isotopic measurement and could therefore not be used for regression analysis.

Figure 1a shows one example of the difference between the original estimate of Evaristo *et al.*[1] (green triangle) and our revised estimate (orange triangle) of the plant water source (site ID 26). For most of the sites, our estimates differ considerably from those of Evaristo *et al.*[1] (see Extended Data Figs 1, 2 and 3).

Extended Data Figs 1, 2 and 3 also reveal that the data set is extremely heterogeneous in terms of the number of sampled points for plant and groundwater, with sometimes inconsistent data leading to unrealistic values of $\delta^{18}O_{intersect}$ and $\delta^{2}H_{intersect}$ for certain sites. Therefore, we applied two non-exclusive criteria to assess the consistency of the calculated intersection values: criterion 1, $\delta^{2}H_{intersect} < \max(\delta^{2}H_{plant})$; and criterion 2, $\delta^{2}H_{intersect} > -200‰$. The first criterion implies that $m < a$, while the second criterion evaluates whether the plant water source hydrogen isotopic composition value is realistic. Eleven sites failed at least one of the two criteria (IDs 3, 7, 8, 17, 20, 21, 23, 24, 32, 36 and 44). Figure 1b shows one example of an inconsistent data set (site ID 3).

Results of this analysis (summarized in Table 1) show that at 26 sites, where data were consistent, $\delta^{2}H_{GW}$ was statistically different from $\delta^{2}H_{intersect}$ using the non-parametric Wilcoxon rank sum test ($\alpha = 0.05$). In conclusion, rainfall segregation (as defined by Evaristo *et al.*[1]) could be observed for only 57% of the sites of the authors' data set and at 74% of the sites with consistent estimates of the intercept as defined by our two criteria.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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

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Evaristo *et al.* reply

REPLYING TO M. Javaux, Y. Rothfuss, J. Vanderborght, H. Vereecken & N. Brüggemann *Nature* **536,** http://dx.doi.org/10.1038/18946 (2016)

In the accompanying Comment[1], Javaux *et al.* correct a mistake in equation (2) of our work[2]; as they point out[1], the mistake does not impact the central conclusion of our paper that ecohydrological separation is widespread[2]. However, equations (2) and (3) in ref. 2 calculate the source precipitation value of xylem water as the point where the xylem water evaporation line (EL) intersects the local meteoric water line (LMWL). In so doing, Javaux *et al.*[1] note that the mistake affects our finding[2] that "at 80% of the sites, the precipitation that supplies groundwater recharge and streamflow is different from the water that supplies parts of soil water recharge and plant transpiration".

There are two key points in our response.

(1) We recognize the mistake now noted in equation (2) and thank Javaux *et al.*[1] for this correction. These authors[1] find that rainfall segregation could be observed at only 74% of the sites (as defined by the two criteria in ref. 1), and not 80% as we originally reported[2].

(2) Our work[2] presented evidence for ecohydrological separation based on a meta-analysis of isotopic dual liquid water isotope data ($\delta^{2}H$ and $\delta^{18}O$) from 47 studies. This conclusion is supported by studies that analysed water vapour isotope data from the Tropospheric Emissions Spectrometer aboard NASA's Aura satellite[3] and by global differences between annual precipitation and groundwater isotope compositions[4,5]. These global-in-scale lines of evidence support earlier field evidence[6,7] that ecohydrological separation (defined as plants using water of a character different to that of mobile water found in soils, groundwater and streamflow) is widespread, and is the rule rather than the exception. Ecohydrological separation was calculated using equation (1) in ref. 2.

It must be understood that equation (1) in ref. 2 is independent of the source precipitation analysis, which was calculated using equations (2) and (3) in that paper. Therefore, any issue with equation (2) in our paper, like the one raised by ref. 1, does not affect the ecohydrological separation conclusion.

We hope that this exchange will generate further interest in the use of stable O and H isotopes in plant water relation studies.

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