Bamboo Water Transport Assessed with Deuterium Tracing

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Received: 18 June 2019; Accepted: 23 July 2019; Published: 26 July 2019

Abstract: Bamboo water transport comprises the pathway rhizomes-culms-leaves as well as transfer among culms via connected rhizomes. We assessed bamboo water transport in three big clumpy bamboo species by deuterium tracing. The tracer was injected into the base of established culms, and water samples were collected from leaves of the labeled culms and from neighboring culms. From the base of labeled culms to their leaves, the average tracer arrival time across species was 1.2 days, maximum tracer concentration was reached after 1.8 days, and the tracer residence time was 5.6 days. Sap velocities were high (13.9 m d⁻¹). Daily culm water use rates estimated by the tracer method versus rates measured by a calibrated sap flux method were highly correlated ($R^2 = 0.94$), but the tracer estimates were about 70% higher. Elevated deuterium concentrations in studied neighbor culms point to deuterium transfer among culms, which may explain the difference in culm water use estimates. We found no differences in deuterium concentrations between neighbor-established and neighbor freshly sprouted culms of a given species. In two species, elevated concentrations in both neighbor-established and neighbor freshly sprouted culms were observed over an extended period. An applied mixing model suggests that five neighbor culms received labeled water. In contrast, for the third species, elevated concentrations in neighbor culms were only observed at the earliest sampling date after labeling. This could indicate that there was only short-term transfer and that the tracer was distributed more widely across the rhizome network. In conclusion, our deuterium tracing experiments point to water transfer among culms, but with species-specific differences.

Keywords: Bambusa vulgaris; Dendrocalamus asper; Gigantochloa apus; sap velocity; water residence time; water storage; water transfer; water use

1. Introduction

In plants, water transport occurs mainly along a pathway from soil water uptake via roots to transport through the stem to transpiration into the atmosphere at the leaf level [1,2]. This classic framework is extended by foliage water uptake [3,4], inverse flow from leaves to roots [2,5], hydraulic redistribution by roots [6–8], and water transfer among interconnected plants through roots or rhizomes [9,10]. These fluxes may further interact with internal water storage mechanisms [11–13]. Water transport within and among plants is of importance for many eco-physiological processes, e.g., regulating stomatal control by transporting root-produced abscisic acid to the leaves [14], supporting high transpiration rates with water previously stored in stems [11,15], or relieving water shortage by water redistribution among interconnected plants [9,10].

Bamboos, very fast-growing monocots, have different culm anatomical properties than dicotyledonous trees, which may lead to unique characteristics regarding water transport. Unlike dicotyledonous trees with a clearly separated xylem and phloem, bamboos have vascular bundles distributed across the bamboo culm walls that are comprised of xylem and phloem. Another difference
to dicotyledonous trees is the lack of secondary growth in monocots such as bamboos; xylem is thus not renewed over the life of a bamboo culm, while dicot trees produce new xylem every year [16]. In old bamboo culms, embolisms may thus substantially limit water use [17]. Compared to trees, bamboo species in previous studies had comparable [18,19] or higher [20,21] maximum sap flux densities. High sap flux densities of bamboos could potentially be due to large culm hydraulic conductivity and low sapwood capacitance, as previous findings across several tree species indicate that sap velocity and thus sap flux density is positively correlated to stem hydraulic conductivity, and negatively to sapwood capacitance [12]. If bamboos indeed have low sapwood capacitance, this should be reflected in a short residence time of a given molecule of water in the stem [12]. However, residence time was found to be longer in one bamboo species, *Bambusa blumeana*, than in reference tree species in a previous study [22]. There is a lack of further (multi-species) studies on bamboos to evaluate whether this finding is case-specific or represents a common trend across species.

Another distinctive characteristic of bamboos is their underground rhizome network. Dicotyledonous trees, in contrast, usually have clearly separated individual root systems. Bamboo rhizome systems have been reported to be a pathway of resource translocation among culms [23]. For *B. blumeana*, elevated deuterium (D$_2$O) concentrations were observed in the leaves of culms neighboring culms that had been labeled with D$_2$O [19]. When using thermal dissipation probes (TDP, [24]) in clumps of *Bambusa vulgaris*, measurements in established culms, freshly sprouted culms, and the connecting rhizomes indicated water transfer from established culms to newly sprouted culms in the period of sprouting [25]. For Moso bamboo (*Phyllostachys pubescens*), water transfer among culms was also assumed, and after cutting rhizomes between neighboring culms water use rates decreased by 20% [10]. However, given the large diversity in bamboos, these findings need to be tested further, on more species, and preferentially, with non-destructive methods. An example for such a non-destructive method is deuterium tracing. It has previously been applied for measuring sap velocity and water residence time in the stems of trees [11,12,26], and for estimating daily water use rates [19,22,27,28]. In these studies, deuterium water is first injected into the stem base of trees or bamboos (‘labeling’), and subsequently transpired water from leaves is periodically sampled to trace the D$_2$O movement. Deuterium tracing can also be an effective method for studying water transfer among connected neighboring plants, e.g., among bamboo culms via rhizomes.

In a botanical garden in Bogor (Java), Indonesia, we applied the deuterium tracing method and simultaneous sap flux measurements with species-specific calibration [21] on three tropical big, tall, clumpy bamboo species, *B. vulgaris*, *Gigantochloa apus*, and *Dendrocalamus asper* (Table 1). Considering the similar sizes and clumpy characteristics, we expected similar water transport characteristics among the three studied bamboo species. The objectives of our study were to assess bamboo water transport characteristics including sap velocities, water residence time, water use rates, and water transfer between culms.

### Table 1. Basic information on culms of the bamboo species *B. vulgaris*, *G. apus*, and *D. asper* (four culms for *B. vulgaris*, three culms for *G. apus* and *D. asper*) for the deuterium study: length (m), diameter at breast height (DBH, cm), culm wall thickness (cm), daily water use estimates as based on TDP measurements (WU$_{TDP}$, kg day$^{-1}$), and contribution of culm water storage to daily water use (C$_{WS}$, %). Means and standard errors. Note that the length of each culm was obtained by measuring the length of the culm from base to top after cutting down the culm at the end of the experiment.

| Species     | Length (m) | DBH (cm) | Culm Wall Thickness (cm) | WU$_{TDP}$ (kg day$^{-1}$) | Culm Water Storage/Daily Water Use (C$_{WS}$, %) |
|-------------|------------|----------|--------------------------|----------------------------|-----------------------------------------------|
| *B. vulgaris* | 17.9 (0.4) | 7.0 (0.2) | 1.3 (0.1)               | 8.1 (1.8)                  | 15.2 (3.4)                                    |
| *G. apus*    | 16.2 (1.3) | 7.9 (0.6) | 1.2 (0.1)               | 7.0 (0.8)                  | 9.1 (1.7)                                     |
| *D. asper*   | 21.1 (0.5) | 10.7 (0.5) | 2.4 (0.1)               | 8.0 (1.2)                  | 8.8 (0.6)                                     |
2. Materials and Methods

2.1. Study Site and Species

The study was conducted in the botanical garden in Bogor Agricultural University (IPB), Indonesia (6°33′40″ S, 106°43′27″ E, 182 m asl). The garden is designed for gathering varying species including many bamboo species, but there are not many individuals for each species. For the studied three bamboo species in our study, there were only one or two clumps available for each species, which is why we limited the studied clump number to one in our study design. The Rainfall in Bogor is 3978 mm per year and the mean annual temperature is 25.6 °C [29]. A relatively dry period occurs between June and September, whereas a wet season lasts from October to May. During the experimental period (8 March to 7 April 2012), daily mean soil moisture in the garden ranged from 0.28 to 0.39 cm3 cm−3, with an average value of 0.33 ± 0.03 cm3 cm−3. Of the 31 days in the study period, 15 were rainy days (>1 mm day−1), adding up to 279 mm over the study period. Two days with substantial rainfall (>20 mm) occurred at the beginning of the experiment (Figure A1).

During the wet season, new shoots of bamboos sprout from the soil and grow to their full length. Three bamboo species (B. vulgaris, G. apus, and D. asper) were selected for this study (Table 1). For each species, the selected bamboo culms were in one clump and located at the edge of the clump (Figure 1). The culm basal areas of the clumps were 18, 20, and 6 m2, and the ground projection areas of the canopies were 346, 427, and 63 m2 for B. vulgaris, G. apus, and D. asper, respectively.

Figure 1. (A) Experimental scheme for applying deuterium tracing and thermal dissipation probes (TDP) measurements, (B) the locations of labeled culms and their neighbor culms in a clump of B. vulgaris (n = 3 pairs of culms). The red, yellow, and green filled circles represent the labeled-established, the neighbor freshly sprouted, and neighbor-established culms, respectively. The white empty circles represent other, non-studied culms.

The anatomy of bamboo culms may change over time, which might lead to different sap flux densities or water use rates among culms of different ages. To assure that the culms we chose for our study were as even-aged as possible within each sampling category (i.e., species, established versus newly sprouted), we only chose directly neighboring culms at clump edges (see Figure 1A). We further
chose culms of similar culm color and also considered other visual appearance characteristics (e.g., regarding culm leaves) as well as culm length. The connections of the selected newly sprouted and neighboring established culms to the labeled culms were confirmed by direct observation of rhizomes after carefully removing the topsoil. For the clumping bamboo species in our study, we observed that each given labeled culm was directly connected to two culms via rhizomes, i.e., a ‘mother’ culm and a ‘child’. Together, such connections form a large underground rhizome systems, which may enable resource exchange even among culms that are not directly connected [16]. Crowns of labeled culms and their neighboring culms were equally exposed to the sun, with no apparent differences regarding environmental conditions.

2.2. D$_2$O Tracing

We focused on edge culms in our study because of their interesting role in promoting clump horizontal growth, which may go along with water (or other resource) transfer between established and newly sprouted culms at the clump edge. Three (G. apus and D. asper) or four (B. vulgaris) culms per species located in the edge of the clumps were labeled (Figure 1B; Table 1). The labeled culms were injected with D$_2$O (99.90%, euroiso-top, Gif sur Yvette, France) between 6:00 and 7:00 on 8 March 2013 (Figure 1). Choosing this injection time was based on the following considerations: (1) Due to reported high-root pressure of bamboos during the night [30], the injection should be applied after sunrise, so that injected deuterium water will not be pushed back out of the culms before it can be assimilated into the stream, and (2) If the injection is applied too late, i.e., when daytime bamboo transpiration is already high, cavitation of conduits could occur, which could also hinder the absorption of injected deuterium.

We followed the injection procedure as described by Dierick et al. [19]. First, plastic tubes were fixed at an angle of 45° to the bamboo culms (50 cm above the ground). For the smaller B. vulgaris and G. apus, four tubes were circumferentially distributed equally around each labeled culm, and for the larger D. asper, the amount of tubes was increased to six. The tubes were then filled with 40 mM KCl solution, and holes were drilled into the culm walls with the drill-bit submerged. The holes were subsequently flushed by injecting more KCl solution. Then, the remaining solution in the holes was extracted with a syringe and replaced by D$_2$O (1.5–3 g). As the D$_2$O slowly dissipated into the transpiration stream of the labeled culms, more KCl solution was added into the holes over several hours to ensure a full assimilation of D$_2$O. After sunset on the day of labeling, the remaining water in tubes and holes was collected, weighed, and a sample of 1.5 mL was stored at 4 °C for isotope analysis.

Following the approach developed by Calder [27], D$_2$O samples were collected from leaf condensate water by installing transparent bags (Figure 1). On each sampling day between 7:00 and 8:00, i.e., just before the morning rise of transpiration, ten self-sealing transparent bags were installed on five different sun-exposed regions of each labeled bamboo’s canopy (sealing 5–6 leaves per bag). The bags were collected at the same time the next day, so that we captured all the water transpired within 24 h. All ten condensate samples from a given culm were then combined into a mixed sample, and 1.5 mL were stored as described above. Sampling was conducted every day in the first ten days after labeling, and in four- to ten-day intervals for another three weeks. The sampling intervals in our study were based on reports from a previous study [27], where most of the injected D$_2$O was transpired within 10 days and only marginal amounts were collected in the days thereafter.

To detect whether there was D$_2$O retention in the studied bamboos, on 16, 17, or 20 April 2013, all labeled bamboos were harvested during the early morning hours (5:00–7:00). For every culm, three water samples each were extracted from leaves, branches, each 2 m segment along the culm, and rhizomes. The respective samples were sealed in transparent plastic bags immediately after cutting and left in the sun for approximately 4 h until enough water had collected in the bags to extract a 1.5 mL sample, which was stored as described above. The outside diameter, wall thickness, and height of each segment were further recorded to calculate the fresh volume ($v_f$, cm$^3$). Samples collected from each organ were weighed immediately after sampling ($w_f$, g) with a balance (KB2400-2N, KERN & SOHN GmbH, Germany) and dried at 100 °C in an oven, 24 h for leaves and branches and 48 h for rhizomes.
and culms. Then, the dry weight ($w_d$, g) was established. The water content of each segment was derived as $(w_f - w_d)/w_d$. The D$_2$O concentration in the collected leaf samples (40 days after labeling) was used as background values to calculate the amount of D$_2$O retention in the studied bamboos.

To test for D$_2$O transfer among bamboo culms, three established and three freshly sprouted neighbor culms, which were 30–50 cm distant to the established labeled culms (Figure 1), were sampled in 3–5-day intervals. The established culms were similar to the labeled ones regarding length, diameter, and leaf cover. The young culms had emerged 4–5 months before the tracer study began and had already reached a length of around 10 m or longer. However, they still had some brown sheaths attached to the culms and were not yet fully leafed. At approximately 6:00, small holes were drilled into the culm walls of the neighbor young and established culms at approximately 1 m height, and water samples (1.5 mL) were collected directly from the holes (Figure 1). The D$_2$O concentrations in culm segments of cut-down labeled culms (at the end of the experiment) were used as background values for comparing the D$_2$O concentrations in the culm samples of the labeled and neighbor culms.

2.3. D$_2$O Laboratory Analysis

The isotope analysis was carried out in the Center for Stable Isotope Research and Analysis (KOSI) at the University of Göttingen, Germany. The samples were measured in a Delta V Plus isotope ratio mass spectrometer (Thermo-Electron Cooperation, Bremen, Germany) coupled with a high-temperature conversion/elemental analyzer (TC/EA, Thermo Quest Finnigan, Bremen, Germany). As in previous deuterium studies (e.g., [19,31]), deuterium enrichment ($\delta^D$, %) is referenced to the Vienna Standard Mean Ocean Water standard and expressed in per mil [32]. The measurement precision for $\delta^D$ based on our approach is $\pm 2\%$.

2.4. D$_2$O Tracer Movement and Derived Velocity and Water Use

Several D$_2$O-related variables were used to describe water uptake and storage characteristics [12] of the studied bamboos. Arrival time ($T_{arrival}$, days) was defined as the time it took for the leaf D$_2$O concentration to surpass 10% of its maximum concentration for the first time. $T_{max}$ (days) was defined as the time when D$_2$O concentration reached its maximum value. Tracer residence time ($T_{residence}$, days) was estimated as the accumulated time when D$_2$O concentration was above 10% of its maximum. Lastly, tracer velocity ($V_{D_2O}$, m day$^{-1}$) was derived as the distance between the location of tracer injection (base) and sampling (leaves) divided by the arrival time.

To estimate water use rates with the deuterium tracing method ($WU_{D_2O}$, kg day$^{-1}$), $\delta^D$ values were converted to mass concentrations of the tracer [27] with the following equation:

$$WU_{D_2O} = \frac{M}{\sum_{i=1}^{T} c_i \cdot \Delta t_i}$$

where, $c_i$ is the mass concentration (g kg$^{-1}$) in the $i^{th}$ time increment, $\Delta t_i$ is the duration of the $i^{th}$ time increment (days), and $T$ is the last time increment. $M$ (g) is the total mass of injected D$_2$O. This function is usually used under two assumed conditions: (1) injected D$_2$O is fully mixed into the transpiration stream, and (2) no tracer loss in other ways except transpiration [33].

2.5. Sap Flow Measurements and Water Use Estimation with the Thermal Dissipation Probes (TDP) Method

On each labeled bamboo culm (three culms per species, four for B. vulgaris), sap flux density ($J_s$, g cm$^{-2}$ h$^{-1}$) was measured with self-made 1 cm length thermal dissipation probes (TDP) from 8 March to 6 April 2013 (i.e., encompassing the deuterium sampling period). Sensors were inserted into the bamboo culms at breast height [21] and below the lowest branches at around 7 m height. At each location, three pairs of TDP were circumferentially distributed equally around the culm. The signals from the three TDP sensors were connected in parallel to obtain averaged voltage values. The TDP signals were sampled every 30 s and stored as 1-minute averages using data loggers and multiplexers.
CR1000, AM16/32, Campbell Scientific Inc., Logan, UT, USA). $J_s$ was subsequently calculated with species-specific calibrated formulas for these bamboos [21].

For a comparison to tracer velocity derived by deuterium tracing ($V_{D2O}$, m day$^{-1}$), daily accumulated $J_s$ ($J_{s-d}$, kg cm$^{-2}$ day$^{-1}$) derived from the TDP method was converted into sap velocity ($V_{TDP}$, m day$^{-1}$). Water use ($WU_{TDP}$, kg day$^{-1}$) of each bamboo was derived by multiplying $J_{s-d}$ with the culm wall cross-sectional area at the location of sensor installation. Because $WU_{D2O}$ was not the arithmetic mean of daily water use during the experiment, mean TDP-derived WU and $WU_{D2O}$ cannot be directly compared [27]. Therefore, a weighted TDP-based WU was further calculated:

$$WU_{TDP-weight} = \frac{\sum WU_i \cdot c_i}{\sum c_i}$$

(2)

where, $WU_i$ is the daily water use (kg day$^{-1}$) in the $i^{th}$ period (day), and $c_i$ is the mean mass concentration of D$\textsubscript{2}$O in condensate water in the $i^{th}$ period.

To assess the influence of stem water storage on $WU_{D2O}$, the relative contribution of culm water storage to transpiration ($C_{WS}$, %) was calculated following the procedure by Goldstein et al. [15]. The underlying assumption is that the daily water use measured on the lower (near breast height) and upper culm (under first branches) is equal for each day. First, adjusted half-hourly water use at the upper culm was derived by multiplying its half-hourly water use with the ratio between daily water use at the lower culm and at the upper culm. Then, the difference between half-hourly water use at the lower culm and the upper culm was calculated, and the positive differences over each day were integrated, reflecting contributions of culm water storage to daily transpiration. Finally, the derived water storage contributions were divided by the according daily transpiration values to obtain $C_{WS}$.

### 2.6. Water Transfer among Culms
#### 2.6.1. The Receiving Culm

For a specific target culm (established or freshly sprouted) in our study, water uptake was assumed to potentially be from three different sources: water directly absorbed from the soil via roots, from neighbor labeled culms via rhizomes, and rhizome transfer from unlabeled neighbor culms. For the analysis, the contribution percentages of the three sources to the whole culm sap flow were summed to 100%. To estimate the contribution of each part, the isotope mixing model SIAR was used (Stable Isotope Analysis in R; [34]):

$$\delta D_{\text{neighbor}} = P_{\text{soil}} \times \delta D_{\text{soil}} + P_{\text{label}} \times \delta D_{\text{label}} + P_{\text{otherUnlabel}} \times \delta D_{\text{otherUnlabel}}$$

(3)

where, $\delta D_{\text{neighbor}}$ are the $\delta D$ values in the target culms neighbor the labeled culms, they were obtained from the fifth day since labeling, with subsequent 4–5-day intervals over the course of the deuterium tracing period. $\delta D_{\text{soil}}$ is the $\delta D$ value in the soil, which was assumed to equal to and thus substituted with averaged $\delta D$ values in the three to four cut-down labeled culms of each species. $\delta D_{\text{label}}$ is the $\delta D$ value in transpired water of the labeled culms, sampled from leaves from the fifth day since labeling to the 30th day. $\delta D_{\text{otherUnlabel}}$ is the unknown $\delta D$ value of the other, unlabeled culms. It was assumed to be within the range of the $\delta D$ values of all sampled culms. $P_{\text{soil}}$, $P_{\text{label}}$, and $P_{\text{otherUnlabel}}$ were the respective contribution percentages of each source. The trophic enrichment factor was set to 0 for each source.

The SIAR model outputs the probability density distribution of the contribution percentage for each culm, from which we calculated the 95% highest density region. Additionally, the mode (with the highest density) of each contribution percentage was extracted.
2.6.2. The Donating Culm

To estimate the maximum number of culms that may receive D$_2$O from a given labeled culm ($N_{\text{max}}$), we assumed that (1) any observed difference between WU$_{D2O}$ and WU$_{TDP}$ was caused solely by D$_2$O transfer, and (2) such water transfer took place continuously over the duration of the experiment. We applied the following equation to estimate $N_{\text{max}}$:

$$N_{\text{max}} = \frac{M_{\text{D-inject}} - WU_{\text{TDP-weight}} \cdot \sum c_i\text{-label} \cdot \Delta t_i}{WU_{\text{TDP-weight}} \cdot \sum c_i\text{-neighbor} \cdot \Delta t_i}$$  \hspace{1cm} (4)

where, $M_{\text{D-inject}}$ is the total injected D$_2$O mass (g), $WU_{\text{TDP-weight}}$ (kg day$^{-1}$) is the weighted water use of an individual culm measured by TDP, $c_i\text{-label}$ (g kg$^{-1}$) is the mean mass concentration of D$_2$O in condensate water of the labeled culm in the $i$th period, $c_i\text{-neighbor}$ (g kg$^{-1}$) is the mean mass concentration of D$_2$O in extracted culm water of the neighbor culm in the $i$th period, and $\Delta t_i$ is the duration of the $i$th time increment (days).

2.7. Data Analysis and Statistics

Differences in D$_2$O tracer movement and derived velocity and water use of the three bamboo species were examined with ANOVA after testing for homogeneity of variance. The D$_2$O signature in the different organs of the cut-down labeled culms were tested with the same procedure. Where ANOVA indicated differences among species or organs, the difference was further examined with a Gabriel’s test (designed for unequal sample sizes). Linear regressions were applied to explore relationships between WU$_{D2O}$ and WU$_{TDP}$, between $T_{\text{residence}}$ and WU$_{TDP}$, and between $T_{\text{residence}}$ and $C_{\text{WS}}$. The distributions of the $N_{\text{max}}$ were plotted in histograms and kernel density distributions were fitted, from which the mode was derived. All data analysis and plotting were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Tracer Movement, Sap Velocities, and Water Use Rates

On most of the studied bamboo culms, the D$_2$O concentrations showed a two-peak pattern during the experiment, with a drop in the second day after labeling, which was a rainy day (Figure A2). $T_{\text{arrival}}$ from the culm base to the crown was one day on most of the labeled bamboos, and three days on one culm of B. vulgaris (Table 2). Maximum D$_2$O concentrations in transpired water of labeled culms ($T_{\text{max}}$) appeared after 1–3 days (Table 2, Figure 2A, and Figure A2). $T_{\text{residence}}$ was on average 5.5, 6.3, and 5.0 days for B. vulgaris, G. apus, and D. asper (Table 2). The according sap velocities derived from deuterium tracing ($V_{D2O}$) were 12.5, 13.3, and 16.1 m day$^{-1}$ (Table 2). Daily water use estimates (WU$_{D2O}$ versus WU$_{TDP}$) were 11.3 versus 8.1, 13.2 versus 7.0, and 16.0 versus 8.0 kg day$^{-1}$ for B. vulgaris, G. apus, and D. asper, respectively (Tables 1 and 2).

| Species     | D$_2$O Injected (g) | $T_{\text{arrival}}$ (days) | $T_{\text{max}}$ (days) | $T_{\text{residence}}$ (days) | WU$_{D2O}$ (kg day$^{-1}$) | $V_{D2O}$ (m day$^{-1}$) |
|-------------|---------------------|-----------------------------|-------------------------|-------------------------------|---------------------------|-------------------------|
| B. vulgaris | 5.8 (0.8)           | 1.5 (0.5)                   | 2.5 (0.5)               | 5.5 (0.6)                     | 11.3 (2.8)                | 12.5 (2.4)              |
| G. apus     | 5.7 (0.1)           | 1.0 (0.0)                   | 1.3 (0.3)               | 6.3 (0.3)                     | 13.2 (1.6)                | 13.3 (0.3)              |
| D. asper    | 8.0 (0.7)           | 1.0 (0.0)                   | 1.7 (0.7)               | 5.0 (1.0)                     | 16.0 (3.6)                | 16.1 (2.2)              |

Table 2. Water transport characteristics of the bamboo species B. vulgaris, G. apus, and D. asper as based on deuterium tracing on four culms for B. vulgaris and three culms for G. apus and D. asper: amounts of injected D$_2$O (D$_2$O injected, g), times when deuterium arrived in the canopy ($T_{\text{arrival}}$, days), reached the maximum concentration ($T_{\text{max}}$, days), and amount of time it remained in the bamboos ($T_{\text{residence}}$, days). Derived daily water use (WU$_{D2O}$, kg day$^{-1}$) and daily sap velocity ($V_{D2O}$, m day$^{-1}$). Means and standard errors.
Based on the ANOVA test on 3–4 culms per species, we did not find significant differences among the three studied clumps (one clump per bamboo species) with regards to any of the studied water transport characteristics. There was a significant correlation between WU$_{\text{D}_2\text{O}}$ and WU$_{\text{TDP}}$ estimates ($R^2 = 0.94$, $P < 0.05$; Figure 3A). However, the deuterium tracing method gave, on average, 70% higher values than the TDP method. $T_{\text{residence}}$ did not significantly correlate with the contribution of culm water storage to daily transpiration ($C_{\text{WS}}$; Figure 3B). During the experiment, $C_{\text{WS}}$ was on average 15%, 9%, and 9% for B. vulgaris, G. apus, and D. asper, respectively (Table 1).

**Figure 2.** Deuterium signature ($\delta$D, ‰, mean ± standard error) in transpired water of labeled culms of *B. vulgaris*, *G. apus*, and *D. asper* over the course of the experiment (first row). Comparison among $\delta$D values in transpired water of labeled culms and in culm water of neighbor-established (Neighbor Es) and freshly sprouted culms (Neighbor Fs), starting five days after labeling (second row). Day 0 is the day of deuterium injection. The background value (labeled culm) was derived by averaging culm $\delta$D values of cut-down labeled bamboos after the experiment.

**Figure 3.** (A) Water use of the bamboo species *B. vulgaris*, *G. apus*, and *D. asper* estimated by the TDP method (WU$_{\text{TDP}}$, kg day$^{-1}$) versus water use determined by deuterium tracing (WU$_{\text{D}_2\text{O}}$, kg day$^{-1}$). The solid line represents the regression for the pooled values of all three species, dotted lines are species-specific regressions and the 1:1 line appears dashed. (B) Residence time (days) of deuterium in transpired water versus WU$_{\text{TDP}}$. 

| Species     | $\delta$D (‰) | Residence time (days) |
|-------------|----------------|-----------------------|
| B. vulgaris | 41.7 ± 1.4     | 14 ± 3                |
| G. apus     | 43.1 ± 1.3     | 15 ± 3                |
| D. asper    | 43.7 ± 2.3     | 12 ± 2                |

Table 2. Water transport characteristics of the bamboo species *B. vulgaris*, *G. apus*, and *D. asper* estimated by the TDP method (WU$_{\text{TDP}}$, kg day$^{-1}$).
3.2. D$_2$O Retention

In the cut-down bamboo tissues, the D$_2$O signature (δD, ‰) showed an increasing trend from culm base to leaves and rhizomes for *B. vulgaris* and *G. apus*, but not for *D. asper* (Table 3). In *B. vulgaris* and *G. apus*, the δD values were significantly higher in leaves than in the culm (*P* < 0.05, *t*-test). The remaining D$_2$O in the whole bamboos (including leaves, branches, culm, and rhizomes) varied between 0.005 to 0.075 g. A retention of such a small amount influences the estimate of differences between WU$_{D_2O}$ and WU$_{TDP}$ by only about 1% (Table 3).

**Table 3.** Deuterium signature (δD, ‰) in different organs of four *B. vulgaris* and three *D. asper* and *G. apus* where culms had been labeled 40 days earlier: signature in rhizomes, from different culm lengths, and from branches and leaves. The bamboos were cut down for the experiment. Means and standard errors. For each species, the different lowercase letters behind δD values indicate significant difference among organs.

| Species            | Rhizome | 0 m  | 2 m  | 6 m  | 10 m | Branch | Leaf  |
|--------------------|---------|------|------|------|------|--------|-------|
| *B. vulgaris*      | −32.9   | (2.8)a | −40.9 | −42.8 | −43.3 | −43.7 | −41.7 | −33.8 |
| *G. apus*         | −38.5   | (0.6)a | −42.4 | −44.3 | −43.1 | −41.7 | −38.4 | −30.2 |
| *D. asper*        | −44.9   | (1.3)a | −32.5 | −38.0 | −40.9 | −42.4 | −39.0 | −52.3 |

3.3. D$_2$O Transfer

Compared to the background δD values, significantly higher δD values were found in the transpiration stream of neighbor-established and freshly sprouted culms for almost the entire measurement period for *B. vulgaris* and *G. apus*, but only over the first six days for *D. asper* (*P* < 0.05, *t*-test; Figure 2). Such elevated δD values may be related to water transfer through rhizomes between the labeled and neighbor culms.

Water transfer among culms, especially among established ones, may happen in a number of different ways (continuous or intermittent, unidirectional or bidirectional flow). In our study, we only modeled one of these scenarios (continuous unidirectional flow) due to a lack of further data and the high complexity of modeling the other two scenarios. Based on the assumption that water transfer occurred continuously during the experiment, the total amount of transferred D$_2$O to a single neighbor culm varied between 0.36 to 0.93 g, accounting for 2%–12% of the injected D$_2$O. Results from the SIAR model suggested a considerable contribution of transferred water to daily transpiration of newly sprouted culms, i.e., on average 9%, 10%, and 12% for *B. vulgaris*, *G. apus*, and *D. asper*, respectively (Figure 4).

As the D$_2$O retention was negligible, the difference between WU$_{D_2O}$ and WU$_{TDP}$ can tentatively be assumed to be caused by water transfer among culms. We estimated the maximum number of culms that may receive D$_2$O from a given labeled culm (*N*$_{max}$) based on this assumption. *N*$_{max}$ ranged from 0–8.7, 3.5–5.3, and 8.5–40.9 for *B. vulgaris*, *G. apus*, and *D. asper*, respectively. The mode of the distribution of *N*$_{max}$ (the value that is most likely to appear), which represents the amount of culms that may receive D$_2$O from a given neighboring labeled culm, were 5.1 for *B. vulgaris* and 4.7 for *G. apus* (Figure 5), whereas results for *D. asper* were less conclusive (22 culms), corresponding to its often not significantly elevated deuterium concentrations in the prior analysis.
Using interval sampling, the peak values that were used to derive
Additionally, di
culms that may receive D<sub>2</sub>O from a given labeled culm (Figure 5), whereas results for
transferred water from the labeled culms (Figure 5). The distribution of the maximum amount of neighbor culms (N<sub>max</sub>) which may receive transferred water from the labeled culms (B. vulgaris, G. apus, and D. asper). Data of four established and four freshly sprouted culms were used for B. vulgaris, while three established and three freshly sprouted culms were used for G. apus and D. asper. The black lines represented fitted kernel distributions.

4. Discussion

4.1. Sap Velocity

Compared to the three bamboo species in our study (B. vulgaris, G. apus, and D. asper; Table 2), B. blumeana in the Philippines showed much longer T<sub>arrival</sub> (3.1 days) and T<sub>max</sub> (4.8 days), but smaller V<sub>D2O</sub> (8.5 m day<sup>−1</sup>) and V<sub>TOP</sub> (0.9 m day<sup>−1</sup>, [19,22]). Such differences among the bamboo species in the two studies may be attributed to species-specific anatomical structures, different sampling methods, or varying environmental conditions at the respective sites. Bamboos have highly variable sizes and shapes of vascular bundles among species [35], which may affect water transport velocities. Additionally, different sampling methods applied in our study and the study on the Philippines might further contribute to the observed differences. In the Philippine study, leaves were collected twice a day (interval sampling), while we installed transparent bags on the branches to gather water for a whole day [36]. Using interval sampling, the peak values that were used to derive T<sub>arrival</sub> and V<sub>D2O</sub> could potentially have been missed. Further, differences in soil properties among the study sites or climatic conditions during the respective measurement periods could be responsible for the observed
divergences. As such, soil moisture and climatic driving variables such as radiation and vapor pressure deficit have been shown to substantially affect water use characteristics of tree and bamboo species in previous studies [19,21]. Anyhow, given the similar sizes of studied bamboos in the Philippines and in our study and the similar oceanic tropical island climate, it is likely that we found species-specific differences. In our study, we did not find significant influences of culm dimensions (as presented in Table 1) on tracer velocity or residence time across the three bamboo species ($P > 0.05$, linear regression). This is likely due to the relatively small differences in dimensions among the three species.

The averaged $V_{D2O}$ of the three bamboo species was 5.6 times higher than $V_{TDP}$. Such large differences between $V_{D2O}$ and $V_{TDP}$ are in line with previous studies [11,12,19]. Actual $V_{D2O}$ in our study could potentially be even higher than derived from our D$_2$O sampling methodology: the arrival time ($T_{arrival}$, ~1 day for all but one culm) was derived based on a sampling interval of once a day and could thus theoretically be (much) shorter for a higher sampling frequency (e.g., every four hours). Thus, the $T_{arrival}$ values presented in our study can be considered the maximum D$_2$O transit times from base culm to canopy, while the derived $V_{D2O}$ are thus minimum estimates of sap velocity [11,12]. In previous studies, much higher $V_{D2O}$ compared to $V_{TDP}$ was found for one bamboo species (B. blumeana) and five tropical tree species [22] as well as for temperate conifers [12], and the authors [12] explained that sap velocity derived from sap flux density by a mere unit change is not equivalent to actual sap velocity in the conduits. Herein, the ratio of conduit area to the whole cross-sectional area may influence the calculation. In another study, sap flux density was described as the product of sap velocity, wood porosity, and water density [37]. Therefore, we estimated wood porosity from the easily obtainable variables, dry wood density and fresh wood volume [38], and subsequently recalculated $V_{TDP}$. This reduced the large relative deviation between $V_{D2O}$ and $V_{TDP}$ from 5.6 times to 0.86 times. However, this correction could also introduce errors and would benefit from a more accurate calculation of porosity. The different measurement scales of the D$_2$O tracing and the TDP measurements could be another reason for the observed large differences between $V_{D2O}$ and $V_{TDP}$. $V_{TDP}$ only represents the velocity between the installation heights of the TDPs (approximately 1.3 and 7 m), while $V_{D2O}$ is a mean value along a larger hydraulic path, from the stem base to the crown. Compared with other studies in bamboos [19,22], our observed $V_{D2O}$ are high.

4.2. D$_2$O Residence Time and Water Storage

Species-averages of $T_{residence}$ varied between 5.0 and 6.3 days for the three studied bamboo species (Table 3). In a previous study in the Philippines, $T_{residence}$ was reported to be 11.5 days for another bamboo species, B. blumeana, and 4.7 days for five reference tree species [22]. $T_{residence}$ of bamboos thus are shorter than the ~20 days reported for two tropical tree species in Panama [11] and much shorter than the 36–79 days for two coniferous species in North America [12]. $T_{residence}$ on trees was found to be positively correlated to normalized maximum crown-base sap flow, which implies the relative reliance of transpiration on water storage [11]. $T_{residence}$ was further found to correlate with sapwood capacitance, which reflects the water exchange capacity between transporting lumens and surrounding tissues [12]. It was therefore assumed that $T_{residence}$ could be an indicator of the relative reliance of transpiration on stem water storage [11]. In contrast, in our study, $T_{residence}$ had no significant relationship with either normalized maximum crown-base sap flow or daily $C_{WS}$ (Figure A3). The generally limited contribution of cult water storage to the relatively high daily transpiration of bamboos can be attributed to the substantially smaller volume of available storage tissue due to the hollow culms with only thin culm walls in comparison to, for example, trees of equal dimensions. In our study, average daily $C_{WS}$ during the experiment from 8 March to 6 April 2013 was 15%, 9%, and 9% for B. vulgaris, D. asper, and G. apus, respectively (Table 1). In contrast, for previously studied tree and palm species, 10–50% of daily transpiration was reported to be contributed by stem water storage [15,39–42]. Even though $T_{residence}$ was not significantly correlated to $C_{WS}$, it showed a slight (and non-significant) increasing trend with decreasing $V_{D2O}$ (Figure A4). This may indicate that high axial water transport efficiency reduces the residence time of water in bamboo culms. These findings
could support the hypothesis that a trade-off between water transport efficiency and water storage capacity contributes to a safe water status in the crown [11,43]. In bamboos, culm water storage may be relatively less important for maintaining leaf water status than the high water-transport efficiency. Such a line of argument is supported by findings of higher maximum sap flux densities in bamboos than in neighbor tree species [20,21].

4.3. Water Use Rates Derived with the Deuterium Tracing Method

Water use derived from the deuterium tracing method (WU\textsubscript{D2O}) was higher compared to the calibrated TDP method (WU\textsubscript{TDP}; Figure 3). On average, the deuterium tracing method gave 41%, 99%, and 87% higher values for \textit{B. vulgaris}, \textit{D. asper}, and \textit{G. apus}, respectively. Higher water use (11%–43%) derived from the D\textsubscript{2}O method was also found for \textit{Eucalyptus grandis} when compared to the heat pulse method [44], however, 7%–26% lower values were found for the same species with the same reference method [28]. For the only previously studied bamboo species (\textit{B. blumeana}) [22], on average, 813% higher water use was found compared to TDP. These substantial discrepancies between the D\textsubscript{2}O method and reference methods were assumed to stem from uncertainties associated with both methods [22,44]. As such, the commonly applied TDP sap flux method, when not calibrated, was found to yield on average 66% lower estimates across four bamboo species than simultaneous measurements with the stem heat balance method, the latter of which is believed to not require species-specific calibration [21]. Regarding the D\textsubscript{2}O method, the two primary potential sources of error are tracer loss (retention in labeled plants or redistribution to soil or neighbor plants) and incomplete mixing of D\textsubscript{2}O in the stem before flowing to different branches or crown levels. Both would violate the underlying assumptions of the applied deuterium methodology [22,44,45]. In our study, we reduced the effects of incomplete mixing by injecting deuterium at multiple positions around each culm.

4.4. D\textsubscript{2}O Retention

Remaining D\textsubscript{2}O was found in the labeled bamboos after cutting them 40 days after the D\textsubscript{2}O labeling (Table 2), but the amounts were too small to significantly affect the performed water use estimations. The total retained D\textsubscript{2}O was, on average, only 1% of the injected D\textsubscript{2}O, which could correspondingly lead to 1% higher estimate of WU\textsubscript{D2O}. In accordance with previous studies, this suggests that D\textsubscript{2}O retention does not affect water use rates estimates if the period since labeling is sufficiently long [11,22,45]. As such, substantial amounts of tracers were still found in \textit{Cordia alliodora} trees eight days after labeling [11], while no tracers were found in branches of \textit{Theobroma cacao} and \textit{Gliricidia sepium} three weeks after labeling [22]. Potential differences among species should further be taken into account when deciding for length of study period in D\textsubscript{2}O studies.

Although the retained D\textsubscript{2}O was too little to influence water use estimated by the D\textsubscript{2}O method significantly, it showed an interesting, reoccurring distribution within the labeled bamboo culm that had been cut down after the experiment. For \textit{B. vulgaris} and \textit{G. apus}, the retained D\textsubscript{2}O was substantially more concentrated in leaves and roots than in culms, with the lowest concentrations appearing at approximately 2 m culm height (Table 2). This D\textsubscript{2}O distribution may indicate that the injected D\textsubscript{2}O was relocated upwards and downwards from the points of injection. In leaves, the enrichment of D\textsubscript{2}O may also be partly attributed to element fractionation, i.e., a discrimination for regular hydrogen (and against heavy deuterium) in the leaves under conditions of high transpiration during the daytime [46]. Regarding the elevated concentrations in the roots, D\textsubscript{2}O could have been transferred there from the injection points in the culm, via the phloem according to the Münch flow-pressure theory [47]. It has been speculated that such inverse water flows through the phloem could be one possible mechanism to account for increased upstream D\textsubscript{2}O concentrations [19,22,48]. In \textit{Eucalyptus saligna} trees, D\textsubscript{2}O was observed translocating between phloem and xylem through ray parenchyma [49,50]. For bamboos, we would assume that water exchange between phloem and xylem is easier than for trees, as phloem and xylem are located at a close distance within the vascular bundles [51]. Generally, inverse water flow may be more pronounced when leaves are wet (e.g., due to rain, fog, or dew), as water potential
gradients from leaves to rhizomes can be formed under such conditions and facilitate water transport against the usual transpiration pathway of rhizomes-stem-leaves [2,3]. In our study, when rain occurred on the second day after D$_2$O labeling, a pronounced drop in D$_2$O concentration was observed in almost all labeled bamboos across the three species (Figure A2). Possibly, such a drop could also be related to foliage water uptake, which may dilute D$_2$O concentrations in the crown and stem [3,4].

4.5. D$_2$O Transfer between Culms

Water transported by inverse water flow may potentially be released from the roots to the soil, where it would then be available for uptake by neighbor plants [6–8], or it could directly be transferred to other trees or bamboo culms if the plants are connected underground [10,19,45,52]. In our study, we visually confirmed a direct rhizome connection between labeled and neighboring culms by carefully removing the topsoil. We interpreted increased D$_2$O levels in culms adjacent to labeled culms as indications of direct water transfer from donor culms to receiving culms via rhizomes (Figure 2). Previous studies have also given indications of direct water transfer via rhizomes among bamboo culms [25]. However, rather than D$_2$O transport via mass water flow, the elevated D$_2$O levels could potentially also be due to diffusion away from the location of D$_2$O injection in labeled culms, first downward into the rhizome system and then upward into neighboring culms. Such D$_2$O diffusion would likely take place in xylem and phloem, even though some studies have also found diffusion via other cell types (e.g., radial diffusion via rays and parenchyma in trees [11,44]). D$_2$O diffusion would probably occur during the nighttime, when the transpirational upward pull of stem water is marginal. However, due to active nighttime root pressure of bamboos [13,30], upward water flow could exist even during the night, which would likely limit D$_2$O diffusion in conducting pathways to periods when there is no upward flow of water at all. In a recent study on newly sprouted culms [25], the Münch theory was used for interpreting observed water transfer between newly sprouted culms and connected established culms. According to this theory, downward carbohydrate transport in the phloem is achieved by forming a hydrostatic pressure gradient, which relies on drawing and releasing water from surrounding tissues [53]. Yet another driving force for water transfer among connected bamboo culms may be water potential gradients, as it has been reported in previous studies on trees [9,54,55]. Applying these insights to our study, it seems that rather than diffusion, water transport mechanisms involving mass flow were likely responsible for the observed increased D$_2$O levels in culms neighboring labeled culms. This line of interpretation also goes along with the results of a recent study on the same bamboo species as in the present study: in rhizomes connecting freshly sprouted and neighboring established culms, active and substantial nighttime sap flux was observed, with water flowing from established to newly sprouted culms [25].

A model application suggests that the water contribution of labeled culms to the water use of neighbor freshly sprouted and established culms of the three studied bamboo species was 9%–12% (Figure 4). In another recent study on a running bamboo species (P. pubescens), freshly sprouted culms where rhizomes had been cut consumed approximately 20% less water use than control culms with intact rhizomes [10]. The difference was assumed to be related to water transfer through rhizome networks among culms [10]. However, the rhizome-cutting experiment interrupts the entire resource exchange between culms, and decreases in water use of isolated culms are thus not necessarily indicative of water transfer under intact conditions. Here, we see a clear advantage of the applied less-intrusive deuterium tracing method.

The reliance on the water transfer to and from culms may further depend on the age of the culms. Freshly sprouted culms are generally thought to rely to a higher degree on (water) transfer from the rhizome network and connected established culms [10,16]. However, in our study, similarly elevated δD values were observed for both freshly sprouted and established culms neighboring the labeled culms. For all three studied bamboo species, the elevated values were significantly higher ($P < 0.05$ with $t$-test; Figure 2) than background values (cut-down labeled culms after the experiment). The elevated D$_2$O concentration did not differ significantly between the neighbor freshly sprouted and
established culms \( (P > 0.05 \text{ with } t\text{-test}) \). This indicates that water transfer via rhizomes might be a typical water transport characteristic at the edge of bamboo clumps, regardless of culm ages. Such an indistinctive form of water transfer may indicate that D\(_2\)O was first translocated to the underground rhizome system \( (e.g., \text{ by diffusion, hydrostatic pressure gradient, or water potential differential}) \), from where it was redistributed to several culms via transpirational mass flow. However, the rhizome systems of bamboos are complex \([23]\) and understanding the water transfer mechanisms and their drivers among culms is only at the beginning. Water transport of culms located at the clump edge, as in our study, may substantially differ from that of culms in clump centers, where competition for resources and space is much higher. The total D\(_2\)O loss of a given labeled culm also depends on how many neighbor culms receive water via rhizome transfer. As an intuitive minimum, one culm could be directly connected at least to one parent culm and one (or more) offspring culm(s). However, bamboos, and particularly clumpy bamboos such as the studied species, commonly have complex rhizome systems with multiple interconnected culms \([16,23]\), and thus, water transfer likely takes place to more than just two neighbor culms. This is supported by the findings of our study, which suggest that donor culms transfer water to 4–5 neighbor culms \( (\text{for two of the three studied species, Figure 5}) \). Our findings thus provide further indications that clumpy bamboos transport water downwards into their underground rhizome system by means of diffusion, hydrostatic pressure gradient, or water potential differential, and that the water is redistributed to other culms within the clump. Further, water transfer among culms, especially among established ones, may happen in a number of different ways \( (\text{continuous or intermittent, unidirectional or bidirectional}) \), also due to the different conditions and mechanisms mentioned above. In our study, we only modeled one extreme scenario \( (\text{continuous unidirectional flow}) \) due to a lack of data and the high complexity of modeling the other two scenarios. Thus, future studies will be necessary to investigate the mechanics and pathways of bamboo water transfer in more detail.

4.6. Lessons for Future Water Transfer Studies on Bamboos

Based on the experiences drawn from our study, for future studies investigating water transfer among bamboo culms we would recommend the following changes to the applied D\(_2\)O methodology: Firstly, some control culms \( (\text{i.e., culms not directly connected with labeled culms}) \) should additionally be monitored, they can probably only be found in different culms. Secondly, soil moisture and soil deuterium concentration with increasing depth within and around the studied culms should be monitored continuously, synchronized with the deuterium sampling on labeled and associated neighbor culms. Thirdly, we recommend standardizing the water sampling intervals of culm and leaves, particularly during the period directly after labeling, a higher sampling frequency than our daily sampling could further allow for more precise estimates of the studied water transport characteristics. Fourthly, future studies aiming to investigate the number of potential donor culms \( (N_{\text{max}}) \) for a given species could benefit from more spatial replicates than was the case in our study. Lastly, the comparison of freshly sprouted and established neighbor culms could be improved. We found no significant differences between the two, which could partly be due to the already culminated length growth and ongoing leaf flushing of the young culms. It may be interesting to study culms in the very early sprouting stages when culms are completely leafless but length increment remains explosive over weeks. Also, differences in bamboo age may lead to differences in certain anatomical properties and in amount and type of leaves and branches. It is thus recommendable for future studies on bamboo water transport to investigate the anatomical and morphological traits of the involved species in more detail.

5. Conclusions

By applying a deuterium tracing method and a reference sap flux method on three clumps of tropical clumpy bamboo species \( (\text{one clump per species}) \), we may find indications for specific water transport characteristics of bamboos. Compared to one previous study on another bamboo species \( (B. \text{ blumeana}) \), the bamboos in our study had higher sap velocities, which could potentially be even
higher at a higher sampling frequency. The high sap velocities, in combination with relatively short residence times and a generally small contribution of culm water storage to daily transpiration, may indicate that bamboo transpiration relies more on water transport efficiency than on water storage mechanisms. We found indications that at least two of the three studied species transferred water among neighboring culms via underground rhizome networks. Our design may not be sufficient to derive overall conclusions on the ecohydrology of the 3 bamboo species, however, our experiments do give interesting first insights for further exploring water transport within and among individuals of the clumpy bamboos. Our work provides an example of the application of deuterium tracing for assessing water redistribution within bamboo clumps. Such insight may also enhance the water management of bamboos in the future.

**Author Contributions:** Conceptualization, T.M. and D.H.; methodology, T.M. and D.F.; software, D.F.; validation, T.M., D.H. and A.R.; formal analysis, D.F.; investigation, T.M., A.R. and D.F.; resources, D.H.; data curation, D.F.; writing—original draft preparation, T.M.; writing—review and editing, T.M., D.F., D.H. and A.R.; visualization, D.F.; supervision, D.H.; project administration, D.H.; funding acquisition, D.H.

**Funding:** This research was funded by the German Research Foundation (DFG, grant number: HO 2119 and CRC 990-A02). Dongming Fang received a scholarship from the China Scholarship Council (CSC). In the later stages of manuscript preparation, Dongming Fang and Tingting Mei were supported by the State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, China, Zhejiang Province Key Science and Technology Projects (grant number: 2015C03008), Zhejiang Provincial Natural Science Foundation (ZJNSF, grant number: Q19C160022), and Zhejiang Postdoctoral foundation (code number: zj20180155).

**Acknowledgments:** We thank our assistant Wahyu Iskandar for his work and enthusiasm during the field experiments. We also thank the Department of Facility and Property, the Ecology Lab and the Agricultural Faculty of Bogor Agriculture University (IPB), Indonesia, for assisting our field work. We especially thank the IPB Faculty of Forestry for their cooperation in our project.

**Conflicts of Interest:** The authors declare no conflict of interest.

### Appendix A

**Figure A1.** Soil moisture (cm³ cm⁻³) and rainfall (mm day⁻¹) in the study period.

| Date       | Soil moisture (cm³ cm⁻³) | Rainfall (mm day⁻¹) |
|------------|-------------------------|---------------------|
| 08Mar13    | 0.5                     | 100                 |
| 14Mar13    | 0.4                     | 60                  |
| 20Mar13    | 0.3                     | 20                  |
| 26Mar13    | 0.2                     | 10                  |
| 31Mar13    | 0.1                     | 5                   |
| 07Apr13    | 0.0                     | 0                   |

Figure A1. Soil moisture (cm³ cm⁻³) and rainfall (mm day⁻¹) in the study period.
Table A1. Testing result of the homogeneity of variance among the unbalanced data. The tested water concentration (D2O, %).

| Source            | DF | Sum of Squares | Mean Square |
|-------------------|----|----------------|-------------|
| Total             | 9  | 8.90           |             |
| Corrected         | 7  | 3.60           |             |
| Error             | 2  | 4.26           | 0.61        |
| Error             | 6  | 10.07          | 1.44        |
| Error             | 1  | 3.00           | 0.43        |

Figure A2. Deuterium signature (δD, ‰) in transpired water of labeled culms of B. vulgaris, G. apus, and D. asper over the course of the experiment. Each single line stands for one culm. There are 4, 3, 3 culms included for B. vulgaris, G. apus, and D. asper, respectively.

Figure A3. Residence time in relation to the contribution of culm water storage to daily water use (CWS, %).

Figure A4. Residence time in relation to the sap velocity estimated by TDP (V_{TDP}, m day^{-1}).
Table A1. Testing result of the homogeneity of variance among the unbalanced data. The tested water transport characteristics based on deuterium tracing on four culms for *B. vulgaris* and three culms for *G. apus* and *D. asper*: times when deuterium arrived in the canopy (*T*\(_{arrival}\), days), reached the maximum concentration (*T*\(_{max}\), days) and amount of time it remained in the bamboos (*T*\(_{residence}\), days). Derived daily water use (*WU*\(_{D2O}\), kg day\(^{-1}\)) and daily sap velocity (*V*\(_{D2O}\), m day\(^{-1}\)). ‘*P* > 0.05 (*Pr > F*)’ indicate equal variance among species.

| Dependent | Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-----------|--------|----|----------------|-------------|---------|--------|
| *T*\(_{arrival}\) | species | 2 | 1.35 | 0.68 | 1.58 | 0.2724 |
| | Error | 7 | 3.00 | 0.43 | . | . |
| *T*\(_{max}\) | species | 2 | 0.76 | 0.38 | 0.62 | 0.5639 |
| | Error | 7 | 4.26 | 0.61 | . | . |
| *T*\(_{residence}\) | species | 2 | 4.79 | 2.39 | 1.66 | 0.2565 |
| | Error | 7 | 10.07 | 1.44 | . | . |
| *V*\(_{D2O}\) | species | 2 | 1383.1 | 691.6 | 1.37 | 0.3139 |
| | Error | 7 | 3525.1 | 503.6 | . | . |
| *WU*\(_{D2O}\) | species | 2 | 818.52 | 409.3 | 0.99 | 0.4190 |
| | Error | 7 | 2900.6 | 414.4 | . | . |

Table A2. Result of the analysis of variance (ANOVA) among the unbalanced data. The tested water transport characteristics based on deuterium tracing on four culms for *B. vulgaris* and three culms for *G. apus* and *D. asper*: times when deuterium arrived in the canopy (*T*\(_{arrival}\), days), reached the maximum concentration (*T*\(_{max}\), days) and amount of time it remained in the bamboos (*T*\(_{residence}\), days). Derived daily water use (*WU*\(_{D2O}\), kg day\(^{-1}\)) and daily sap velocity (*V*\(_{D2O}\), m day\(^{-1}\)). ‘*P* > 0.05 (*Pr > F*)’ indicate equal variance among species.

| Dependent | Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-----------|--------|----|----------------|-------------|---------|--------|
| *T*\(_{arrival}\) | Model | 2 | 0.60 | 0.30 | 0.70 | 0.5283 |
| | Error | 7 | 3.00 | 0.43 | . | . |
| | Corrected Total | 9 | 3.60 | . | . | . |
| *T*\(_{max}\) | Model | 2 | 2.57 | 1.28 | 1.42 | 0.3040 |
| | Error | 7 | 6.33 | 0.90 | . | . |
| | Corrected Total | 9 | 8.90 | . | . | . |
| *T*\(_{residence}\) | Model | 2 | 2.73 | 1.37 | 0.82 | 0.4787 |
| | Error | 7 | 11.67 | 1.67 | . | . |
| | Corrected Total | 9 | 14.40 | . | . | . |
| *V*\(_{D2O}\) | Model | 2 | 45.62 | 22.81 | 1.35 | 0.3182 |
| | Error | 7 | 117.88 | 16.84 | . | . |
| | Corrected Total | 9 | 163.50 | . | . | . |
| *WU*\(_{D2O}\) | Model | 2 | 38.72 | 19.36 | 0.71 | 0.5225 |
| | Error | 7 | 190.01 | 27.14 | . | . |
| | Corrected Total | 9 | 228.72 | . | . | . |
Table A3. Testing result of the homogeneity of variance among the deuterium signature ($\delta D$, %) in different organs of *B. vulgaris*, *G. apus*, and *D. asper* where culms had been labeled 40 days earlier: signature in rhizomes, from different culm lengths, and from branches and leaves. The bamboos were cut down for the experiment. 'P > 0.05 (Pr > F)' indicate equal variance among species.

| Species     | Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-------------|--------|----|----------------|-------------|---------|--------|
| *D. asper*  | organs | 4  | 380,615        | 95154       | 3.44    | 0.0515 |
|             | Error  | 10 | 276,822        | 27682       | .       | .      |
| *G. apus*   | organs | 4  | 62.44          | 15.61       | 0.86    | 0.5176 |
|             | Error  | 10 | 180.60         | 18.06       | .       | .      |
| *B. vulgaris* | organs | 6  | 1746.4         | 291.1       | 2.17    | 0.0896 |
|             | Error  | 20 | 2682.0         | 134.1       | .       | .      |

Table A4. Result of the analysis of variance (ANOVA) among the deuterium signature ($\delta D$, %) in different organs of *B. vulgaris*, *G. apus*, and *D. asper* where culms had been labeled 40 days earlier: signature in rhizomes, from different culm lengths, and from branches and leaves. The bamboos were cut down for the experiment. 'P > 0.05 (Pr > F)' indicate equal variance among species.

| Species     | Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|-------------|--------|----|----------------|-------------|---------|--------|
| *D. asper*  | Model  | 6  | 555.02         | 92.50       | 0.60    | 0.7289 |
|             | Error  | 12 | 1863.8         | 155.3       | .       | .      |
|             | Corrected Total | 18 | 2418.8 | . | . | . |
| *G. apus*   | Model  | 6  | 402.56         | 67.09       | 10.87   | 0.0003 |
|             | Error  | 12 | 74.05          | 6.17        | .       | .      |
|             | Corrected Total | 18 | 476.61 | . | . | . |
| *B. vulgaris* | Model | 6  | 491.48         | 81.91       | 7.95    | 0.0002 |
|             | Error  | 20 | 206.04         | 10.30       | .       | .      |
|             | Corrected Total | 26 | 697.52 | . | . | . |

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