Revisiting ecohydrological separation

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Brief Communication

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Revisiting ecohydrological separation

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Abstract

The ecohydrological separation (EHS) paradigm, frequently compared with the translatory flow (HH), opens up a new understanding of terrestrial water cycles. Recent finding of cryogenic vacuum extraction (CVE) bias and associated correction no longer supports the EHS but goes back to HH. However, we found systematic similarities in plant and soil water lines for a global database, whereby the CVE-bias correction may not shift the EHS and/or HH.

Keywords: cryogenic vacuum extraction; ecohydrological separation; translatory flow; uncertainties

There are two frequently discussed isotope-based paradigms: a) "ecohydrological separation (1; henceforth EHS)", which states vegetation and streams return different pools of water to the hydrosphere, and b) "translatory flow" by Hewlett and Hibbert (2; henceforth HH), the process through which soil water is rapidly displaced through piston-flow by new precipitation infiltration and later contributes to streamflow. Differentiating EHS and HH paradigms is critically important to understand the underlying processes of the terrestrial water cycles, e.g., by determining plant water sources and elucidating soil water transport and mixing dynamics.

Both paradigms were derived from the isotope-based data (^{2}H, ^{18}O) that relied mainly on the decade-old cryogenic vacuum extraction (CVE) for water extractions from soils and plants. However, Chen and colleagues (3) found a large deuterium bias in tree water obtained from the "gold-standard" CVE method (i.e., CVE-bias). They further proposed a $\delta^{2}H$ offset to correct the CVE-bias inherited from a global meta-analysis by Evaristo et al. (4) (74% CVE-based dataset; 1,079 out of 1451 samples) that the EHS was generalized as a global phenomenon, following up the findings of Brooks et al. (5). Ultimately, they found that the correction data no longer supported the EHS but HH. Consequently, many water isotope-based studies have refocused on reducing CVE-bias for (dis)proving EHS and/or HH (6-9).

Chen et al. (3) was highly appraised for critical "missing piece" finding of hydrogen exchange during the extraction process (i.e., CVE-bias); however, the resulted paradigm-shifting from EHS to HH is not reasonable. Their suggested correction factor may not be the same for different plants and their stem water contents (6; which will be discussed later). Likewise, their direct finding of CVE-bias may not be the only cause for $\delta^{2}H$ offsets between plant water and its source waters. For instance, when only the sap water was sampled—which excluded the nonconductive xylem water— the previous δ^2H offset had disappeared (7). Besides, the EHS and/or HH paradigms may not be directly comparable (8). Neither HH nor EHS describes whether incoming precipitation mixes thoroughly with pre-event soil water. The partial displacement of pre-event soil water by incoming new precipitation (HH paradigm) is entirely consistent with the EHS: Vegetation may access immobile soil water, which is not mixing and is isotopically different from mobile soil water (8). Consequently, while the EHS may still withstand closer scrutiny, there is cause to question whether, and if so, how to correct the "isotopic separation" patterns in the original publication (4).

For clarity, we define the EHS as 1) mobile and immobile water are isotopically different from one another ('hydrological separation' or subsurface water compartmentalization); and 2) plants primarily take up immobile soil water ('ecological separation' or plant water selection). Based on our conceptual diagram (Fig. 1), Types 2 and 4 water pools relate to ecological and hydrological separation (i.e., plants primarily use immobile or mobile water). However, only type 2 fulfills the definition of EHS as the Type 4 means plants primarily use mobile soil water (e.g., in the riparian environment). Therefore, only type 2 must undergo data correction to evaluate whether uncertainties are large enough to shift the plant water line (ratio between $\delta^{18}O$ and δ^2H) closer to the mobile soil water line (i.e., affecting the EHS conclusion). The correction of types 1 and 3 are not associated with the EHS. Note that any correction of plant water isotope does not influence the HH since it fundamentally does not include plant water uptake (i.e., Type 1).

To confirm whether the EHS is biased from the CVE-based extraction techniques, we reanalyzed the "raw" data (4) by separating the whole dataset into two types of a) with and b) without CVE-based measurements and found that the averaged precipitation offsets illustrated systematic similarities in plant and soil water, regardless of the dataset (Fig. 2). Such proportionally isotopic differences in compartmentalized water pools excluded the effect of individual extraction techniques. Further comparisons of CVE and other extraction methods (e.g., azeotropic distillation) confirmed that the extraction methods were not statistically different (4). As such, except for a systemic bias identified throughout the dataset, it is puzzling why only the CVE-based dataset needs a bias correction, but the others do not.

Moreover, the suggested correction factor (3) does not change the statistical significance of compartmentalized water pools (i.e., the slope of the plant water line), except that the plant water isotopes would plot closer to the local meteoric water line (i.e., the intercept of the plant water line) (Fig. 3b). Therefore, this is not applicable for an unbiased assessment of the plant's source water used in Evaristo et al. (4). Similarly, another species-averaged correction factor (9) — which covered more diversified plant species (i.e., ~6.1‰)— also did not change the statistical significance (Fig. 3c). Only species/biome-differentiated correction factors (6) changed (Fig. 3d), indicating the "local/site-specific" correction function is needed for a potentially effective paradigm-shifting.

Indeed, a universal correction function might not exist. A typical argument of the EHS is its derivation was based on the "raw" meta-data (4) that bear substantial measurement uncertainties/bias under various locations, seasons, biomes and soils, and different water extraction techniques. Ultimately, differences in the seasonal depth of plant water uptake, active growing season, different rooting depth, and plant water storage types contribute to natural, regional disparities in the plant xylem's water isotopic composition. Besides, the CVE-based results have been largely affected by the residual water content, soil texture, effective pore pressure, temperature, wettability, tension, etc. (10). Such uncertainties call for a detailed soil wetting-drying and plant hydration-dehydration experiment to deliver the underlying mechanisms of CVE bias and ensure accurate correction.

A key to better coupled ecohydrological understanding would be elucidating the temporal dynamics of draining and refilling of soil water by plants. It is essential to know the sampled water status for soils, plants, or both in data correction or matching of water sources. Most of the extracted soil water inherits some CVE-bias, controlled by water-retention characteristics (WRC; 11), which databases are easily accessible globally. This implies that by establishing isotope-retention characteristics (12) as an analog to WRC, it might be possible to expand the CVE-bias correction extensively. Consequently, this water potential-based approach may provide the dearth of information needed to obtain accurate source/xylem water information.

Last but not least, Song et al. (13) showed the δ^2H in several water pools (ground, soil, and plant) are significantly different (Supp. Table 1), implying that all water is disconnected, i.e., neither HH nor EHS applies. Does not our statistical method rigorously work for those water sourcing analyses, e.g., identifying the common characteristics while excluding the individual differences? Otherwise, the mechanisms cannot be explicitly specified, provided that water mixes too irregularly such that the discoverable implication underscores the nature of water inextricably linking in an ecosystem.

Coincidentally, there are increasing reports that plant water isotope can not be ascribed to any potential water sources in a dual-space analysis (14). Beyond the arguments of isotopic fractionation during root water uptake, it was generally explained by mismatches such that partial sap water that used to identify the plant's source water (7), the CVE-based soil water cannot be distinguished between mobile and immobile fractions (10), and sampled pore spaces do not reflect the actual tension applied by plants (15). However, it was not necessarily to be. Such uncertainties may have prevented the plant water source analysis. Water isotopes have many advantages for tracing water sources (i.e., snapshots; "what and where" questions), whereas they are more complex and error-prone when reflecting spatio-temporal dynamic processes of water movements (i.e., slow motion; "when and why" questions). Ultimately, the water-isotope-based explanation, a more chemically-altered water source, has often been confounded by a more physical-based water mixing and/or cycling process.

It is still debatable whether a consistent and combined CVE-bias correction methodology exists, and further studies exploring the underlying mechanisms of how plants regulate the EHS from the subsurface to the atmosphere are needed. Clarifying the mechanisms of EHS requires detailed soil physical knowledge and concurrent in-situ monitoring of water isotopes combined with deuterium labeling approaches for process validation. Otherwise, plant water isotope studies should be optimally designed so that their conclusions will not be overly sensitive to known or suspected biases and uncertainties. We suggest the water potential-based approach (16) applied for the CVE-bias correction to reduce uncertainties for (dis)proving EHS and/or HH.

Materials and Methods

Source data used for calculating globally-averaged xylem water offset from precipitation (hereafter denoted by d_{xylem}) in Evaristo et al. (2015) were downloaded from https://www.nature.com/articles/nature14983#Sec11. We reanalyzed those "raw" data by separating the whole dataset into two types of 1) with and 2) without CVE-based water extraction techniques.

In Evaristo et al. (2015), d_{xylem} was calculated as:

$$d_{xylem} = [\delta^2H_{xylem} - a\,\delta^{18}O_{xylem} - b] / S \quad (1)$$

where a and b are the slope and intercept of LMWL respectively, and S denotes the measurement uncertainty for both δ^2H_{xylem} and $\delta^{18}O_{xylem}$, which according to Evaristo et al. (2015) is 1.02.

Out of 1,451 compiled data entries, 1,079 (i.e., comprising ca. 74% of the entire dataset) are involved in the use CVE method for determining xylem water isotope ratios. For each of these 1,079 CVE-based data, we corrected the d_{xylem} by taking assumed CVE-bias in δ^2H_{xylem} into consideration. The corrected d_{xylem}, hereafter denoted as d_{xylem_c}, can be described using the following equation:

$$d_{xylem\_c} = [(\delta^2H_{xylem} - \eta) - a\,\delta^{18}O_{xylem} - b] / S \qquad (2)$$

where η denotes the deuterium offset.

η is uncertain as a function of stem relative water content data, which is currently unavailable to determine each data point. Here we tested η with three types: a) the species-averaged wetness correction by Chen et al. (3) (i.e., ~8.1‰); b) a new species-averaged correction factor by Allen and Kirchner (9) which covered more diversified plant species (i.e., ~6.1‰); and c) biome-differentiated correction factors by Zhao (6) (i.e., from 6.1‰ to 10.1‰).

After data correction, a global average of d_{xylem} for every three types was recalculated and compared against groundwater, stream water, and soil water offset to re-evaluate whether EHS is widespread. These CVE-based correction data were further tested with data without CVE-based water extraction techniques to evaluate whether the CVE-bias is a systematic error. For better visualization, the dual-isotope space for each dataset was figured out. We also explored the relationship between water extraction techniques and found that the CVE and other extraction methods (e.g., azeotropic distillation) are not statistically different.

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Author contributions

Y.Z. and N.O. conceived the study. Y.Z. carried out the analyses. Y.Z., N.O., Y.W and J.L. contributed discussions. Y.Z. and N.O. wrote the article.

Competing interests

The authors declare no competing interests.

Figures

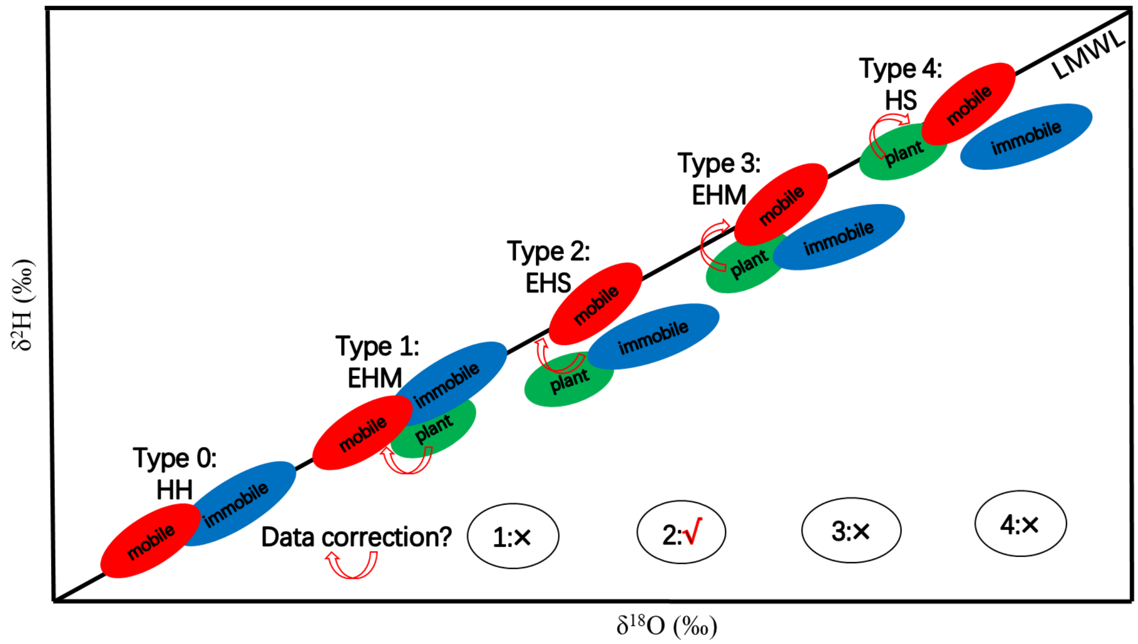

Fig. 1 | Conceptual diagram showing several positions of soil and plant water lines and the requirement of CVE-data correction. Red, blue, and green circles symbolize mobile and immobile soil water, and plant water, respectively; the global meteoric water line (GMWL) and a water line relate to ecological and hydrological separation. HH: translatory flow, EHM: not ecohydrological separation, EHS: ecohydrological separation, HS: hydrological separation. Whether a CVE-data correction is needed is indicated at the bottom of the plot.

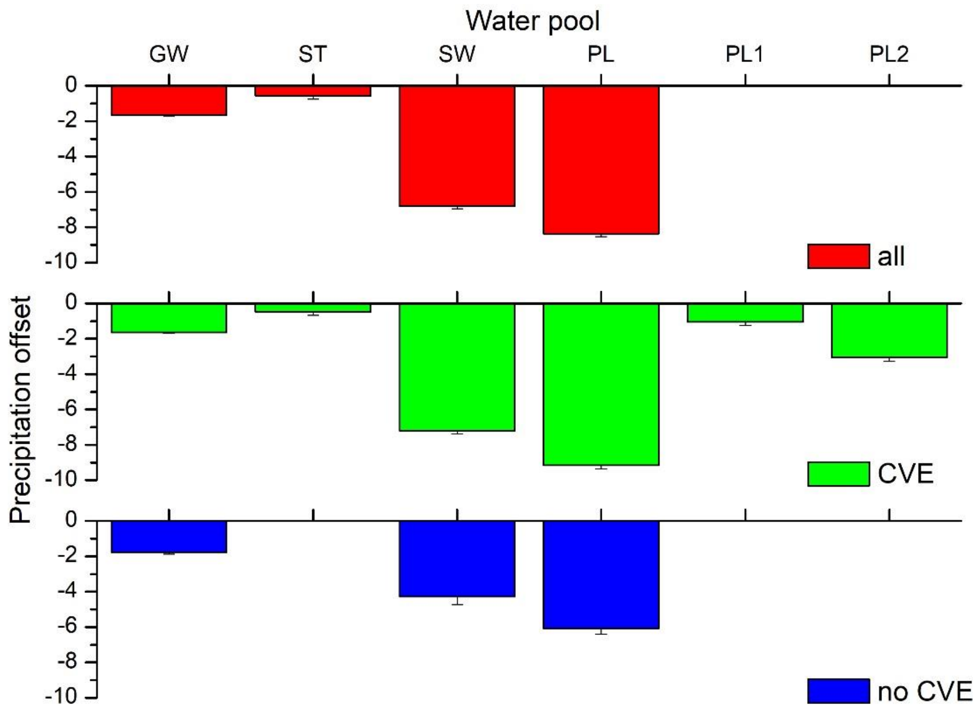

Fig. 2 | Globally averaged precipitation offsets of groundwater (GW as labeled in the figure), stream water (ST), **soil water** (SW), and **xylem water** (PL, original; PL1, Chen et al.'s (3); PL2, Allen & Kirchner's (9)) **with and without considering cryogenic extraction-caused bias under three types of datasets** (i.e., all, with and without the CVE-based measurements). The values presented were calculated from an extensive global compilation of data (4). Bars indicate the standard errors. Data is omitted for no CVE-based ST since only one data is in the statistics.

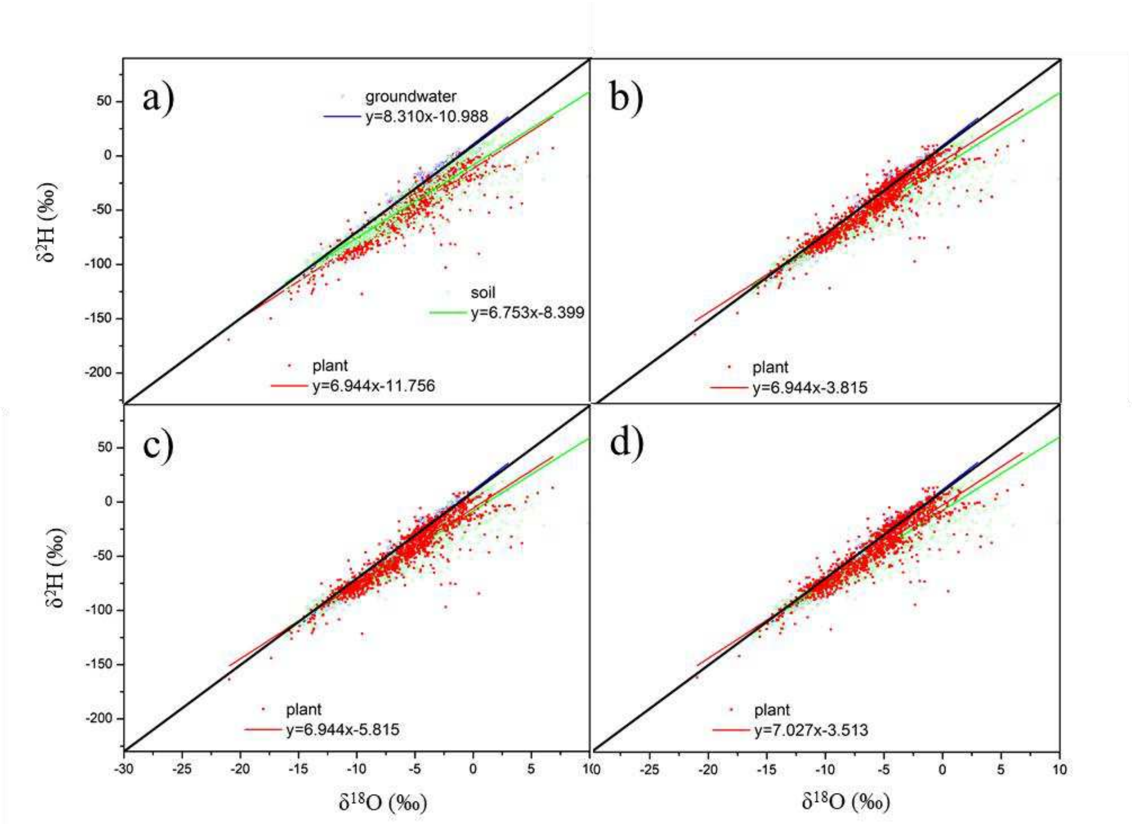

Fig. 3 | $\delta^{18}O$ and $\delta^{2}H$ values of groundwater, plant xylem water and soil water from the globally distributed sites (only where the CVE method was used) of Evaristo et al. (4) in comparison to the results before a bias-correction **a**) and after **b**) data bias-correction following Chen et al. (3), **c**) incorporating a bias-correction suggested by Allen & Kirchner (9), and **d**) incorporating a bias-correction suggested by Zhao (6). Also shown are the GMWL and the linear regression lines of each water pool in the subplots, respectively.

Supplement file (Referenced to Song et al., 2021 (13))**:**

Table 1. ***P* values for a nonparametric test (Kruskal–Wallis multiple comparison test; $\alpha = 0.05$) for differences among precipitation offsets of different water types in the globally compiled dataset in ref. 1**

	d_{stream}	d_{soil}	d_{xylem}	d_{xylem_c}
d_{gw}	<0.001	<0.001	<0.001	<0.001
d_{stream}		<0.001	<0.001	<0.001
d_{soil}			<0.001	<0.001
d_{xylem}				<0.001

Here d_{gw}, d_{stream}, d_{soil}, d_{xylem_c}, and d_{xylem} refer to precipitation offsets of groundwater, stream water, soil water, and xylem water with and without taking cryogenic extraction caused bias into consideration, respectively. Whole-dataset averaged offset value for each water type can be found in figure 5 in ref. 3.