LETTER

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REPLY TO EVARISTO ET AL.:

Strong evidence for the need of correcting extraction bias in an early study of ecohydrological separation

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In their reexamination of an earlier study (1) in which water isotope data from globally distributed sites were compiled, Evaristo et al. (2) show that, after accounting for extraction artifacts, precipitation offsets of xylem water (d_{xylem_c}) are still statistically distinct from those of stream (d_{stream})/groundwater (d_{gw}) in a majority of the sampling sites. In agreement, our own analysis on the dataset of ref. 1 also revealed a significant d_{xylem_c} versus d_{gw}/d_{stream} difference at the global scale (Table 1). However, we would like to point out that these analyses miss a crucial point: By removing the ~8‰ stem water extraction-associated error, there is as much "ecohydrological separation" from xylem water to groundwater as there is between stream and groundwater.

In our opinion, assessment of ecohydrological separation based solely on statistical significance in "offset" differences—as done here by Evaristo et al. (2)—is problematic, as it ignores the fact that, upon artifact correction, the originally large deviation in xylem water offset from groundwater/stream offset becomes very small (i.e., globally averaged d_{xylem_c} differs from d_{gw} by only 0.7‰; figure 5 in ref. 3), comparable in magnitude even to deuterium measurement precisions typically reported in literature (4, 5). Note that similarly minor and significant difference also exists between d_{gw} and d_{stream} (Table 1), but Evaristo et al. (1) do not use this small difference to argue that groundwater and steam water are hydrologically disconnected; rather, they correctly assume the opposite. As a matter of fact, the small degree of between-water pool isotopic segregation could arise from many common vagaries associated with field observations (i.e., different water pools may be subject to variable degrees of evaporative enrichment, or may be more or less of a reflection of a growing season versus annually averaged isotope signal), and so should not be taken as a sign for ecohydrological separation. It is for this reason that we chose not to focus on statistical significance, but to emphasize that the xylem water offset after artifact correction "is much closer to the offsets of groundwater and stream water than to the offset of soil water" in our discussion about the implication of extraction artifact correction for ecohydrological separation.

In our study, we show that the determined magnitudes of deuterium depletion during stem water cryogenic extraction broadly agree with those reported previously in various plants under diverse settings (i.e., including many nonsandy, field-based settings) (6–10). Notwithstanding, as acknowledged in refs. 3 and 11—and also in agreement with the criticism in ref. 2—our use of a species-averaged Δ value determined from a single experiment to correct for a metadataset spanning many species/environments is certainly not the most ideal way of accounting for deuterium biases. As further noted in ref. 11, we have explicitly pointed to the illustrative nature of this artifact correction practice, that is, by stating that the analysis "was intended to illustrate the need for measurement artifact correction" in studies of ecohydrological separation, rather than to draw a definitive conclusion about the validity of the "ecohydrological separation" concept per se.

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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.

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