Can deuterium tracing be used for reliably estimating water use of tropical trees and bamboo?

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Summary Reliable estimates of water use by trees and other woody plants are crucial for an improved understanding of plant physiology and for water resource management. Since the 1980s, the thermal dissipation probe (TDP) method has been widely applied in trees, proved to be fairly accurate but is challenging in remote areas. Also in the 1980s, the deuterium (D2O or deuterium oxide) tracing method was proposed, which so far has less often been applied. However, deuterium tracing requires less sophisticated equipment in the field and new analytical methods reduce costs and increase sample throughput. The objectives of this study were (i) to compare plant water use estimates of the TDP and D2O method and (ii) to determine whether the D2O method is appropriate for assessing absolute magnitudes of plant water use. The two methods were employed on five tropical tree species and a bamboo species growing in a reforestation stand in the Philippines and an agroforestry system in Indonesia. For bamboo, an increase in D2O values in neighbouring, non-labelled culms suggests that injected D2O was partly redistributed among culms, which would seriously limit the accurate estimation of water use for the target culm. For trees, water use estimates resulting from the D2O tracing method were proportional to the TDP results (r² = 0.85, P < 0.001), but absolute values were, on average, about seven times higher. This overestimation may be due to the assumptions underlying the D2O tracing method, such as the conservation of tracer mass, not being met. Further, it cannot be excluded that underestimation of water use by the TDP method contributed partly to the observed difference. However, when considering known sources of error, a large part of the observed difference remains unexplained. Based on our results, the use of the D2O tracing method cannot be recommended without further experimental testing if absolute values of whole-plant water use are a major goal. However, the D2O tracing method appears suitable for answering other questions, such as relative differences in water use among trees, water redistribution among neighbours and internal water transport and storage processes in plants.

Keywords: cacao, dipterocarps, gliricidia, Indonesia, mahogany, Philippines, sap flux density, stable isotopes, thermal dissipation probe method.

Introduction

Quantitative estimates of plant water use are essential for an improved understanding of plant physiology and for the management of water resources (Wullschleger et al. 1998, Farley et al. 2005, van Dijk and Keenan 2007). Techniques employed to estimate plant water use include the thermal dissipation probe (TDP) method (Granier 1985) and deuterium tracing (Calder et al. 1986) each with their respective strengths and weaknesses.

A thermoelectric method has already been described by Huber (1932). Innovations and technical progress improved applicability, making TDP a widely used method for the past 20 years. The TDP method is based on the relation between xylem sap flow and heat dissipation away from needle-like probes (typically 20 mm long and 2 mm diameter; Granier 1985) inserted radially into the sapwood. The temperature difference between the upper heated probe and a reference probe that measures the background stem temperature is related to sap flux density using an empirical calibration equation (Granier 1987). Plant water use is estimated from sap flux density and sapwood cross section, taking into account radial changes in sap flux density across the conducting sapwood (Hatton et al. 1990, Lu et al. 2004, Meinzer et al. 2005). The TDP method has been demonstrated to yield water use estimates of good quality (James et al. 2002, Lu et al. 2002). However, some studies indicate that sap flux
density might be underestimated using the TDP method (Lundblad et al. 2001, Roupsard et al. 2006). Major advantages of the TDP method are the high temporal resolution of sap flux measurements, the reliability and the simple sap flux calculations (see the reviews of Wullschleger et al. 1998, Lu et al. 2004). A problem is that this method is based on an empirical calibration function which should be verified for each species studied (Smith and Allen 1996, Lu et al. 2004), which is often not done. Further, the TDP technique is difficult to maintain, especially in remote areas, as a constant power source for probe heating and data recording is required and trained personnel are needed for equipment maintenance. The dependence on energy and the need to keep cable length low also limits the selection of study plants and often leads to a clustering of sample trees. This may become a problem if more sophisticated statistical approaches are envisaged for studies at the landscape level.

The deuterium tracing method is based on the movement of tracers (chemicals, radioisotopes or stable isotopes) in steady-state systems (Hull 1958, Kline et al. 1970, Calder et al. 1986). Deuterium oxide (D2O), a commonly used tracer, is injected into the base of a plant and transpired water is sampled periodically to construct a curve of tracer concentration in transpired water as a function of time. Assuming that the tracer passed through the plant over the course of the experiment, the plant water use is inversely proportional to the area under the curve of tracer concentration (Calder 1991). Studies conducted with young eucalyptus trees (Kalma et al. 1998) and potted trees (Dugas et al. 1993) indicate that plant water use estimated from the D2O method (WUD2O) was higher (up to 113%) compared with the TDP method and gravimetric measurements. In contrast, Dye et al. (1992) reported that WUD2O of two Eucalyptus grandis trees was 7 and 26% lower than water use based on the TDP method. Besides estimates of individual plant water use, tracing methods can also provide information on absolute sap velocity, internal water storage and retention of water in plants (James et al. 2003, Meinzer et al. 2006). It can further be used to independently test for diameter water use relations as, in contrast to the TDP method, diameter does not enter the calculation. Unlike TDP, the deuterium tracing method neither depends on a power source nor does it require specialized equipment in the field, which makes its application at remote sites and for extensive spatial sampling designs feasible. Further, the development of new techniques for stable isotope analyses such as continuous flow of δD and δ18O measurements of water samples (Gehre et al. 2004) and cavity ring-down spectroscopy (Lis et al. 2008, Gupta et al. 2009) increased throughput of samples and lowered the analytical costs. In contrast to the TDP method, water use cannot be characterized on a timescale of <1 day or even several days using the tracing method (Smith and Allen 1996). Further, sampling is labour intensive, especially for taller trees. Still, for specific questions or under certain circumstances, the deuterium tracing method might provide a suitable alternative to the TDP method.

It appears that both methods have their advantages and progress in analytical methods and cost reductions may lead to a more frequent application of the D2O tracing method. However, information is required as to whether individual plant water use estimates from the TDP and D2O tracing method are comparable over a range of plant life forms, species and sizes. The objectives of this study were (i) to compare plant water use estimates of the TDP and D2O method and (ii) to determine whether the D2O method is appropriate for assessing absolute magnitudes of plant water use. The two methods were employed on five tropical tree species and a bamboo species growing in a reforestation stand in the Philippines and an agroforestry system in Indonesia.

Materials and methods

Study sites and plant species selection

In the Philippines, we worked on the island of Leyte in the Eastern Visayas. The study site was located near the village of Patag (10°36′ N, 124°80′ E) at an elevation of 40 m a.s.l. Annual rainfall in the region amounts to 2753 mm year−1 and is relatively evenly distributed throughout the year (PAGASA 2007). Mean annual air temperature is 27.5 °C. The natural vegetation in the region is species-rich lowland dipterocarp forest (Langenberger 2006). After deforestation and intermittent cultivation, the degraded site was reforested with a mixture of native species, promoting the in-

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**Table 1. Characteristics of the trees and bamboos studied at the Philippine and Indonesian sites and an indication of the methods applied.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Method used</th>
<th>Family</th>
<th>Study location</th>
<th>Trees/culms studied (n)</th>
<th>DBH (m)</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Theobroma cacao</em></td>
<td>TDP</td>
<td>Malvaceae</td>
<td>Indonesia</td>
<td>18</td>
<td>0.10 (0.02)</td>
<td>4.5 (0.8)</td>
</tr>
<tr>
<td><em>Theobroma cacao</em></td>
<td>D2O</td>
<td>Malvaceae</td>
<td>Indonesia</td>
<td>6</td>
<td>0.10 (0.01)</td>
<td>4.2 (0.6)</td>
</tr>
<tr>
<td><em>Gliricidia sepium</em></td>
<td>TDP</td>
<td>Fabaceae</td>
<td>Indonesia</td>
<td>18</td>
<td>0.15 (0.03)</td>
<td>10.9 (2.1)</td>
</tr>
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<td>D2O</td>
<td>Fabaceae</td>
<td>Indonesia</td>
<td>6</td>
<td>0.13 (0.02)</td>
<td>10.6 (3.3)</td>
</tr>
<tr>
<td><em>Shorea contorta</em></td>
<td>TDP/D2O</td>
<td>Dipterocarpaceae</td>
<td>Philippines</td>
<td>5</td>
<td>0.18 (0.07)</td>
<td>16.1 (3.5)</td>
</tr>
<tr>
<td><em>Shorea polystyphera</em></td>
<td>TDP</td>
<td>Dipterocarpaceae</td>
<td>Philippines</td>
<td>5</td>
<td>0.14 (0.02)</td>
<td>13.3 (1.7)</td>
</tr>
<tr>
<td><em>Swietenia macrophylla</em></td>
<td>TDP/D2O</td>
<td>Meliaceae</td>
<td>Philippines</td>
<td>5</td>
<td>0.15 (0.01)</td>
<td>14.2 (1.5)</td>
</tr>
<tr>
<td><em>Bambusa blumeana</em></td>
<td>TDP/D2O</td>
<td>Poaceae</td>
<td>Philippines</td>
<td>4</td>
<td>0.10 (0.01)</td>
<td>19.8 (0.7)</td>
</tr>
</tbody>
</table>

**Notes:**
- DBH (Diameter at Breast Height) and height values are presented as the means ± 1 SD.
- The Family and Location columns indicate the study sites and plant species selection.
- The trees were studied in Indonesia and the Philippines.
- The study sites include an agroforestry system in Indonesia.

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corporation of fruit trees and combining fast-growing trees with shade-tolerant species (Margraf and Milan 1996). At the time of the field study (July to October 2007), the stand was 12 years old and stem density was 1367 stems per hectare. Within this stand, three tree species (Shorea contorta S. Vidal, Shorea polysperma Merr. and Swietenia macrophylla King) with five individuals per species were selected for simultaneous deuterium labelling, TDP and stem heat balance (SHB) water use measurements. The bamboo species Bambusa blumeana J.A. & J.H. Schultes was represented by a total of four culms selected in pairs from two separate clumps. Tree and bamboo structural characteristics are summarized in Table 1. Most of these species were also part of a wider study on plant water use in reforestation stands (Dierick and Hölscher 2009).

In Indonesia, our study site was located in Central Sulawesi in the vicinity of the village of Marena (1.552° S, 120.020° E) at 560 m a.s.l. Annual rainfall at a close-by climate station is 2090 mm year⁻¹ (2002–06) and shows a weak bimodal pattern. The mean annual air temperature is 25.5 °C. The agroforest stand (~1 ha) was established in December 2000 on former upland rice and maize fields by planting Theobroma cacao L. saplings and Gliricidia sepium (Jacq.) Kunth ex Steud. cuttings. G. sepium trees, which served as shade trees, were at the time of the study 11 m high, while T. cacao grew in its understorey with a mean height of 4.5 m (Köhler et al. 2009). The stem density was 1030 stems per hectare for T. cacao and 325 stems per hectare for G. sepium. The agroforest stand was divided into six plots (35 m × 40 m). In each plot, three randomly selected T. cacao and G. sepium trees (in total 18 individuals per species) were selected for other studies using the TDP method (Köhler et al. 2009, Schwendenmann et al. 2010). To prevent potential side effects of drilling several holes into the trunk for tracer injection on these sap flux trees, a second set of T. cacao and G. sepium (with one individual per species and plot, in total six T. cacao and six G. sepium) were chosen for the deuterium tracer experiment (Table 1).

Table 2. Sap flux density measured by the TDP method

<table>
<thead>
<tr>
<th>Species</th>
<th>Study location</th>
<th>$J_s$ (m$^3$ m$^{-2}$ day$^{-1}$)</th>
<th>Normalized $J_s$ (Depth 1) (%)</th>
<th>Normalized $J_s$ (Depth 2) (%)</th>
<th>WU$^{TDP}$ (kg day$^{-1}$)</th>
<th>WU$^{TDP}$ weighted (kg day$^{-1}$)</th>
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<tr>
<td><em>Theobroma cacao</em></td>
<td>Indonesia</td>
<td>1.35 (0.32)</td>
<td>81.7 (22.4)</td>
<td>n.d.$^1$</td>
<td>8.7 (1.6)</td>
<td>8.7 (1.6)$^2$</td>
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<tr>
<td><em>Gliricidia sepium</em></td>
<td>Indonesia</td>
<td>1.46 (0.39)</td>
<td>61.7 (29.4)</td>
<td>n.d.$^3$</td>
<td>11.4 (1.6)</td>
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<td><em>Shorea contorta</em></td>
<td>Philippines</td>
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<td>36.3 (28.1)</td>
<td>7.0 (4.4)</td>
<td>18.6 (13.9)</td>
<td>20.3 (14.6)</td>
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<tr>
<td><em>Shorea polysperma</em></td>
<td>Philippines</td>
<td>0.75 (0.13)</td>
<td>44.0 (13.6)</td>
<td>11.0 (3.4)</td>
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<td><em>Swietenia macrophylla</em></td>
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<td>1.73 (0.20)</td>
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<td><em>Bambusa blumeana</em></td>
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$^1$Not determined because of small diameter.

$^2$For T. cacao and G. sepium, two different sets of trees were used to apply the D$_2$O and TDP method. The WU$^{TDP}$ weighted was derived from species-specific relationships between WU$^{TDP}$ and tree diameter (DBH) and approximated by the average water use over the last 14 days of the tracer experiment (26 January to 8 February 2007) when TDP data was available.

$^3$Not determined because of heartwood formation.

$^4$Not determined because of hollow culm.

Plant water use estimated from the TDP method (WU$^{TDP}$)

Sap flux density measured with the TDP method was scaled to tree water use rates based upon radial profiles of $J_s$ within the conductive sapwood for the different species (Table 2). Radial profiles were determined by measuring sap flux densities at one (Depth 1: for Indonesia, 25–50 mm; for the Philippines, 20–45 mm) or two (Depth 2: for the Philippines, 40–65 mm) additional depth intervals for several days and normalizing the results with respect to sap flux density at a reference depth (0–25 mm). For details, we refer to Dierick and Hölscher (2009) and Köhler et al. (2009). Sap flow was determined in ring-shaped cross sections, taking into account the cross-sectional area of the ring corresponding to the respective installation depth, $J_s$ as measured at the reference depth at the outer xylem and the normalized profile of $J_s$ for the species considered. Contributions of

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$^4$Not determined because of hollow culm.
up to three cross sections were added to estimate water use rates and summed over a day to give daily tree water use (WUTDP, in kilogrammes per day) (Hatton et al. 1990, Meinzer et al. 2005). For bamboo, sap flux densities were scaled to culm water use, assuming that sap flux density was uniform over the culm cross section, while in adjacent culms, it was verified that the void in the culm at installation height was negligible or absent (Dierick et al., in revision).

TDP method estimates of plant water use (WUTDP) cannot be compared directly to estimates of plant water use obtained from deuterium tracing (WUD2O, in kilogrammes per day; see below) as the tracing method does not simply average plant water use over the period where the tracer is present in transpired water. Rather, as Calder (1991) demonstrated, the tracer method gives an estimate that equals weighted mean water use during the period the tracer is present where the weights are the observed tracer concentration at a given time (Figure 1A–D). Therefore, WUTDP values should be weighted before comparing them with WUD2O values. If estimates of tracer levels are available at regular intervals, the following simplified equation applies:

$$WUTDP\text{ weighted} = \frac{\sum q_i C_i}{\sum C_i}$$

where WUTDP weighted is the weighted mean water use (in kilogrammes per day), $q_i$ is the instantaneous water use measured with the TDP method and $C_i$ is the tracer concentration at time interval $i$. The simplified equation is appropriate as estimates of tracer concentrations are available at a half hourly time step through linear interpolation (see below). Over the duration of the tracer experiment ($t = 0–31$ days), the tracer concentrations were summed and half hourly tracer concentrations were divided by this sum to give weights according to tracer concentration. The sum of all weights equals one, and summing beyond the point where tracer was last observed does not influence the result as tracer concentration and

Figure 1. Illustration of the procedure used to determine weighted water use from the TDP method (WUTDP weighted) for comparison with water use estimates of the deuterium method (WUD2O). Instantaneous water use rates $q$ (A) are weighted proportionally to the deuterium concentrations observed at the leaf level (B), whereby weights are calculated such that the sum of weights equals unity (C). The resulting weighted water use rates (D) are summed over the duration of the experiment to yield weighted WUTDP or WUTDP weighted (in kilogrammes per hour).
thus weights essentially become zero. Half hourly measurements of WUTDP were multiplied by the weights at the corresponding time and summed to give the average weighted water use WUTDP weighted which can be compared with the water use estimate from the tracer experiment.

For *T. cacao* and *G. sepium,* it was not possible to weight WUTDP as two different sets of trees were used to apply the D2O and TDP methods (see above) and TDP measurements started only in the second half of January 2007. Species-specific relationships between WUTDP and tree diameter at breast height (DBH) were established and used to derive WUTDP of trees used in the D2O experiment, based on their diameter (Köhler et al. 2009). For each individual, weighted WUTDP was averaged over a 2-week period (26 January to 8 February 2007) for comparison with WU_D2O.

Estimates of WUTDP or WUTDP weighted were compared with WU_D2O using simple linear regression. All calculations and statistical analyses were done with R version 2.6.2 (R Development Core Team 2009).

To assess the performance of the TDP method, simultaneous measurements of plant water use were conducted using the SHB method (Dynagage SGA100-WS; Dynamax Inc., Houston, TX) for 5 days in four culms of *B. blumeana* and two individuals of *S. contorta,* *S. polysperma,* *S. macrophylla,* and an additional species *Parashorea malaanonan* Merr. SHB gauges were installed following the procedures outlined by the manufacturer (Dynamax 2005) at 3–4 m above the thermal dissipation probes to avoid thermal interference between the two systems. Two extra thermocouples monitored the temperature of the enclosed stem/culm segment thus making it possible to include heat storage in the heat balance for improved accuracy (Grime et al. 1995).

### Deuterium tracing method

Deuterium oxide (D2O, 99.90% D; Euroiso-Top, Gif sur Yvette, France) was injected into holes drilled into the trunk of trees and bamboo. The holes (four to eight holes per tree, two holes in bamboo; 2.5–5 mm diameter, 5 cm deep) were drilled at an angle of 30–45° and were spaced regularly around the trunk. Holes were 0.2 m (*B. blumeana,* 0.8 m (*G. sepium*) and between 0.2 and 0.3 m (*T. cacao,* *S. contorta,* *S. polysperma* and *S. macrophylla*) above ground level. The holes were immediately filled with D2O and the amount of D2O was evenly distributed between the holes. For the experiment in Indonesia, a syringe was used to inject deuterium into the transpiration stream of *T. cacao* and *G. sepium.* Injections were done between 11 am and 3 pm local time. The first week after deuterium labelling was characterized by mostly sunny days with <10 mm rainfall. Micrometeorological data such as global radiation and vapour pressure deficit were not available for this period.

In the Philippines, a slightly different procedure was applied. Small plastic tubes were glued to the stem/culm under an angle of 30–40°. These were filled with water and holes were drilled under water, thus minimizing embolism of the vessels. Excess water was then removed from the tube and replaced by deuterium. Tracer application took place between 9 am and 5 pm. Most tracer was taken up quickly (within a few hours), but uptake was slow in *S. polysperma,* probably because of resin formation in the holes. *S. contorta* and *S. polysperma* were labelled on 16 June 2007 while *S. macrophylla* and *B. blumeana* were labelled on 8 September 2007. During the first week of the experiment performed in June, conditions were mostly sunny with a total precipitation of 26.5 mm. Mean vapour pressure deficit and global radiation were 0.9 kPa and 16.8 MJ m⁻² day⁻¹, respectively. During the first week of the experiment performed in September, weather conditions were more cloudy, with mean vapour pressure deficit and global radiation of 0.7 kPa and 12.7 MJ m⁻² day⁻¹, respectively. Total precipitation was 20.8 mm.

Transpired water was collected in situ from *T. cacao* and *G. sepium* following the approach of Calder (1991). Clear plastic bags were placed on the ends of three to five branches located in the upper part of the canopies. Each bag contained approximately two to three leaves and was sealed with insulation tape. Samples from labelled trees and control trees (see below) were always collected around midday and new plastic bags were placed on different branches with each sampling campaign. After the injection of D2O (Day 0), transpired water was collected daily over the first 10 days and then every 2–4 days over two more weeks.

All condensate samples for a tree on a given day were combined to provide a single sample from which an aliquot (1.5 ml) was transferred into a glass vial and stored at 4 °C until stable isotope analysis could take place. The variability of deuterium values within the canopy was evaluated by
occasionally measuring all replicates obtained from different branches separately.

For the species studied in the Philippines (S. contorta, S. polysperma, S. macrophylla and B. blumeana), a somewhat different approach, comparable to the one used by James et al. (2003), was used to recover tracer. For each sample, a total of eight leaves were collected from four cardinal directions in the upper sun-exposed part of the crown. Collected leaves were enclosed in a transparent plastic container and left out in the sun for 2–3 h and 1–2 ml of the formed condensate was collected in glass vials. Samples were collected twice daily (typically 9 am to noon and 1 pm to 4 pm local time) on the first 5 days following labelling if weather conditions were favourable and less frequently, once per day (9 am to noon) later on. The last sample was taken 30 days after labelling.

The baseline D$_2$O signal in transpired water was determined by collecting samples of transpired water 1 or 2 days prior to labelling and from four to six controls per species which were monitored for background deuterium concentrations. Control trees or culms were measured in total four times, 1 or 2 days before tracer injection and later at 4- to 10-day intervals.

The quantity of transpired water recovered differed among species and between sampling methods and ranged between 0.5 and 5 ml.

Deuterium in xylem water

Stem (5 mm diameter wood cores, 3–4 cm long) and branch samples were taken from two labelled T. cacao trees to assess the spatial distribution of D$_2$O over the course of the experiment. Wood cores from the lower stem were collected only once (1 day after deuterium injection), wood cores from the upper stem were taken a few hours after tracer injection, 1 and 2 days after tracer injection and branch segments were sampled 2, 7, 22 and 25 days after deuterium labelling. To measure whether any D$_2$O remained in the xylem tissue, branch samples were taken from all labelled T. cacao and G. sepium trees at the end of the sampling period (26 days after labelling). Bark was removed from sampled stem or branch material to avoid contamination of xylem water with phloem water. Samples were placed in a glass vial, closed with a Teflon-coated lid, sealed with Parafilm and stored at 4 °C to prevent evaporative fractionation. Water was extracted from the xylem by cryogenic vacuum distillation (Ehleringer and Osmond 1989) and then analysed for deuterium.

Stable isotope analysis

Hydrogen isotopic composition was measured by injecting the transpired and extracted water into a high-temperature conversion/elemental analyser coupled via a ConFlo III interface to a Delta V Plus isotope ratio mass spectrometer (Thermo-Electron Cooperation, Bremen, Germany) following the method developed by Gehre et al. (2004). The stable hydrogen isotope composition was expressed in δ notation (‰) as the deuterium over hydrogen or D/H ratio of the sample ($R_{sample}$) relative to the D/H ratio for the Vienna Standard Mean Ocean Water ($R_{V-SMOW}$), which equals 155.75E$^{-6}$:

$$\delta D = \left( \frac{R_{sample}}{R_{V-SMOW}} - 1 \right) \times 1000$$  

(2)

Measurement precision was 2‰. Analyses were carried out at the Center for Stable Isotope Research and Analysis (KOSI) at the University of Göttingen, Germany.

Figure 3. Maximum deuterium (D$_2$O) concentration in transpired water (in grammes per kilogramme) versus the amount of injected tracer per basal area of the tree (in grammes per square centimetre). Maximum values were positively correlated with the injected mass of deuterium relative to basal area ($r^2 = 0.44$, $P < 0.001$) if G. sepium trees were excluded.

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Table 3. Mass of injected deuterium (D$_2$O), time of D$_2$O arrival in the canopy ($T_{arrival}$), time when maximum D$_2$O concentration in transpired water were measured ($T_{max}$), residence time of deuterium ($T_{residence}$) and comparison of flux velocity and water use between the heat dissipation method (TDP) and the deuterium tracing method (D$_2$O). Values are presented as the means ± 1 SD; for the number of individuals used, see Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>D$_2$O injected (g)</th>
<th>$T_{arrival}$ (days)</th>
<th>$T_{max}$ (days)</th>
<th>$T_{residence}$ (days)</th>
<th>$V_{D_2O}$ (m day$^{-1}$)</th>
<th>$V_{TDP}$ weighted (m day$^{-1}$)</th>
<th>WUD$_{D_2O}$ (kg day$^{-1}$)</th>
<th>WUT$_{D_2O}$ weighted (kg day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theobroma cacao</td>
<td>14.3 (2.8)</td>
<td>1.7 (0.5)</td>
<td>2.3 (0.5)</td>
<td>5.1 (0.9)</td>
<td>2.5 (0.6)</td>
<td>1.5 (0.2)$^1$</td>
<td>27.6 (4.4)</td>
<td>8.7 (1.6)$^2$</td>
</tr>
<tr>
<td>Gliricidia sepium</td>
<td>29.2 (6.6)</td>
<td>1.7 (0.8)</td>
<td>2.3 (0.8)</td>
<td>6.4 (0.8)</td>
<td>6.3 (2.6)</td>
<td>1.5 (0.3)$^1$</td>
<td>95.0 (33.5)</td>
<td>11.4 (1.6)$^2$</td>
</tr>
<tr>
<td>Shorea contorta</td>
<td>28.0 (11.0)</td>
<td>0.9 (0.0)</td>
<td>0.9 (0.1)</td>
<td>4.5 (0.3)</td>
<td>18.1 (4.0)</td>
<td>1.3 (0.4)</td>
<td>185.0 (146.9)</td>
<td>20.3 (14.6)</td>
</tr>
<tr>
<td>Shorea polysperma</td>
<td>0.0 (0.0)</td>
<td>0.9 (0.0)</td>
<td>1.1 (0.0)</td>
<td>3.8 (0.6)</td>
<td>14.9 (4.0)</td>
<td>1.6 (0.2)</td>
<td>162.2 (23.2)</td>
<td>19.9 (2.9)</td>
</tr>
<tr>
<td>Swietenia macrophylla</td>
<td>20.0 (0.0)</td>
<td>0.9 (0.0)</td>
<td>0.9 (0.0)</td>
<td>3.9 (0.3)</td>
<td>15.9 (1.7)</td>
<td>1.6 (0.2)</td>
<td>162.2 (23.2)</td>
<td>19.9 (2.9)</td>
</tr>
<tr>
<td>Bambusa blumeana</td>
<td>10.0 (0.0)</td>
<td>3.1 (1.4)</td>
<td>4.8 (1.6)</td>
<td>11.5 (4.3)</td>
<td>8.5 (6.4)</td>
<td>0.9 (0.3)</td>
<td>63.0 (29.1)</td>
<td>6.9 (2.5)</td>
</tr>
</tbody>
</table>

$^1$For T. cacao and G. sepium, two different sets of trees were used to apply the D$_2$O and TDP method. $^2$The $V_{TDP}$ weighted was estimated as a mean of $n = 18$ individuals per species equipped with sap flow sensors. $^3$The WUT$_{TDP}$ weighted was derived from species-specific relationships between WUT$_{TDP}$ and tree diameter (DBH) and approximated by the average water use over the last 14 days of the tracer experiment (26 January to 8 February 2007) when TDP data was available.

Tracer velocity and residence time

Water transport and storage characteristics of deuterium in trees and bamboo were evaluated using tracer arrival time, tracer velocity and residence time. Tracer arrival time ($T_{arrival}$, in days) was defined as the time of the first sample exceeding 10% of the maximum deuterium concentration. The velocity of D$_2$O transport ($v_{D_2O}$, in metres per day), which equals maximum sap velocity, was estimated by dividing the total plant height (minus injection height) by the arrival time. Estimated tracer velocity was compared with sap velocity obtained by the TDP method using simple linear regression. The residence time ($T_{residence}$, in days) of the D$_2$O signal was taken as the time at which deuterium concentration dropped below 10% of the observed maximum value.

Plant water use estimated from the deuterium tracing method (WUD$_{D_2O}$)

In order to calculate plant water use from deuterium values, absolute tracer concentrations (in grammes per kilogramme) are required (Calder et al. 1992). Thus, the $\delta$ notation was converted into atom fractions ($R_{sample} = D/H$) with $R_{V-SMOW} = 155.75E^{-6}$:

$$R_{sample} = \left( \frac{\delta D}{1000} + 1 \right) \times R_{V-SMOW}$$  \hspace{1cm} (3)

The $D/H$ ratio was converted into atom fractions of H$_2$O ($f_{H_2O}$, in moles of H$_2$O per mole of mixture) and D$_2$O ($f_{D_2O}$, in moles of D$_2$O per mole of mixture) as follows:

$$f_{H_2O} = \frac{1}{1 + R_{sample}} \quad \text{and} \quad f_{D_2O} = \frac{R_{sample}}{1 + R_{sample}} \quad \text{where}$$

$$f_{H_2O} + f_{D_2O} = 1$$  \hspace{1cm} (4)

The ratio of D$_2$O mass and total mass in 1 mole of mixture gives the mass concentration of tracer ($C$, in grammes of D$_2$O per kilogramme of mixture) with $m_{D_2O} = 20.04$ g mol$^{-1}$ and $m_{H_2O} = 18.02$ g mol$^{-1}$:

$$C = \left( \frac{f_{D_2O} \times m_{D_2O} + f_{H_2O} \times m_{H_2O}}{1} \right) \times 1000$$  \hspace{1cm} (5)

Background values ($\delta$ notation) were averaged over the four to six replicates per species. Mean background values were linearly interpolated between the sampling points and converted into grammes per kilogramme notation for correction of that species. Continuous breakthrough curves were estimated by linear interpolation between measured deuterium concentrations after background correction. Interpolation values were determined at half hourly time steps. This resolution corresponds to that of thermal dissipation sap flux density data, which allows an easy weighing of the measured thermal dissipation water use rate (see below). The deuterium concentration measured 1 or 2 days before tracer injection was assumed to be valid at $t = 0$ and negative interpolation values were set to zero.

Plant water use was then determined from the amount of tracer injected and the surface under the breakthrough curve as (Calder 1991):

$$WUD_{D_2O} = \frac{M}{\sum_i C_i \Delta t_i}$$  \hspace{1cm} (6)

where WUD$_{D_2O}$ is the water use determined using the tracer method (in kilogrammes per day), $M$ is the tracer mass injected in the plant (in grammes), $C_i$ is the deuterium concentration at time interval $i$ (in grammes per kilogramme), $\Delta t_i$ is the duration of time interval $i$ (in days) and 1...T is the time interval between Day 0 and the end of the experiment at Day 31.

Results

Sap flux density and plant water use

Average sap flux densities ranged between 0.75 and 1.46 (in cubic metres per square metre per day) and differed among
Figure 4. Deuterium concentration (D₂O in grammes per kilogramme above background level) in transpired water of *T. cacao*, *G. sepium*, *S. contorta*, *S. polysperma*, *S. macrophylla* and *B. blumeana* over the course of the experiment. Day 0 is the day of deuterium injection. Note the differences in scale.
species (Table 2). However, direct comparisons among species may be misleading as species were studied at different times and under different conditions. The \( J_s \) in Depth 1 below the cambium was 36–82% of \( J_s \) measured in the outermost sapwood section (reference depth). Normalized \( J_s \) in Depth 2 below the cambium was <26% (Table 2). In Gliricidia trees (>14 cm in diameter), only the outer 0–30 mm was conductive sapwood, indicating that heartwood formation occurred. This observation was also supported by differences in wood colour and staining (Köhler et al. 2009).

We found a good linear relationship between plant water use measured with the TDP method and the SHB method (slope = 1.07, intercept = −1.28, \( r^2 = 0.65, P = 0.002 \); Figure 2). However, the comparison with SHB suggests that TDP underestimated tree water use on average by 17% (excluding S. macrophylla). For bamboo, the WU_TDP was on average 13% lower than WU_SHB (Dierick et al., in revision).

**Deuterium patterns in transpired water**

The background \( \delta D \) values in transpired water from trees for both sites ranged from −10 to −70‰ and did not show a trend over the course of the experiment. In contrast, in B. blumeana culms, we measured a steady rise of background \( \delta D \) from −30‰ at the onset of the experiment to −7‰ after 30 days.

The maximum \( \delta D \) values in transpired water of labelled trees (corrected for background values) varied between 100 and 1800‰, which corresponded to 0.02 and 0.32 g \( D_2O \) kg\(^{-1}\), respectively (Figure 3). Maximum values were positively correlated with the injected mass of deuterium relative to the basal area (\( r^2 = 0.44, P < 0.001 \)) if G. sepium trees were excluded (Figure 3). The coefficient of variation of the \( \delta D \) values within the canopy was on average <1% for background values and 20% for maximum \( \delta D \) values measured in transpired water of T. cacao and G. sepium trees.

For all tree species, deuterium arrived (Tarrival) in the canopy on average 0.9–1.7 days after labelling (Table 3, Figure 4). For B. blumeana, Tarrival was 3.1 days (Table 3, Figure 4). Maximum \( D_2O \) concentration (Tmax) was measured 0.9–2.3 days after labelling for trees and after 4.8 days for B. blumeana. In general, the decline of \( D_2O \) concentration after the peak was more gradual compared with the increase after labelling (Figure 4). Average Tresidence varied between 4.3 days (S. macrophylla) and 12.5 days (B. blumeana) (Table 3, Figure 4). At the end of the experiment (26–30 days after labelling), the deuterium concentrations of all labelled trees/culms were not significantly different from background values (Figure 4).

**Deuterium in xylem water**

Deuterium was detected in xylem water extracted from the upper stem of two T. cacao trees within 3–5 hours after deuterium injection. Maximum \( \delta D \) values (26,208‰) were measured in the lower stem on the day following labelling (Figure 5). Deuterium values in branches declined strongly later on and were close to background values 3 weeks after labelling (Figure 5). The \( \delta D \) values in xylem water extracted from branches of T. cacao and G. sepium at the end of the experiment ranged from −42 to −69‰. These values were similar to values found in T. cacao and G. sepium trees that were not subjected
to D₂O labelling (from −48 to −62‰; Schwendenmann et al. 2010).

Comparison of velocities and water use estimates

The tracer velocity (v\textsubscript{D₂O}, in metres per day) averaged over the replicates ranged from 2.5 m day\textsuperscript{−1} for T. cacao to 18.1 m day\textsuperscript{−1} for S. contorta (Table 3). The sap flux velocities (v\textsubscript{TDP}, in metres per day) derived from the thermal dissipation measurements were considerably lower compared with the velocity obtained from the deuterium tracing experiment, especially for the tree species investigated in the Philippines (Table 3, Figure 6). No significant correlations were found between v\textsubscript{D₂O} and v\textsubscript{TDP}.

The deuterium tracing method gave 230–790% higher estimates for water use than the TDP method (Table 3, Figure 7). However, W\textsubscript{UTDP} and W\textsubscript{UD₂O} showed a strong positive relationship (r\textsuperscript{2} = 0.86, P < 0.001).

There was a positive correlation between W\textsubscript{UD₂O} and DBH (r\textsuperscript{2} = 0.77, P < 0.001). An even stronger relationship was found between W\textsubscript{UTDP} and DBH (r\textsuperscript{2} = 0.82, P < 0.001).

Discussion

The observed variation in background δD values in transpired water from trees over time (−10 to −70‰) is probably the result of (i) changing transpiration rates due to varying environmental conditions, (ii) isotopic dilution resulting from enclosing wet leaves after rainfall (Kline et al. 1970, Dye et al. 1992) and/or (iii) shifts in δD of the tree’s water sources at the time of sampling. In bamboo (B. blumeana), the increase of background δD over the course of the experiment suggests that a redistribution of water between interconnected culms within a clump occurred (Dierick et al., in revision). This would be in line with previous observations that showed that nutrients and photosynthates in bamboo can be transported between culms (Kleinhenz and Midmore 2001). Thus, the requirement that a known amount of injected tracer passes through the culm during the course of the experiment is violated (see Kline et al. 1970, Calder et al. 1986) as part of the tracer leaving the culm could not be quantified and W\textsubscript{UD₂O} in B. blumeana will, therefore, most likely be overestimated. Redistriution of tracer may also occur in multiple-stem trees (Marc and Robinson 2004).

We found a positive correlation between maximum D₂O concentrations in transpired water and the amount of tracer injected per unit basal area when omitting G. sepium trees from the analysis (Figure 3). Such a correlation was also found by Meinzer et al. (2006) for conifers. In contrast, the D₂O maximum in G. sepium tended to be lower than expected from the injected tracer mass relative to basal area. This may be attributed to heartwood formation in G. sepium trees (Köhler et al. 2009), leading to temporary storage and subsequent gradual release of tracer into the transpiration stream (Kalma et al. 1998).

The tracer concentration in transpired water collected from S. contorta, S. polysperma and S. macrophylla increased rapidly and reached a maximum between 0.9 and 1.1 days after labelling. Maximum deuterium enrichment after a single day was also detected in transpired water of E. grandis (DBH = 0.11 m, height = 8 m) in South Africa (Dye et al. 1992) and Cordia alliodora (DBH = 0.34 m, height = 26 m) in Panama (James et al. 2003). For T. cacao and G. sepium and other tree species of similar (or smaller) size, the maximum concentration was measured 2–6 days after labelling (Calder et al. 2010).

Figure 6. Velocity estimated by the TDP method (v\textsubscript{TDP}) versus velocity measured by deuterium tracing (v\textsubscript{D₂O}). Including only species studied in the Philippines, a weak positive linear correlation was found between v\textsubscript{D₂O} and v\textsubscript{TDP} (r\textsuperscript{2} = 0.327, P = 0.0105).
Our study showed that path length was not a good predictor for $T_{\text{max}}$. Similar results were found by James et al. (2003), injecting $D_2O$ into different tree species of similar height (22–28 m) resulted in tracer arrival from 1 to 5 days. A gradual decline in $D_2O$ concentrations after the peak seems to be a common feature as it is reported from other studies in which deuterium was used as a tracer for a range of tree species and tree sizes (e.g., Kalma et al. 1998, James et al. 2003) and can be expected from theoretical considerations (Calder 1991). Differences in $T_{\text{residence}}$ among species might be explained by differences in their internal water storage capacity and the capacitive exchange of water between the transpiration stream and storage compartments in the stem (James et al. 2003, Meinzer et al. 2006). Species with longer tracer residence time are assumed to have a greater diurnal water storage capacity (James et al. 2003). A strong positive correlation between $v_{D_2O}$ and $v_{TDP}$ if each species is considered separately. In contrast, including all species studied in the Philippines, a weak positive linear correlation was found between $v_{D_2O}$ and $v_{TDP}$ ($r^2 = 0.327, P = 0.0105$). A positive correlation between $v_{D_2O}$ and $v_{TDP}$ was also found for conifers (Meinzer et al. 2006).

The finding that estimated plant water use was strongly related to plant diameter is in agreement with previous studies (Becker 1996, Meinzer et al. 2001). When establishing this relationship, one should be aware that $WU_{TDP}$ and DBH are not independent variables, as DBH enters the $WU_{TDP}$ calculations (Meinzer et al. 2001). In contrast, the $D_2O$ tracing method does not suffer this problem and still confirms the strong dependency of plant water use on DBH.

The deuterium tracing method gave 230–790% higher estimates for water use than the TDP method (Figure 7). Kalma et al. (1998) reported that $WU_{D_2O}$ was 11–113% higher compared with $WU_{TDP}$ for 5-year-old $E.\text{grandis}$ trees. The $WU_{D_2O}$ was also generally higher than tree water use measured gravimetrically in $Eucalyptus\text{gunnii}$ and $Prunus\text{serrulata}$ trees (Dugas et al. 1993). In contrast, Dye et al. (1992) reported that $WU_{D_2O}$ of two $E.\text{grandis}$ trees was 7 and 26% lower than $WU_{TDP}$.

The following errors associated with either the deuterium tracing method or the TDP methods may account for some
of the differences found between WU_{D,O} and WU_{TDP} estimates.

(i) Underestimation of plant water use derived from the TDP method. Potential errors associated with the TDP method are thermal gradients (Do and Rocheteau 2002). Especially in bamboo where the sap flux sensors were placed close to the ground, thermal gradients are a possible source of error. However, monitoring thermal gradients on all culms showed that they were largely within the range (<0.2 °C) put forward by Do and Rocheteau (2002). In addition, radial gradients of sap flux density (Clearwater et al. 1999), uncertainties as a result of empirical calibrations (Smith and Allen 1996) and errors associated with the integration of sap flux over the conducting wood area (Wullschleger et al. 1998) are likely sources of errors. As mentioned above, the performance of the TDP method was evaluated with the SHB method for a number of species. The WU_{TDP} was on average between 17% (trees) and 13% (bamboo) lower than water use estimated with the SHB method. Even larger underestimations of sap flux density of about 60% using the TDP method with the original calibration equation were revealed during a gravimetric calibration of home-made probes (Roupard et al. 2006). Around 50% lower estimates of the TDP method compared with the tissue heat balance method were reported by Lundblad et al. (2001). However, even underestimations of water use by the TDP method in this range would not explain the considerable differences between WU_{TDP} and WU_{D,O} encountered in our study.

(ii) Overestimation of plant water use derived from the D_{2}O method and other potential errors. Estimating WU_{D,O} is based on two conditions: (i) all of the tracer passes through the plant with the transpiration stream during the course of the experiment (mass conservation) and (ii) complete mixing of the tracer with the tissue water (Kline et al. 1970, Calder et al. 1986). The D_{2}O concentration–time curves showed that tracer concentrations approached background levels (Figure 4), which by other authors was taken as a sign that all of the tracer had passed through the plants (Dugas et al. 1993). We further measured high δD values in xylem water extracted from the stem and branches of two T. cacao trees in the first days after labelling (Figure 5). Other studies found significant concentrations of deuterium in the heartwood of various tree species after tracer injection, suggesting radial tracer transport (Kalma et al. 1998, James et al. 2003). The diffusion of deuterium from the sapwood through rays and axial parenchyma into the heartwood would slow the release of deuterium to the transpiration stream (Kalma et al. 1998). Although we did not detect deuterium in xylem water extracted from T. cacao and G. sepium branches at the end of the experiment, we cannot exclude that deuterium might have remained in the stem, particularly in the heartwood. An earlier study has shown that G. sepium has considerable heartwood formation (Köhler et al. 2009). This way, small amounts of tracer might still be released from the heartwood over a longer time, thus leaving the system while being indistinguishable from background values. Assuming background signatures of ~50% and levels of deuterium in transpired water of ~30% would mean that 0.5 g of D_{2}O would leave a plant in the course of 14 days, assuming a daily transpiration of 10 kg day^{-1}. However, the undetected loss of tracer is small compared with the total amount of D_{2}O injected per individual (10–40 g) and thus unlikely to explain the high WU_{D,O}.

The assumption of mass conservation may have been violated due to the spillage and evaporation of D_{2}O during the injection. Tracer may also have gotten lost to the root system especially in those trees where D_{2}O was injected in the lower part of the trunk missing the main ascendant flux (Marc and Robinson 2004). A loss of 10% would have resulted in a 10% overestimate of plant water use. However, this correction is also insufficient to account for the difference between WU_{D,O} and WU_{TDP}.

The surface under the deuterium curve may have been underestimated especially for the tree species in the reforestation stand due to low sampling frequency. Although leaves were sampled twice a day at the beginning of the experiment, this might be insufficient to accurately capture the peak of the breakthrough curve. Further, leaf collection followed by ex situ condensation gives only instantaneous values of D_{2}O concentration. In contrast, the in situ collection of transpired water provides time averaging of the concentrations. Missing the maximum concentration would lead to an underestimation of the area under the breakthrough curves and thus an overestimation of the WU_{D,O}.

The damage caused by drilling may affect the uptake of the tracer due to cavitations of water columns in vessels near the holes (Dye et al. 1992, Kalma et al. 1998). However, for the plants where the TDP and D_{2}O tracing methods were employed simultaneously (S. contorta, S. polyperma, S. macrophylla and B. blumeana), disruption to xylem flow paths caused by the deuterium injection holes did not significantly alter measured sap flux. No discernible effects of drilling on sap flux density were reported by Kalma et al. (1998) and Meinzer et al. (2006). Further, Kline et al. (1970) stated that short-term anomalies during tree-trunk injections do not affect the final result.

Lack of complete mixing of the tracer is violating one of the conditions required (Kline et al. 1970, Calder et al. 1986). The high coefficient of variation in transpired water deuterium values among samples taken across the T. cacao and G. sepium canopies at the beginning of the experiment (when deuterium concentrations were high) indicated incomplete mixing. High sampling errors due to incomplete mixing may result in inaccurate estimates of transpiration (Kline et al. 1970). The heterogeneity in deuterium values within the canopy may be due to preferential flow from injection holes to specific points in the canopy and differences in radiation leading to variations in stomatal behaviour and transpiration. However, large sampling errors in the first days after label-
ling have little effect on the computed area of the curve as the slope of the rising curve is high.

The deuterium tracing method is based on the movement of tracers (chemicals, radioisotopes or stable isotopes) in steady-state systems (Hull 1958, Kline et al. 1970, Calder et al. 1986). Under field conditions where changes in radiation and vapour pressure deficit may lead to fractionation, isotopic steady-state conditions may be absent for a time (Roden and Ehleringer 1999). Evaporative deuterium enrichment was \( \sim 20\% \) at our Indonesian study site. For the days when control and labelled trees were sampled simultaneously, we can expect a good correction for fractionation. For intermediate days, fractionation may be different than the fractionation we assumed. The interpolation of background values probably captures slow changes (e.g., due to different soil water signature) rather well, but maybe not fast changes like fractionation caused by changes in environmental conditions and transpiration.

Sampling transpired water only from the upper part of the canopy might have biased our plant water use estimates. The coefficient of variation of tree water use measured from three canopy layers (bottom, mid and top) in a 3.5- and 5-year-old *E. grandis* tree was 7 and 9%, respectively (Kalma et al. 1998). Although differences in water use among canopy layers tend to be small, variation occurs and may influence how fast deuterium moves in different layers of the crown. We have too little data to assess how it would have influenced our results, but we recommend further work on this aspect.

Overall, the large difference between the methods cannot be fully explained. Either we overlooked sources of errors or the magnitude for one or more source errors may not have been estimated correctly. The WUD\(_{2O}\) values gave unrealistically high water use estimates for some species. Interestingly, some high water use values compiled by Wullschleger et al. (1998) were also obtained with tracer methods (140 kg day\(^{-1}\) for a 27-cm tree, 100 kg day\(^{-1}\) for a 13-cm tree and 94 kg day\(^{-1}\) for an 18-cm tree). Also, the highest value reported in Wullschleger et al. (1998) (1180 kg day\(^{-1}\), no diameter given) was obtained with tracers. This may imply that some of the underlying assumptions in using tracer might not be accurate.

Conclusions

Water use estimates derived from the deuterium tracing method were proportional to the TDP method. For some questions, in particular applied ones, this may be a satisfying level of information. In contrast, for many other purposes, one may require quantitative precise information, and there were large discrepancies (on average, factor 7) between the methods. Based on our results, the use of the D\(_2O\) tracing method cannot be recommended without further experimental testing if absolute values of whole-plant water use are a major goal. Building upon a rigorous assessment of reference plant water use, future experimental work should include concurrent monitoring of spatial (considering aboveground and belowground plant tissue) and temporal changes in xylem water deuterium signals, measuring transpiration water from the lower canopy stratum and assessing the evaporative enrichment factor in the leaf tissue. Future research may also aim at covering a broader range of tree sizes and thus water use rates also within a species. Our study suggests that water use estimation for bamboo by the D\(_2O\) tracing method is more difficult than for trees due to a potential redistribution of water and tracer among culms. However, water redistribution may also occur in trees having multiple stems or even among neighbouring individual stems. Therefore, the D\(_2O\) tracing method can be very helpful in revealing water redistribution. At this stage, the D\(_2O\) tracing method appears suitable for answering questions, such as relative differences in water use among trees, water redistribution among neighbours and internal water transport and storage processes in plants.

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