. Thus, new studies that address TWW need high temporal and spatial resolution of below-ground liquid, vapor fluxes and plant xylem water, ideally measured in situ. This approach should target sampling throughout the uptake pathway including soil immediately surrounding fine roots, mycorrhizal associations, large suberized roots, and xylem (specific) water. Ideally sampling should occur across time scales from seconds to hours to determine whether fractionation occurs across any of these interfaces or varying time lags exist on the basis of species, location, or climate. The utilization of labeling with artificially enriched water may prove particularly fruitful in testing the TWW hypothesis at finer temporal resolutions. Higher spatial and temporal resolution in sampling would also assist in understanding when soil water across depths falls on or off the MWL and allow researchers to more precisely target the source water utilized in plants. It is also important that extraction methods are developed incorporating both liquid and water vapor into the bulk sample as these fluxes are likely to affect fractionation processes. Recent advances by Volkmann, Kühnhammer, Herbstritt, Gessler, and Weiler (2016) provide a method for high frequency measurement of isotopic composition of xylem water in situ. Although the results remain to be tested for replicability and the method for dependability, these are advances that will contribute to furthering our understanding of the temporal variations in plant water uptake.

4.3 | Test sampling and methodological errors

There is an urgent need to find out whether cryogenic extraction is effectively sampling all bulk soil water or, alternatively, identifying exactly where errors exist in this methodology. This needs to be tested across numerous soil types at various water contents to determine when potential errors dramatically bias results. Equally important, technological progress is needed in the analysis of water isotope composition of soil water at discrete tensions along the soil moisture release curve. At present, we can only sample either the most mobile water via suction lysimeters or effectively “all the water” through various distillation methods (−10 to −15 MPa) or hydraulic squeezing (−41 MPa; Evaristo et al., 2016; Sprenger, Herbstritt, & Weiler, 2015).

Additionally, there is a concern that the sampling of plant tissue may be prone to cavitation errors. In cavitation research, sampling is done very carefully with a cut (and recut) of the sample under water to prevent cavitation or air entry in the system (e.g., Cochard et al., 2013). When a stem core is used for cryogenic extraction, cavitation will occur as soon as the stem core is extracted from the tree. Depending on the time lag between stem core extraction and sealing, water will evaporate and fractionate the water in the sample. It could then be hypothesized that the longer the stem core is exposed to air upon sampling, the more cavitation will occur and the more the data point will plot below the MWL line. A similar error might take place with soil samples extracted this way, which could explain why these cryogenically extracted soil and plant samples plot below the MWL line.

The destructive nature of sampling related to these extraction techniques eliminates the opportunity to account for effects on soil properties by soil microfauna and microflora (Hallett, Mooney, & Whalley, 2013) and vice versa (Kravchenko et al., 2013). Given the incongruence in both space and time between our soil water extraction techniques and plant (root/mycorrhizal) water uptake mechanisms, we need to develop, fundamentally, new extraction approaches that are able to interrogate water sources and root water uptake mechanisms at matching scales.

4.4 | Final considerations

The TWW hypothesis has stirred significant debate in recent years as it is simplistic, yet confounds many basic paradigms of both watershed hydrology and plant biology. Isotopes have proven to be a valuable tool in landscape hydrology, but their coarseness in this analysis leaves us with more questions than answers. Ultimately, focused, hypothesis-driven studies are needed to explore alternative explanations of the divergent isotopic signatures from MWL. In this paper, we have provided some of these alternative explanations and proposed key aspects where new research is urgently needed. Finally, we need new techniques that allow for improved spatial and temporal sampling of soil and plant water. Such techniques may draw on emerging tools from other disciplines that are seldom used in water stable isotope ecohydrology such as nanoscale secondary ion mass spectrometry, neutron radiography, and X-ray tomography. If high-resolution in situ techniques for xylem and soil water sampling become available, neutron radiography technique could prove instructive. Perhaps, it is long overdue that we apply more pointed techniques from complementary disciplines to increase our precision and understanding of ecohydrological processes.

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