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Demystifying stable hydrogen isotope offsets between plants and source waters

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Stable isotopes are widely used to investigate plant-water interactions, yet many studies report differences in deuterium (δ^2H) ratios between plants and source waters (termed δ^2H offsets). These δ^2H offsets challenge the assumption that water uptake and transport in plants do not alter the isotopic composition of water. Here, we develop a conceptual framework that distinguishes three soil water pools and two plant water pools based on water potential. We synthesize data from 110 published studies across 212 field sites worldwide and reanalyze 6333 δ^2H offset measurements using possible source water lines. We find that δ^2H offsets are absent when using appropriate water pools (e.g., use sap flow water rather than bulk xylem tissue water to represent plant water), with mean offsets not statistically different from zero. We argue that accurately separating correct water pools from bulk water pools in soils and plants is key to reconciling the observed δ^2H offsets.

The global flux of water through terrestrial plants (i.e., transpiration) accounts for about two-thirds of land evapotranspiration and shapes ecosystems, climates, and natural resources[1–3]. Understanding where water comes from and how water moves through the soil-vegetation-atmosphere continuum is important for predicting the impacts of global climate change and anthropogenic activities on critical hydrological processes and ecosystem services[4,5]. One of the most effective tools for quantifying plant water uptake patterns and linking plant ecology to hydrologic flows is the analysis of stable isotope ratios in environmental water pools and fluxes[6–10]. Global isotopic databases of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) in different environmental water stores accumulated over the past six decades have enabled examinations of local, regional, and global-scale land-atmosphere interactions[11,12], evapotranspiration partitioning[1,13–15], and plant water source identifications[16].

Although Lin and Sternberg[17] and Ellsworth and Williams[18] noted that hydrogen isotope fractionation did occur for halophytic species that relied heavily on the symplastic root water uptake pathways in saline environments, it is generally acknowledged that the biological processes of root water uptake and water transport within terrestrial plants do not result in isotope fractionation[19]. However, over the three decades following Lin and Sternberg's work, significant δ^2H offsets between plant and source waters have been increasingly documented in non-halophyte species across both natural settings and controlled experiments (hydroponic or soil-potted), with far fewer corresponding reports for $\delta^{18}O$ values[10,20–28]. Such δ^2H offsets between plant and source waters challenge the reliability of isotope methods in ecohydrological studies based on the assumption of isotopic consistency during plant water uptake and transport and subsequent water extraction processes[4,10,20–22,27–30]. For instance, the widespread observations of δ^2H offsets raise concerns about the accuracy of previously reported global and seasonal patterns of plant water uptake[16,31–33] and their underlying drivers[12,16,34], as these studies often did not account for such offsets. The reporting of δ^2H offsets has also renewed the unresolved debates regarding whether the isotope ratios of soil water accessible to plants remain separated from those of water rapidly bypassing soils and recharging open channels (i.e., the "ecohydrological separation" or "ecohydrological connectivity" hypothesis)[1,4,35,36]. Given the widespread practice of using the isotopic signatures of bulk woody xylem water as proxies for those of plant-available soil water, the observed δ^2H separation between soil water accessible to plants and preferential soil water recharging streams could also result from δ^2H changes during plant water use processes (i.e., δ^2H offsets) in addition to "ecohydrological separation" within soils. Additionally, isotope-based evapotranspiration partitioning methods typically leverage isotopic differences between transpired water vapor, consistent with source waters, and evaporated water vapor, often exhibiting a δ^2H-depletion compared to source waters[37]. This isotopic difference between transpiration and evaporation provides a basis for separating their contributions to total evapotranspiration[1,37–39]. However, deviations of transpired vapor from the isotopic composition of source waters (i.e., δ^2H offsets) may result in an increasing or decreasing disparity in isotope values between transpiration and evaporation, consequently leading to inaccurate estimations of transpiration's contribution to overall evapotranspiration. As such, it is urgently necessary to rigorously examine the fidelity of isotope methods used in ecohydrological investigations.

The δ^2H offset between plant and source waters may result from (1) inherent δ^2H changes deriving from isotope fractionation during root water uptake and subsequent water transport from roots to leaves, (2) deceptive δ^2H changes due to methodological artifacts imparted during the water

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extraction process, (3) inaccurate δ^2H signature measurements due to improper water pool extractions or mixing of distinct water pools within soils and plants, or potentially all three factors combined. A series of greenhouse experiments[20,40,41] and a xylem sap bleeding experiment for the field-grown *Populus euphratica*[21] demonstrated that the within-stem isotope heterogeneity, which can be derived from isotope fractionation during plant water use, could be the primary cause for δ^2H offsets between plants and source waters[20,21,40,41]. In contrast, another greenhouse experiment reported that kinetic hydrogen fractionation during cryogenic extraction is the primary cause of δ^2H offsets, resulting from dynamic exchange between organically bound δ^2H and liquid water during extraction[26]. An extensive catchment observation[27] and a global meta-analysis[28] encompassing different plant species, climate zones, and water extraction methods quantified a highly variable δ^2H offset between bulk xylem water and its sources, ranging from −57.2‰ to 36.3‰, with the mean value of −5.1‰ ($p < 0.05$) and −3.0‰ ($p < 0.05$), respectively. They further identified that such significant δ^2H offsets with high variability cannot be attributed solely to methodological artifacts, but also to various environmental factors, such as air temperature and relative humidity, which can affect leaf physiology and, consequently, hydrogen isotope fractionation processes within plants across different climate zones. More recently, a field study quantified contributions of methodological artifacts and within-stem isotope heterogeneities to plant-source water δ^2H offsets, accounting for 75.1% and 24.9%, respectively[10]. Overall, we posit that it is essential to eliminate deceptive δ^2H offsets resulting from both methodological artifacts and mixing water pool measurements before questioning the reliability of isotope methods in ecohydrological investigations and undertaking attribution analysis for the inherent δ^2H offsets induced by the within-plant isotope fractionation. Failure to do so compromises the reliability of interpretations regarding the causes and fidelity of the observed δ^2H offsets.

The goal of this study is to thoroughly evaluate the fidelity and causes of δ^2H offsets. Here, we test a key hypothesis that δ^2H offsets do not occur if the correct water pools, with accurate δ^2H signatures, are collected using appropriate extraction methods in isotope studies. We will first construct a water potential-based framework that accounts for water flow and corresponding isotope heterogeneities within the soil-vegetation-atmosphere continuum (Fig. 1). Plant waters can be separated into two distinct pools with unique isotope signatures: the non-fractionated sap flow water and the fractionated non-conducting xylem tissue water (termed the "two plant water pools" concept, Fig. 1A). It is worth noting that the "two plant water pools" concept simplifies isotopic heterogeneities among various non-conducting xylem tissue water pools, which may arise from isotopic fractionation during processes, such as membrane transport, differences in water residence time, and internal water mixing dynamics[23,42,43]. Similarly, we identify three distinct soil water pools with unique isotope signatures within the "three soil water pools" concept (Fig. 1B): gravitational soil water, which drains freely through the soil profile; plant-available soil water, retained in mesopores and micropores and accessible to roots; and hygroscopic soil water, which forms thin films tightly bound to soil particles and is unavailable to plants. This framework will guide us to accurately separate water pools within plants and soils for isotope-based ecohydrological investigations (Fig. 1C). To test this framework, a global re-quantification of δ^2H offsets between plant water and the "possible-source" water line (PWL) is performed using a linear regression that integrates the isotopic signatures of all possible water sources across different combinations of three soil water pools. Both the proposed conceptual framework and the re-evaluation of the global δ^2H offset dataset presented in this study will help comprehensively assess and verify the robustness and reliability of isotope methods in ecohydrological investigations.

Establishing a new framework

A new "three soil water worlds" concept to understand the actual sources of plant water use

An essential prerequisite for obtaining the actual sources of plant water use is to correctly extract all possible source waters. For natural terrestrial plants, all the environmental waters, including precipitation, soil water at different depths, groundwater, river water (for riparian plants), fog, dew, and water vapor, can be possible water sources. However, most recent δ^2H offset-related studies have considered only a subset of below-ground water sources (i.e., soil water and groundwater) as possible sources for plant water use[10,21,27,44–47]. Precipitation was often combined with surface soil water to represent one water source with highly variable isotope signatures. Water uptake from above-ground plant parts (e.g., leaves and bark) is often overlooked in δ^2H offset-related studies[1,4,16,20,23,48]. Occult moisture, such as fog, dew, and water vapor in the air, has been observed to provide critical water sources for plants across a range of biomes[49–57]. Therefore, the absence of isotopic signatures of these critical water sources may lead to deceptive δ^2H offsets[27,48].

More importantly, water flow heterogeneity within soils is a product of complex factors, such as tension variations, storage time, and the manner in which water flows through soil pores[58,59]. Such heterogeneous water flow within soils could result in isotopic heterogeneities on the soil pore scale[36]. The currently recognized "ecohydrological separation" hypothesis is conceptualized as a classification of soil isotopic heterogeneities between fast-flowing soil water recharging into open channels and slow-flowing soil water accessible for plant transpiration[9,59]. To tackle the challenge of accurately extracting the plant-available soil water pool, the urgency lies in quantitatively determining the range of soil water potential and pore diameter for the plant-available soil water. It is widely recognized that the field capacity of soil and the permanent wilting point are the upper and lower limits of plant-available soil water, between −0.01 and −1.5 MPa in soil water potential and between 0.2 and 30 μm in soil pore diameter[60–63], though variations in wilting points have been observed across biomes[64,65] and plant species[66–68]. Measurements of the sap flow in plant xylem conduits suggest that roots prefer to take up plant-available soil water in fine pores[1,9,20,21,60]. The gravitational water pool in soil water potential > −0.01 MPa (i.e., gravitational water between maximum water-holding capacity and field capacity) drains rapidly and is typically unavailable for plant uptake. The hygroscopic water pool in soil water potential < −1.5 MPa (i.e., hygroscopic water below the permanent wilting point) exists as thin films tightly bound to soil particles and cannot be accessible for plant roots[60,69]. Revisiting the physical conceptualization of different water pools in isotope-based ecohydrological studies helps address the inherent limitations of the "two water worlds" hypothesis in soils, which we argue inaccurately characterizes the isotopic dynamics of plant-available soil water. Instead, we advocate for a "three soil water pools" concept that encompasses the gravitational soil water pool, the plant-available soil water pool, and the hygroscopic soil water pool (Fig. 1B). This three-pool concept offers a simplified framework for what is likely a continuous spectrum of water potentials within soils. Nevertheless, the transition from a two-pool to a three-pool soil water concept effectively encapsulates the diversity of soil water stores. Although some previous isotope-based ecohydrological studies reported the heterogeneous isotopic signatures of soil water in terms of the "two water worlds" hypothesis[20,23,70], only a few studies have directly identified the isotopic compositions of plant-available soil water pool from the perspective of the "three soil water pools" concept[20,26,71]. The vague or non-specific soil water pool extraction could also be a primary reason for the observed δ^2H offsets between plants and source waters.

Some comparative studies encompassing different extraction methods (e.g., lysimeters, wick samplers, mechanical squeezing, centrifugation, distillation, cryogenic vacuum distillation, CO_2/hydrogen equilibration, and direct water vapor equilibration) have systematically summarized sampled pore-size distributions and extracted different water pools at the pore scale[60,72–75]. While these methods have greatly advanced our understanding of soil water extraction, growing evidence suggests that many of them may not reliably separate the plant-available soil water pool (Fig. 1B). From the perspective of the "three soil water pools" concept, most of the pore water stable isotope analysis methods, including lysimeters, wick samplers, and mechanical squeezing, were often used to extract the gravitational soil water pool and were not able to collect the entire plant- available soil water pool due to the limitation of their maximum tension of 0.05–0.07 MPa[60]. Some

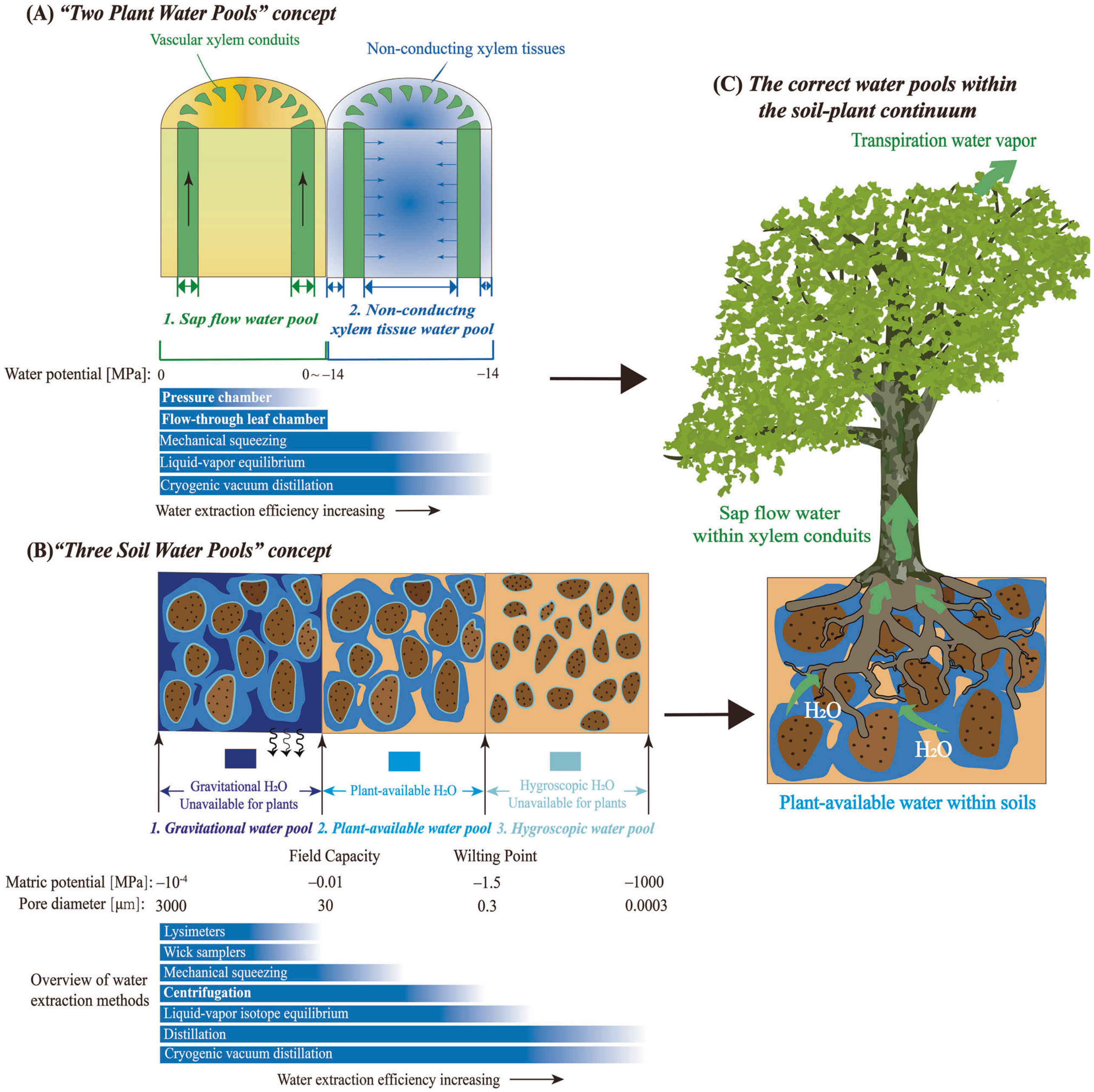

Fig. 1 | Conceptual representation of water pools within the soil–plant continuum and associated water extraction methods. A The "two plant water pools" concept; **B** The "three soil water pools" concept;, **C** The correct water pools within the soil-plant continuum deriving from diverse flow heterogeneities. The water extraction methods and their corresponding extraction range concerning water potential, pore diameter, and different water pools. The soil water extraction methods, along with their corresponding ranges of matric potential and pore diameter, were adapted from Sprenger et al.[60]. The "two plant water pools" concept refers to the sap flow water pool within xylem conduits and surrounding non-conducting xylem tissue water. The "three soil water pools" concept refers to gravitational soil water, plant-available soil water, and hygroscopic soil water. The plant-available soil water pool and the sap flow water pool within plant xylem conduits are recognized as appropriate water pools for isotope-based ecohydrological investigations.

distillation methods, such as azeotropic distillation and cryogenic vacuum distillation, were initially designed to extract bulk soil water (i.e., all three soil water pools)[60,76]. When gravitational water is present, these methods may struggle to accurately isolate the plant-available soil water pool. Conversely, when soil is at or below field capacity (i.e., lacking gravitational water), the extracted water may include both plant-available soil water and hygroscopic soil water. It is worth noting that the CO_2/hydrogen equilibration method, which typically captures soil water within a potential range of −0.01 to −1 MPa, may effectively exclude hygroscopic water and instead separate the plant-available water pool when gravitational soil water is absent[77,78]. In addition, to identify isotopic signatures of the plant-available soil water pool, some existing hydroponic- and sandy-soil-potted experiments have established well-mixed water conditions by using hydroponic water or, in soil experiments, achieving near-complete replacement of soil water with irrigation water[17,18,20,24,26,41,79,80]. They simultaneously prevent evaporation-derived water isotopic heterogeneities within possible water sources for plants. These potted experiments can offer precise and reliable isotopic characteristics of the plant-available soil water pool. However, many soil- and plant-isotope studies conducted under natural conditions remain unresolved. Over the recent decade, in situ measurements based on liquid-vapor isotope equilibrium have been recognized as a consistent, integrated (soils and plants) methodology to address these issues[81–83]. This approach has been used to target the gravitational water fraction held at relatively low tensions, typically within a water potential range of approximately 0 to −10 MPa[81,82,84,85]. Notably, it can also differentiate the plant-available water pool in the absence of gravitational soil water. Nevertheless, accurately identifying the exact water potential threshold at which vapor–liquid isotopic equilibrium occurs remains a challenge. Centrifuge approaches, with rotation speeds up to 14,000 rpm, have been documented to extract the plant-available water pool within a certain range of soil water potential by controlling centrifugation speed[60], indicating that the plant-available water pool can be separated from the bulk soil water[20,30,86]. However, this requires detailed site-specific soil properties, including soil water potentials at field capacity (the upper limit of plant-available water) and permanent wilting point (the lower limit of plant-available water), for heterogeneous soil profiles. Until now, only a few studies have successfully separated the exact plant-available water pools from bulk soil water using the centrifugation method[10,29]. Here, we emphasize the importance of moving toward community-accepted standardization, such as the potential use of centrifugation, the CO_2/hydrogen equilibration method, and in situ measurements based on the liquid-vapor isotope equilibrium, while acknowledging that many methodological details remain unresolved[10,20,29,71].

A new "two plant water worlds" concept within plants

Evidence of heterogeneous water flows within plants dates back to 2010[21,38,87,88]. These pioneering studies showed that the isotopic signatures of sap flow water within conductive xylem conduits and the steady-state transpiration vapor remain consistent with the designed water sources of plants, whereas bulk xylem water does not show such consistency[10,20,21,26,79,80]. Upward sap flows from roots to leaves are driven by the passive transpiration process, which is tightly controlled by the energy difference between soil water with a higher potential and water in the atmosphere with a lower potential[89–92]. These sap flows that support transpiration loss have been documented to account for more than 95% of total plant water uptake[93–96] and are able to stably reflect the isotopic composition of source water due to the well-mixed, homogeneous water pool within xylem conduits[10,20,21]. In addition to the xylem sap flow water, three other non-conducting xylem tissue water pools might also exist within woody stems, including intracellular water within living cells (such as growing xylem cells, phloem, and parenchyma), capillary water within apoplastic intercellular spaces between xylem cells, and fiber water within cell walls[97,98]. Both biological processes, including downward transport of enriched leaf water, radial water transport across different membrane boundaries, and internal water residence time, and environmental cues, have been recognized as critical factors in isotopic heterogeneities within woody stems[10,20,69,99,100]. Nevertheless, the sap flow within xylem conduits for transpiration would not displace or dilute non-conducting xylem tissue waters[20,21]. To ensure isotopic consistency during plant water uptake and transport in future isotope-based ecohydrological investigations, a simplified "two plant water pools" concept is proposed in this study. This concept highlights the isotopic heterogeneities between non-fractionated sap flow water directly used for transpiration and fractionated non-conducting xylem tissue water used for plant growth and metabolism. In the meantime, this concept simplifies the isotopic heterogeneities between various non-conducting xylem tissue water pools, integrating them into a unified fractionated non-conducting xylem tissue water pool. This framework enables a clearer distinction between sap flow water and bulk xylem water in isotope-based ecohydrological studies.

Thus far, the concept of sap flow water within plant xylem conduits has not yet been widely adopted as a standard water pool in isotope-based ecohydrological studies[10,101]. Most previous studies have relied on bulk woody xylem water extractions, which can obscure isotopic heterogeneities between sap flow water and surrounding xylem tissue water. While these pools may equilibrate over longer time scales[102], treating them as a single isotopic reservoir under dynamic conditions may compromise the integrity of δ^2H interpretations related to root water uptake and internal transport, potentially leading to confounded or misleading conclusions. The absence of a standardized plant water extraction method may hamper robust, spatiotemporal examinations of δ^2H offsets between plants and source waters[20,41,103]. We emphasize this not to discredit previous approaches, but to highlight a promising direction for improving the accuracy and interpretability of isotope-based ecohydrological studies. The biological water potential across plant organs varies widely in response to factors, such as soil water availability, atmospheric water status, plant species, light intensity, and physiological characteristics[20,104–107]. The upper and lower thresholds of the sap flow water pool vary dynamically across temporal and spatial scales (Fig. 1A), making it difficult to establish a universal extraction criterion. Therefore, accurately and quantitatively separating the "two plant water pools"—sap flow water within xylem conduits versus surrounding non-conducting xylem tissue water—through the plant water potential method remains a significant technical challenge[108,109]. For example, distillation-based methods may often extract from both water stores, including sap flow water pool within plant xylem conduits and non-conducting xylem tissue water[10,20,26,86,110]. Recently, some studies applied a constant empirical value in plant water potential to separate sap flow water pool from bulk xylem water using a centrifuge or pressure chamber[20,41,71,86]. While this approach offers a practical approximation, it does not fully capture the dynamic nature of plant water potentials, which vary over time, across species, and in response to environmental conditions[111]. As a result, using a fixed threshold may not accurately isolate the entire sap flow water pool, potentially leading to partial or inconsistent extractions across different temporal and spatial contexts. Our previous study[10] proposed that the most mobile part of the sap flow water pool, which is preferentially released from bulk xylem water pools during water extraction, can represent the entire sap flow water pool in isotope-based ecohydrological investigations due to the well-mixed, isotopically homogeneous water pool within xylem conduits. It is worth noting that as little as 0.5 mL of sap flow water is sufficient for stable isotope analysis. Consequently, the most mobile fraction of xylem sap flow water, amounting to just 0.5 mL and under the lowest tension, could serve as a representative proxy for the entire sap flow water pool. However, its small volume makes it more susceptible to evaporative enrichment and more difficult to isolate accurately. Direct water vapor equilibration and in situ measurements based on liquid-vapor isotope equilibrium were applied to collect sap flow water at lower tension within a water potential range of approximately 0 to −10 MPa[81,82,84,85]. However, it remains uncertain whether the water potential threshold at which vapor–liquid isotopic equilibrium occurs aligns precisely with, or falls within, the threshold for xylem sap flow, given the dynamic nature of plant water potentials. This raises an important question: to what extent can vapor equilibration-based methods reliably isolate the true sap flow water pool without inadvertently integrating woody non-conducting xylem tissue water? Additionally, flow-through chambers have been widely recognized as a direct method for quantifying the stable isotope composition of plant transpired water vapor, which is a powerful indicator of the whole sap flow water pool within the xylem conduit[38,87].

Midday plant water potential reflects the peak tension exerted by plants to access water under maximum transpiration demand and thus serves as a physiologically meaningful upper limit for identifying the portion of soil water accessible to plants[112]. However, operationalizing this conceptual threshold requires detailed site-specific information, including soil water retention curves to accurately link plant water potentials to corresponding soil matric potentials and pore size distributions. In heterogeneous field conditions, particularly in soils with layered textures, high gravel content, or variable structures, such information is often incomplete or uncertain. Additionally, midday plant water potential exhibits substantial variability across species, ecosystems, and climatic conditions, complicating the development of a universally applicable protocol. Despite these challenges, we emphasize that aligning soil water extraction thresholds with the measured plant water potential represents a critical advancement toward integrating plant physiological function with hydrological process understanding. As sensor technologies, in situ monitoring techniques, and modeling frameworks improve, future research will enable the development of standardized yet adaptable methodologies for isolating physiologically meaningful water pools in both soils and plants. Such approaches could significantly improve the accuracy and comparability of isotope-based ecohydrological studies.

Examining δ^2H offsets using the new framework

To address the conflicting δ^2H offset observations and test our key hypothesis—that δ^2H offsets do not occur when correct water pools are used

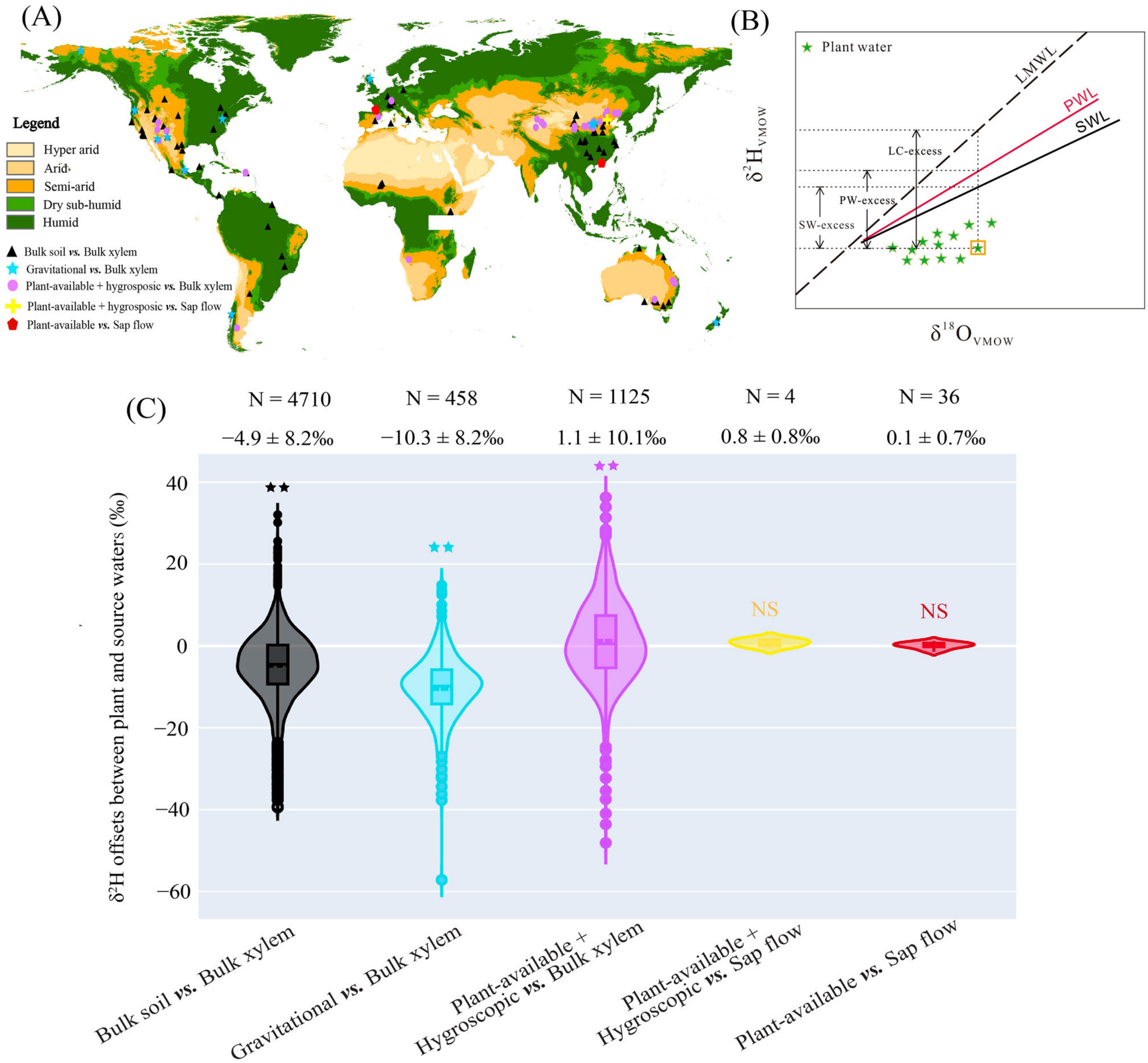

Fig. 2 | The re-quantified δ^2H offsets between plant water and "possible source" water line (PWL) across 212 globally distributed field sites sourced from 110 published studies. **A** The global distribution of past research sites in dryland and non-dryland ecosystems is evaluated here. The aridity index (AI), defined as the ratio of precipitation to potential evapotranspiration, was used to classify climate zones as follows: hyper-arid ($AI < 0.03$), arid ($0.03 \le AI < 0.2$), semi-arid ($0.2 \le AI < 0.5$), dry sub-humid ($0.5 \le AI \le 0.65$), and humid ($AI > 0.65$). The AI data and base map were derived from the Version 3 product of the global aridity index and potential evapotranspiration (ET0) Database[116]. Sites overlap due to multiple samples or water extraction methods in a small area. **B** Schematic diagram of the quantification method of PW-excess, SW-excess, and LC-excess. The line-condition excess (LC-excess) describes the δ^2H offset of the water samples from the local meteoric water line (LMWL)[117]. The SW-excess is defined as the δ^2H offset between plant water and the soil water line (SWL)[22]. The PW-excess is proposed to quantify the δ^2H offsets between plant water and the PWL[48]. PW-excess could provide more accurate information on isotope offsets between plants and source waters because PWL considers more possible water sources that plants use than both LMWL and SWL. **C** Violin plots of the distribution of five-scenario δ^2H offsets considering different water pool combinations within soils and plants. Violin widths represent kernel density distributions. Box plots within violins show means (centre lines), medians (two square dots), interquartile ranges (boxes) and whiskers extending to 1.5 times the interquartile range, and outliers (points). Values above each violin indicate sample size (*N*) and mean ± standard deviation. "**" above each violin represents there is significant difference between δ^2H offsets and zero ($p > 0.05$), while "NS" indicates offsets not statistically different from zero ($p > 0.05$). δ^2H offsets in the "bulk soil vs. bulk xylem" scenario represent δ^2H offsets between the PWL based on bulk soil water (i.e., three soil water pools) and bulk woody xylem water (i.e., two plant water pools). δ^2H offsets in the "gravitational vs. bulk xylem" scenario represent δ^2H offsets between the PWL based on gravitational soil water and bulk woody xylem water. δ^2H offsets in the "plant-available + hygroscopic vs. bulk xylem" scenario represent δ^2H offsets between the PWL based on the combination of plant-available and hygroscopic soil water (i.e., two water pools including plant-available soil water and hygroscopic soil water) and bulk xylem water. δ^2H offsets in the "plant-available + hygroscopic vs. sap flow" scenario represent δ^2H offsets between the PWL based on the combination of plant-available and hygroscopic soil water and sap flow water. δ^2H offsets in the "plant-available vs. sap flow" scenario represent δ^2H offsets between the PWL based on plant-available soil water and sap flow water.

Table 1 | Summary of soil and plant water pools and associated extraction methods

	Water worlds/pools	Water extraction method	Specific conditions	Number of sites	Proportions of all sites (%)
Soil	Gravitational soil water	Suction lysimeters	With a max tension of 50–70 kPa	14	6.6
		Passive-wick soil water samples	–	1	0.47
	Bulk soil water	Cryogenic vacuum distillation	Gravitational soil water is available	144	67.92
		Azeotropic distillation		11	5.19
		Direct liquid-vapor equilibrium method		4	1.89
	Plant-available + hygroscopic soil water	Cryogenic vacuum distillation	Gravitational soil water is not available	34	16.04
		Azeotropic distillation		2	0.94
	Plant-available soil water	Cryogenic vacuum distillation	Well-mixing and homogenous soil water in soil-potted experiments	2	0.94
		Centrifuge set with a certain range of soil potential	Suitable for both field and potted conditions	None	–
		CO_2/H_2 equilibration method	Gravitational soil water is not available	–	–
Plant	Bulk xylem water	Cryogenic vacuum distillation	–	207	97.64
		Azeotropic distillation	–	2	0.94
	Sap flow water	A high-speed centrifuge equipped with a cavitation flow rotor and custom-made sap flow water collectors	The empirical threshold between sap flow water and non-conducted tissue waters (−2 MPa for *F.sylvatica* branches) is provided	1	0.47
		A flow-through whole-plant chamber coupled with a water vapor isotope ratio infrared	–	1	0.47
		Scholander pressure chamber	The sap flow water with a total of 0.5 ml is collected	1	0.47

This table summarizes different types of water pools within soils and plants identified using various water extraction methods, based on data from 110 published studies spanning 212 field sites worldwide.

in isotope studies—we conducted a global synthesis to quantify the magnitude of isotope offsets between plants and possible water sources (Fig. 2A). We also examined the extent to which these offsets are caused by both methodological artifacts and improper water pool extractions, which are supposed to be deceptive and eliminable. Our data synthesis shows that the isotope signatures of groundwater, four combinations of the three soil water pools, and two combinations of the two plant water pools have been documented (Supplementary Information Fig. 1). Specifically, the global isotopic datasets consist of hydrogen (^{2}H/^{1}H) and oxygen (^{18}O/^{16}O) isotope ratios for bulk soil water ($n = 6430$), gravitational soil water ($n = 539$), the combined plant-available and hygroscopic soil water ($n = 2034$), groundwater ($n = 695$), plant-available soil water ($n = 13$), xylem sap flow water ($n = 20$), and bulk xylem water ($n = 5759$) across 212 globally distributed field sites (Supplementary Information Fig. 1). Globally, gravitational soil water and groundwater plot approximately along the global meteoric water line (GMWL) (Supplementary Information Fig. 1B, D). While the bulk soil water, the combined plant-available and hygroscopic soil water, and bulk xylem water plot both along and below the GMWL (Supplementary Fig. 1A, C, F). The plant-available soil water matched well with the xylem sap flow water (Supplementary Information Fig. 1E). Ultimately, a dataset comprising 6,333 PWL-based δ^{2}H offsets was recalculated and categorized according to distinct water pools extracted from bulk soil and plant xylem waters, based on the proposed framework of "three soil water pools" and "two plant water pools" (Fig. 2 and Table 1).

Based on our global data synthesis, a total of five δ^{2}H offset scenarios of different water pool combinations within soils and plants have been categorized to quantify the magnitude of δ^{2}H offsets (Fig. 2): (1) bulk soil water vs. bulk xylem water, (2) gravitational soil water vs. bulk xylem water, (3) combined plant-available and hygroscopic soil water vs. bulk xylem water, (4) combined plant-available and hygroscopic soil water vs. sap flow water, and (5) plant-available soil water vs. sap flow water. Our findings showed that the δ^{2}H offsets between bulk xylem water and the PWL based on bulk soil water (-4.9 ± 8.2‰), between bulk xylem water and the PWL based on gravitational soil water (-10.3 ± 8.2‰), and between bulk xylem water and the PWL based on the combination of plant-available and hygroscopic soil water (1.1 ± 10.1‰) were significantly different from zero ($p < 0.01$, Fig. 2C). In contrast, the mean δ^{2}H offsets between sap flow water and the PWL based on the combination of plant-available and hygroscopic soil water (0.8 ± 0.8‰, $p = 0.14$), as well as between sap flow water and the PWL based on plant-available soil water (0.1 ± 0.7‰, $p = 0.62$) are close to zero (Fig. 2C and Table 2). The observed differences in δ^{2}H offsets across the five scenarios with distinct water pools suggest that improper water-pool extractions or mixing water-pool measurements can result in significant, highly variable δ^{2}H offsets (Fig. 2C). It is worth noting that δ^{2}H fractionation induced by methodological artifacts during cryogenic vacuum distillation extraction processes could also contribute to δ^{2}H offsets, potentially amplifying the effects of mixed water-pool measurements. Nevertheless, our results reveal a more pronounced negative δ^{2}H offset associated with gravitational soil water pools compared to both bulk soil water pools and the combined plant-available and hygroscopic soil water pools ($p < 0.05$, Fig. 2C). This suggests that improper collection of gravitational soil water pools likely play a more significant role in driving substantial δ^{2}H offsets, compared to δ^{2}H fractionation induced by methodological artifacts. In contrast, the inappropriate collection of the hygroscopic soil water pool has a smaller effect on δ^{2}H offsets, which may be attributed to the smaller volume of hygroscopic water within the soil profile[113]. The average δ^{2}H offset between sap flow water and the PWL, based on the plant-available soil water, exhibited the minimal value (0.1 ± 0.7‰) with the least deviation among all five scenarios and showed no statistically significant difference from zero[20,26]. This result supports our key hypothesis that no δ^{2}H offset arises when appropriate extraction methods and accurate water pools are used. We argue that accurate separation of the correct water pool from bulk water pools within soils or plants, using appropriate extraction methods, is key to reconciling the observed contradictory δ^{2}H offsets.

The results of two potted experimental studies[20,26] that reported similar hydrogen isotope signatures for the plant-available soil water pool and the sap

Table 2 | The δ^2H offsets between plant-available soil water and sap flow water in controlled experiments

Species	Growth form	δ^2H_plant-available soil water	δ^2H_ sap flow water	δ^2H offsets
Atriplex canescens	Evergreen shrub	−41.4 ± 0.2	−41.6 ± 0.2	−0.2 ns
Populus euphratica	Deciduous tree	−41.1 ± 0.1	−41.9 ± 0.5	−0.8 ns
Tamarix chinensis	Deciduous shrub	−41.6 ± 0.4	−41.3 ± 0.1	+0.3 ns
Aegicerascorniculatum	Evergreen mangrove	−36.9 ± 0.3	−36.7 ± 0.2	+0.2 ns
Avicennia marina	Evergreen mangrove	−31.7 ± 0.1	−31.2 ± 1.1	+0.5 ns
Kandelia obovata	Evergreen mangrove	−32.9 ± 0.8	−33.1 ± 1.1	−0.2 ns
Eucalyptus robusta	Evergreen broadleaf tree	−42.7 ± 0.4	−41.7 ± 0.3	+1.0 ns
Ficus microcarpa	Evergreen oblanceolate leaf tree	−43.0 ± 0.4	−42.3 ± 0.4	+0.7 ns
Taxodium distichum	Deciduous tree	−37.4 ± 0.2	−38.8 ± 0.3	−1.4 ns
Fagus sylvatica L.	Deciduous tree	−38.3	−37.5	+0.8 ns
		−38.7	−37.5	+1.2 ns

This table summarizes δ^2H offsets between plant-available soil water and sap flow water within xylem conduits, based on two soil-potted experimental studies[20,26].
The presence of "ns" indicates that the value of δ^2H offsets is not significantly different from zero.

flow water pool further support the hypothesis of the absence of inherent δ^2H offsets. In addition, a field-grown riparian Populus euphratica[21], reliant solely on groundwater, provides evidence that its sap flow water aligned consistently with its exclusive water source-groundwater. Despite these encouraging findings, the vast majority of published studies to date—98.3% of the 110 studies we reviewed (see Table 1)—have employed water-sampling methods unlikely to reliably isolate the water pools most relevant to transpiration. These legacy approaches, while foundational to the development of ecohydrological investigations, often relied on extraction techniques that do not discriminate against different water pools in either soil or plant tissues. As a result, our global data synthesis and δ^2H offset re-quantifications cast doubt on the reliability of prior interpretations of the global and seasonal patterns of critical ecohydrological pathways. For instance, many recent ecohydrology studies used isotopic differences between gravitational soil water (or stream water) and bulk woody xylem water in dual-isotope space (δ^2H vs. δ^{18}O) to confirm the "ecohydrological separation" hypothesis between gravitational soil water recharging to open channels and plant-available soil water. Indeed, water flow heterogeneities (i.e., our "two plant water pools" concept) within plants can lead to substantial isotopic differences between bulk xylem water and unfractionated sap flow water. Consequently, inferences drawn from the "ecohydrological separation" hypothesis are generally confounded. Therefore, there is an urgent need for a framework that integrates recent technical innovations and physiological evidence to advance community-accepted standardization extraction approaches and more accurately characterize plant water-use patterns. Our work will help guide the ecohydrological community toward more comparable isotope-based investigations by highlighting the limitations of past approaches and identifying best practices for future sampling.

A key limitation of our analysis is the limited number of studies that meet the ideal sampling criteria necessary to distinguish the proper water pools in both soils and plants. Among the existing literature, only a few studies—primarily those involving potted plants—have accurately captured the isotopic signatures of the relevant water pools. However, this scarcity of ideal datasets is not a shortcoming of the conceptual framework we propose, but rather a reflection of the current state of ecohydrological research. This lack of suitable data underscores the urgent need for more physiologically and hydrologically rigorous sampling approaches. Our intention is not to overstate conclusions based on limited data, but to highlight and promote emerging best practices that are foundational for improving future ecohydrological investigations. In addition, we recognize that our reliance on publicly available datasets, originally collected under heterogeneous methodological assumptions and technical constraints, introduces inherent limitations and variabilities. Nonetheless, this data-driven synthesis serves as a critical first step in advancing the field toward a more mechanistic and ecologically meaningful understanding of plant–soil water interactions. By identifying methodological gaps and proposing pathways for improved sampling, we aim to conduct further research that can validate and refine this framework under field-relevant conditions.

Conclusions

Recent observations revealed significant differences in δ^2H values between plants and source waters (termed δ^2H offsets), with a large range of −57.2‰ to 36.3‰. These challenge the fundamental assumption that no isotope fractionation occurs during plant water uptake and transport or during subsequent water-extraction processes. In addition, it is unclear what causes substantial δ^2H offsets and whether they can be eliminated. To account for the isotopic heterogeneities of soils and plants, we propose a water potential-derived framework of the "three soil water pools" concept and the "two plant water pools" concept. This proposed framework will aid in accurately separating the plant-available water pool from bulk soil water, and the sap flow water pool from bulk woody xylem water, which is key to eliminating the "deceptive" δ^2H offsets. We hypothesize that observed δ^2H offsets are likely due to inaccurate δ^2H signature collection caused by methodological artifacts or improper water-pool extraction, rather than δ^2H fractionation during plant water use. To test this, we used 110 published studies across 212 globally distributed field sites to re-quantify 6,333 measurements of δ^2H offsets between plant water and the "possible-source" water line, a linear regression line that integrates isotopic characteristics of all water sources for different combinations of three soil water pools. The δ^2H offsets between sap flow water and "possible-source" water line amount to 0.1 ± 0.7‰ and show no statistical difference from zero ($p > 0.05$). Therefore, our global literature synthesis and δ^2H offset re-quantifications showed that δ^2H offsets would be absent when appropriate extraction methods and accurate water pools were used, supporting our hypothesis. Accurately separating the correct water pools from bulk water pools in soils and plants using appropriate extraction methods is key to reconciling the observed contradictory δ^2H offsets. This framework, which provides a critical lens for interpreting plant-soil water heterogeneity, will help guide the ecohydrological community toward more reliable and comparable isotope-based investigations by highlighting the limitations of past approaches and identifying best practices for future sampling. Our findings are crucial for enhancing our capacity to understand and predict the vegetation responses and feedback to future global water fluxes.

Methods

We built a database from peer-reviewed journal articles published in English during the last four decades (i.e., from 1985 to 2024) using the search engines Web of Science and Google Scholar using "(water AND isotop*) AND (plant OR tree OR vegetat*) AND (dual OR (hydrogen AND oxygen))" as the search query, to ensure that we include all studies using isotopes to determine plant water sources. We selected studies that report (1) dual-isotope values for both plant and source waters, (2) sufficient source water data ($n \geq 3$) to fit a PWL, and (3) soil water status to diagnose different water worlds within soils.

Halophytic species living in saline environments were excluded from the dataset to avoid the influence of δ^2H fractionation induced by the symplastic root water uptake pathway associated with salt tolerance[17,18]. Data were derived directly from tables or figures using the GetData Graph Digitizer software v2.26 (http://getdata-graph-digitizer.com/download.php). The data collection benefited from recent isotope data synthesis efforts to investigate the plant groundwater use and isotope separation between plant transpiration and groundwater/streamwater[4,114,115]. It is noted that the measurement precision for δ^{18}O and δ^2H has not changed significantly since the 1980s, even though measurement speeds are much faster, so earlier publications will not affect the synthesis results.

A total of 110 published studies across 212 globally distributed field sites have been synthesized in this study (Supplementary Information References). The site-specific information, such as latitude, longitude, mean annual precipitation, mean annual temperature, potential evapotranspiration, ecosystem type, climate zone, groundwater level, the range of soil water content, soil bulk density, soil texture, soil water potential, soil water extraction methods, soil depths, plant species, plant water extraction methods, and stable isotopes of various source waters and plant waters was recorded if they are available. The re-classification of three soil water pools and two plant water pools was carefully examined in this study based on the following categorical variables derived from the literature: (i) climatic conditions (e.g., drylands and non-drylands based on the aridity index, the ratio between potential evapotranspiration and precipitation), (ii) groundwater levels, (iii) soil water conditions (e.g., soil depths, soil water contents, soil water potentials, and soil textures), (iv) water extraction methods, and (v) environmental conditions (e.g., hydroponic-potted or sandy soil-potted experiments and natural field experiments). Gravitational soil water would be considered to be available in the site if one of the below situations occurs in the literature: (a) the maximum soil water content within soil profiles is more than the field capacity; (b) irrigation or preferential flow exists; (c) soil water potential exceeds the field capacity threshold; (d) groundwater is regarded as one of the potential water sources for plants; (e) in riparian zones; and (f) gravitational soil water have been extracted and their corresponding isotopic compositions are available. We classified the soil water isotope dataset at the site as representing the "bulk soil water pool" when they were obtained using distillation- or equilibrium-based extraction methods, and when evidence of gravitational soil water was concurrently reported at the same site. Accordingly, we acknowledge that some datasets categorized as representing "bulk soil water pool" in our global synthesis, particularly those collected from different soil depths at the same site, may reflect mixtures of the three conceptual soil water pools (gravitational, plant-available, and hygroscopic soil water), or subset thereof (the combination of plant-available and hygroscopic soil water pools, or the combination of gravitational and plant-available soil water pools).

The "possible-source water excess" (PW-excess), introduced in our previous studies[5,10,48], is utilized in this study to quantify the δ^2H offsets between plant water and the PWL (Fig. 2B). It is defined as PW-excess = $\delta^2\mathrm{H}-a_p\delta^{18}\mathrm{O}-b_p$, where a_p and b_p are the slope and intercept of the PWL determined by a linear regression fit through all possible source waters for different combinations of three soil water pools. We used all available source water data from each study during one growing season to construct the PWL. In short, PW-excess (i.e., δ^2H offsets) is calculated as the vertical deviation of the plant water's isotope value from the PWL (i.e., how far the plant's δ^2H falls above or below the source-water line). PW-excess offers more accurate quantifications of δ^2H offsets between plants and source waters, as the PWL accounts for all possible water sources utilized by plants compared to the local meteoric water line and soil water line typically employed in previous studies[20,22,27,48] (Fig. 2B). We consider different combinations of soil water pools at varying depths as potential sources of water for plant uptake. Additionally, alternative sources such as groundwater, spring water, fog, and dew are considered possible sources, provided their use by plants has been documented in previous studies. In this study, 9.4% of the potential source water isotope datasets had a coefficient of determination (R^2) below 0.5 in the linear regression analysis. To improve the model fit for these datasets, non-linear regression models, specifically, quadratic and cubic equations, were subsequently applied. We compared the performance of linear and non-linear models by examining R^2 values; when R^2 values were comparable, model selection was further guided by the Akaike information criterion (AIC), a widely used metric that balances model fit and complexity. In cases where non-linear models showed a markedly improved R^2 or lower AIC value, we ultimately evaluated differences in the estimated δ^2H offsets by comparing their means and standard deviations between linear and non-linear regression models. This comparison revealed that non-linear regressions often produced higher average δ^2H offsets and greater variability, suggesting that these models may overfit the data in certain cases, particularly when driven by outliers or noise rather than true curvature in the isotopic relationship. Consequently, while non-linear models may capture some complex dynamics, caution is warranted in their application, and linear models may offer more robust and interpretable estimates in δ^2H offset datasets with limited sample sizes or high variance. The underlying compiled dataset is publicly available via Figshare (see Data availability). The one-way analysis of variance (ANOVA) was applied to examine differences between PWL-based δ^2H offsets and zero. All statistical analyses were conducted using Microsoft Excel (v2016) and R (3.4.1).

Data availability

The datasets supporting the findings of this study are publicly available in the Figshare repository at https://doi.org/10.6084/m9.figshare.31076299. Supplementary Information references provide a complete list of the 110 published studies synthesized in this study.

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Author contributions

Yue Li and Lixin Wang designed the research, synthesized the database, conducted the analysis, and wrote the first draft. Stephen P. Good contributed ideas and revised the draft. All authors contributed to the writing and reviewing of the paper.

Competing interests

The authors declare no competing interests.

Additional information

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