Implication of stem water cryogenic extraction experiment for an earlier study is not supported with robust context-specific statistical assessment

Jaivime Evaristo^{a,1}⁽ⁱ⁾, Yusuf Jameel^b⁽ⁱ⁾, and Kwok P. Chun^c

Chen et al. (1) conclude that "the extraction errorcorrected result tends to nullify support for ecohydrological separation as a globally widespread phenomenon" based on an extrapolation of results from a carefully designed experiment under controlled conditions. The extrapolation was performed using global precipitation offset data (2) compiled from field-based studies that showed "widespread occurrence of ecohydrological separation." The precipitation offset describes the difference in the isotopic composition of groundwater, stream water, soil water, and xylem water from that of local (i.e., context-specific) precipitation, which has a precipitation offset of zero. However, the conclusion in Chen et al.'s reanalysis is not supported with context-specific statistical analysis, which, in turn, was the basis for the conclusion in the original study (2).

To establish support for Chen et al.'s (1) conclusion, we ran the same suite of statistical tests that were performed in the original study (2) after incorporating Chen et al.'s recalculated precipitation offset of xylem water that accounts for cryogenic vacuum distillation (CVD)-caused bias (d_{xylem_c}) (n = 1,079; see SI Appendix in ref. 1). We implemented these tests only for the subset of precipitation offset data where CVD was used (37 of 47 studies). Using the original precipitation offset of xylem water (d_{xylem}), we found that groundwater and stream precipitation offsets were statistically different from d_{xylem} in 29 of 37 studies (Table 1). Using Chen et al.'s $d_{xylem c}$, we found that groundwater and stream precipitation offsets were statistically different in 28 of 37 studies (Table 1). These results show that, when assessed on a per study basis, as the case was in ref. 2, Chen et al.'s conclusion with respect to ecohydrological separation is not supported. However, the distribution of $d_{xylem c}$ is different, which may call for rethinking of the original model (Fig. 1).

Notwithstanding, we underline two possible sources of uncertainty in Chen et al.'s (1) generalization. Firstly, of the nine species in Chen et al.'s experiment, only three species are present in the compilation of field-based studies that represented 109 species (2, 3). Secondly, the compilation of field-based studies represents a range of environmental conditions (climate, soil texture, etc.) that are not comparable to the sandy soil and controlled conditions in Chen et al.'s experiment. Chen et al. did not account for these sources of uncertainty in extending the generalizability of their results with respect to ecohydrological separation (2).

Our comment notwithstanding, Chen et al.'s (1) experiment provides compelling evidence that calls for cautious consideration of CVD as a water extraction method. Some researchers find support for Chen et al.'s finding (4), while others refute the same (5).

A diversity of conceptualizations regarding ecohydrological separation (e.g., refs. 6 and 7) have been proposed since the publication of ref. 2. The main objective of our comment is specific to Chen et al.'s (1) claim regarding the implication of their work for ecohydrological separation (2). Using the same statistical tests performed in ref. 2, we have established similar ecohydrological separation outcomes in both original (d_{xylem}) and recalculated (d_{xylem_c}) precipitation offsets: 78% and 76%, respectively, of field-based studies that used stem water CVD extraction provide support for ecohydrological separation.

Data Availability. The data used in the statistical tests are the same data used by Chen et al. (1), which were obtained from Evaristo et al. (2) (https://www.nature.com/articles/nature14983#Sec11) and filtered following Chen et al.'s approach (see SI Appendix in ref. 1).

Downloaded at Universiteitsbibliotheek Utrecht on June 1, 202'

^aCopernicus Institute of Sustainable Development, Utrecht University, 3584 Utrecht, The Netherlands; ^bDepartment of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; and ^cDepartment of Geography, Hong Kong Baptist University, Hong Kong, SAR, China

Author contributions: J.E. performed research; J.E., Y.J., and K.P.C. analyzed data; and J.E., Y.J., and K.P.C. wrote the paper.

The authors declare no competing interest.

Published under the PNAS license.

¹To whom correspondence may be addressed. Email: j.evaristo@uu.nl.

Published April 13, 2021.

Site/paper ID	d _{xylem}		d _{xylem_} c	
	Prob> Z	Prob > ChiSq	Prob> Z	Prob > ChiSq
2	<0.0001	<0.0001	0.2478	0.2468
3	0.0058	0.0055	0.0039	0.0037
4	0.2616	0.2207	0.6098	0.5403
5	<0.0001	<0.0001	0.0002	0.0002
6	0.0016	0.0016	0.6954	0.6839
7	0.0189	0.0143	0.3583	0.3074
8	<0.0001	<0.0001	0.0189	0.0188
9	<0.0001	<0.0001	0.0223	0.0221
10	0.302	0.2664	0.0472	0.039
12	0.0021	0.0019	0.0007	0.0007
13	0.6366	0.5708	0.0107	0.0082
14	0.1694	0.1674	<0.0001	<0.0001
15	0.0038	0.0037	<0.0001	<0.0001
16	<0.0001	<0.0001	0.8696	0.8578
17	<0.0001	<0.0001	0.1966	0.1893
18	<0.0001	<0.0001	0.0086	0.0086
19	0.0027	0.0026	0.1093	0.1063
20	0.0022	0.0018	0.0933	0.0826
21	0.0167	0.0164	<0.0001	<0.0001
22	0.3865	0.2482	0.1489	0.0833
23	<0.0001	<0.0001	0.0094	0.0093
24	<0.0001	<0.0001	0.006	0.006
26	<0.0001	<0.0001	<0.0001	<0.0001
27	<0.0001	<0.0001	<0.0001	<0.0001
29	0.0011	0.0009	0.0128	0.0112
30	0.4211	0.4124	0.001	0.0009
32	0.0171	0.0164	0.0437	0.042
35	<0.0001	<0.0001	<0.0001	<0.0001
36	0.0018	0.0016	0.007	0.0063
40	0.5076	0.5038	<0.0001	<0.0001
41	<0.0001	<0.0001	<0.0001	< 0.0001
42	0.0135	0.0106	0.05	0.0408
43	<0.0001	<0.0001	0.0038	0.0038
44	< 0.0001	<0.0001	0.0021	0.002
45	0.4306	0.4116	0.0001	0.0001
46	< 0.0001	<0.0001	0.0051	0.005
47	<0.0001	<0.0001	0.031	0.0308

Table 1. Results of nonparametric tests on two levels, groundwater/stream and xylem water precipitation offsets

For these two levels, Prob>|Z| and Prob > ChiSq columns show the *P* values of the Wilcoxon (Mann–Whitney) test based on normal distribution approximations and the Kruskal–Wallis test based on one-way ChiSquare (ChiSq) approximations, respectively. The d_{xylem} is based on the precipitation offset values in ref. 2; d_{xylem_c} is based on the CVD error-corrected precipitation offset values using SI Appendix, equation S3 in ref. 1.



Fig. 1. Histograms of (A) d_{xylem} and (B) d_{xylem_c} binned following the biome classification in ref. 2. The dashed red line (precipitation offset = zero) serves as reference for quantifying the offset of environmental waters from local precipitation. Here, only precipitation offsets of xylem are shown comparing the original (d_{xylem}) and recalculated (d_{xylem_c}) values.

- 6 Z. C. Berry et al., The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward. Ecohydrology 11, e1843 (2018).
- 7 M. R. Cain, A. S. Ward, M. Hrachowitz, Ecohydrologic separation alters interpreted hydrologic stores and fluxes in a headwater mountain catchment. *Hydrol.* Processes 33, 2658–2675 (2019).

¹ Y. Chen et al., Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water. Proc. Natl. Acad. Sci. U.S.A. 117, 33345–33350 (2020).

² J. Evaristo, S. Jasechko, J. J. McDonnell, Global separation of plant transpiration from groundwater and streamflow. Nature 525, 91–94 (2015).

³ J. Evaristo, J. J. McDonnell, Prevalence and magnitude of groundwater use by vegetation: A global stable isotope meta-analysis. Sci. Rep. 7, 44110 (2017).

⁴ C. Millar, D. Pratt, D. J. Schneider, G. Koehler, J. J. McDonnell, Further experiments comparing direct vapor equilibration and cryogenic vacuum distillation for plant water stable isotope analysis. *Rapid Commun. Mass Spectrom.* **33**, 1850–1854 (2019).

⁵ S. L. Newberry, D. B. Nelson, A. Kahmen, Cryogenic vacuum artifacts do not affect plant water-uptake studies using stable isotope analysis. *Ecohydrology* 10, e1892 (2017).