Oxygen isotopes as a valuable tool for measuring annual growth in tropical trees that lack distinct annual rings

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Some trees from tropical areas lack visually detectable and consistent annual growth rings. As such, we have measured the radial variation of cellulose oxygen isotopes in trees that grow in seasonally dry forests of Northern Laos to explore the possibility if this method can be used for the identification of annual rings. One disk from a 7-year-old *Styrax tonkinensis* (*S. tonkinensis*) in plantation and two cores from two *Ficus semicordata* var. *semicordata* (*F. semicordata*) in forests were examined. High-resolution cellulose oxygen isotopes of *S. tonkinensis* and *F. semicordata* show clear cycles with amplitudes of 5‰ and 9‰, respectively. To further test if the oxygen isotope cycles that we observed are annual or not, a tree ring cellulose oxygen isotope model is employed. Input data of the model are relative humidity and modeled precipitation $\delta^{18}\text{O}$. The modeling results independently support our detection of oxygen isotope annual cycles. Therefore, we conclude that tree ring cellulose oxygen isotopes have great potential to identify annual rings in tropical trees, which typically lack distinct annual rings in the context of seasonal climate.

Keywords: tropical tree with non-distinct annual rings, cellulose oxygen isotopes, fractionation model, *Styrax tonkinensis*, *Ficus semicordata* var. *semicordata*

INTRODUCTION

Tropical forests contain roughly 25% of the carbon in the terrestrial biosphere, therefore, research on the responses of tropical forests to global change is critical in predicting future global carbon cycling (Bonan, 2008). To date, such research has primarily been focused at the leaf and community level, and studies on long-term changes at the individual tree and population levels are needed (Zuidema *et al.*, 2013). However, tropical trees often fail to develop reliable annual rings, because many species lack visible tree rings or have only indistinct ring patterns. As such, it is difficult to estimate the age and growth rate of tropical trees. This information is essential for understanding the forest development process and forest response to global change, and is also useful for reconstructing past climate. Therefore, developing a method for estimating the annual growth pattern of such trees with non-distinct rings is important. Dendrometer can measure the radial growth rate of tropical trees by continuous measurements of diameter growth (da Silva *et al.*, 2002; Ohashi *et al.*, 2005). However, the dendrometer method cannot provide tree's growth rate

prior to the marking period.

Another method to measure growth of trees is to detect the periodicity of wood formation. Tree ring cellulose oxygen isotopes ($\delta^{18}\text{O}$) are mainly controlled by soil water $\delta^{18}\text{O}$ and relative humidity (Roden *et al.*, 2000). In tropical areas with distinct rainy and dry seasons, the amount of precipitation shows clear seasonality, and generally relative humidity and precipitation $\delta^{18}\text{O}$ has a positive and negative correlation (amount effect) with the amount of rainfall in tropical regions (Araguas-Araguas *et al.*, 1998; Dansgaard, 1964). ^{18}O -depleted precipitation and high relative humidity in the rainy season results in lower tree ring cellulose $\delta^{18}\text{O}$, and vice versa in the dry season. Such seasonal cycles of precipitation $\delta^{18}\text{O}$ and relative humidity may generate seasonal cycles of tree ring cellulose $\delta^{18}\text{O}$, which can be used to identify annual growth (Anchukaitis *et al.*, 2008; Anchukaitis and Evans, 2010; Evans and Schrag, 2004; Poussart and Schrag, 2005). To date, however, no such study has been performed in northern Southeast Asia.

In this study, we measured tree ring cellulose $\delta^{18}\text{O}$ for three cores in total, collected from two species in plantation and natural forest, respectively in northern Laos in order to: (1) further investigate the potential of oxygen isotopes in the identification of annual growth in trees that have no anatomically distinct rings; (2) cross-check the periodicity between different tree species.

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TREE RING CELLULOSE OXYGEN ISOTOPE MODEL

During the process of taking soil water by tree roots there is no isotopic fractionation (White *et al.*, 1985). The xylem water is then transported to the leaves, where leaf water is subject to evaporation that results in isotopic enrichment, which is controlled by the relative humidity of the ambient environment (Barbour *et al.*, 2004; Roden *et al.*, 2000). This process is described by the equation of Craig and Gordon (1965):

$$\delta^{18}\text{O}_l = \delta^{18}\text{O}_s + \varepsilon^* + \varepsilon_k + e_a / e_i (\delta^{18}\text{O}_a - \varepsilon_k - \delta^{18}\text{O}_x) \quad (1)$$

where the subscripts l, s, x, and a refer to leaf water, source water, xylem water and water vapor in the air outside the leaf, respectively, ε^* (ε_k) is the equilibrium (kinetic) isotopic fractionation factor, and e_a (e_i) is the ambient (inter-cellular) vapor pressure. However, the $\delta^{18}\text{O}$ of leaf water is often not as enriched as predicted by the Craig-Gordon model (Allison *et al.*, 1985; Flanagan and Ehleringer, 1991; Flanagan *et al.*, 1991). Barbour *et al.* (2004) improved the Craig-Gordon model to include the potential influence of the Péclet effect, where diffusion of ^{18}O -enriched water away from the sites of transpiration in the leaves back into the ^{18}O -unenriched xylem water leads to lower $\delta^{18}\text{O}$ for bulk leaf water than predicted from a simple calculation based only on the evapotranspiration site. The bulk leaf isotopic composition is predicted by the following equation (Sternberg, 2009):

$$\delta^{18}\text{O}_L = (1 - \alpha)\delta^{18}\text{O}_s + \alpha\delta^{18}\text{O}_l \quad (2)$$

where $\delta^{18}\text{O}_L$ represents the $\delta^{18}\text{O}$ of bulk leaf water and α is the fraction of enriched water.

Then, carbohydrate that records the water $\delta^{18}\text{O}$ is produced in the leaves by combining water and carbon dioxide during the process of photosynthesis. After carbohydrates formation, it is then transported to the trunk where a fraction of the oxygen atoms from sucrose undergo isotopic exchange with xylem water during cellulose synthesis. Tree ring cellulose oxygen isotope is then calculated as:

$$\delta^{18}\text{O}_c = f_0(\delta^{18}\text{O}_s + \varepsilon_o) + (1 - f_0)(\delta^{18}\text{O}_L + \varepsilon_o) \quad (3)$$

where $\delta^{18}\text{O}_c$ represents the $\delta^{18}\text{O}$ of tree ring cellulose, f_0 is the exchanged proportion of carbohydrate oxygen with xylem water, and ε_o is the net biological isotopic fractionation factor between the xylem water and the exchanged oxygen in the carbohydrate.

During the whole process, it is generally assumed that atmospheric vapor is at equilibrium with xylem water and

Fig. 1. The study region, showing the locations of the Kachet village (black spot), the Muang Xai meteorological station (square) and monthly modeled precipitation oxygen isotope grid point (triangle).

that leaf temperature follows the ambient temperature. Based on these assumptions, $\delta^{18}\text{O}_a$ can be equal to $\delta^{18}\text{O}_p - \varepsilon^*$ and e_a/e_i be equal to the relative humidity (h) (Dongmann *et al.*, 1974). The cellulose oxygen isotopic ratio is expressed as (Sternberg, 2009):

$$\delta^{18}\text{O}_c = (\delta^{18}\text{O}_s + \varepsilon_o) + \alpha(1 - f_0)(1 - h)(\varepsilon^* + \varepsilon_k). \quad (4)$$

METHODS AND MATERIALS

Sampling site and local climate

To test the stable isotope chronometer in samples lacking ring structure, but from a region with distinct rainfall seasonality, we collected two cores of *F. semicordata* in

Fig. 2. Monthly relative humidity (black spot), precipitation (gray bar) between 1991 and 2010 at the Muang Xai instrumental station, and monthly modeled precipitation $\delta^{18}\text{O}$ (black triangle) from two grid points (Yoshimura *et al.*, 2008).

natural forests using a 12 mm diameter increment borer at breast height, and one disk of *S. tonkinensis* from a plantation near Kachet village, Luang Prabang, Laos (20.5°N, 102.3°E, 800 m asl; Fig. 1) in August 2011. We select *S. tonkinensis* in the plantation for our research, because we can know the exact age of *Styrax tonkinensis* based on the local villagers. So we can test if the age of *S. tonkinensis* that is measured by our method is right. There is a distance of *ca.* 1 km from the natural forests to the plantation. According to 1991–2010 meteorological data (Fig. 2) from the Muang Xai weather station (20.7°N, 102°E), which is located 40 km northwest of Kachet village, the mean annual temperature is 23°C and the mean annual precipitation is 1460 mm, of which 78% falls in the rainy season (May–September). In addition, relative humidity is higher in the monsoon season (May–September) and lower in the dry season (November–April) (Fig. 2).

F. semicordata (Figs. 3a and b; Ka-018 and -019) has apparent growth rings, whereas *S. tonkinensis* (Fig. 3c; Ka-d) has non-distinct ring boundaries. In order to identify tree ring cellulose $\delta^{18}\text{O}$ cycles, we measured stable isotopes in *S. tonkinensis* at 1 mm resolution. To test whether or not the apparent rings of *F. semicordata* are annual in nature, we analyzed tree ring oxygen isotopes of the two cores in the apparent rings. We can assume that tree ring cellulose $\delta^{18}\text{O}$ of the two trees should be highly correlated if the apparent rings of *F. semicordata* are actual annual rings, based on previous studies of tree ring cellulose $\delta^{18}\text{O}$ of *Fokienia hodginsii* from northern Laos, Vietnam and southern China (Sano *et al.*, 2012; Xu *et al.*, 2011, 2013a, 2013b). We measured 50 and 184 rings for Ka-018 and Ka-019, respectively. The age of the *F. semicordata* from the natural forest is unknown, whereas the *S. tonkinensis* in the plantation is 7 years old.

Fig. 3. Photo of sample for *Styrax tonkinensis* (Ka-d) and *Ficus semicordata* var. *semicordata* (Ka-018 and 019).

Cellulose extraction

To reduce the time required for sample preparation, we used the plate method (Xu *et al.*, 2011) to extract α -cellulose from the tree cores, which generally involves the chemical protocol of the Jayme and Wise method (Green, 1963; Loader *et al.*, 1997). The main feature of the plate method is that α -cellulose is extracted directly from the wood plate, rather than from individual rings (i.e., wood segments or powders). Due to wood shrinkage during the process of chemical treatment, a simple method to maintain the 1 mm resolution of the original sample was employed, whereby we measured the length of the original wood (L_o) and cellulose plates (L_c), then calculated the ratio of shrinkage (L_c/L_o), and subsequently cut samples from the cellulose plate under a microscope with a resolution of L_c/L_o mm.

Isotope measurements

Oxygen isotope ratios ($^{18}\text{O}/^{16}\text{O}$) of the cellulose samples were determined with a continuous flow system using an isotope ratio mass spectrometer (Delta Plus XL and Delta V ADVANTAGE) interfaced to pyrolysis-type elemental analyzers (TC/EA) at the Research Institute of Humanity and Nature (Kyoto, Japan) and Graduate School of Environmental Studies of Nagoya University (Nagoya, Japan). Cellulose $\delta^{18}\text{O}$ values were calculated by comparison to the Merck cellulose laboratory working standard, which was analyzed frequently during the measurement of the tree cellulose samples. Oxygen isotope results are presented in the δ notation as the per mil (‰) deviation from the international stable oxygen isotope standard Vienna Standard Mean Ocean Water (VSMOW): $\delta^{18}\text{O} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R_{sample} and R_{standard} are the $^{18}\text{O}/^{16}\text{O}$ ratios of the sample and standard, respectively. The analytical uncertainties for repeated

Fig. 4. Two input (a: relative humidity, b: precipitation $\delta^{18}\text{O}$) parameters for tree ring cellulose oxygen isotope modeling.

measurements of the Merck cellulose were approximately $\pm 0.16\text{‰}$ ($n = 78$).

Forward modeling

We hypothesize that the isotope composition of precipitation and relative humidity difference between wet season and dry season is sufficient to generate annual isotope cycles in trees (Fig. 2). We used the Sternberg (2009) model (Eq. (4)) to simulate a theoretical monthly stable oxygen isotope time series, based on local meteorological data (Fig. 2). Modeled isotope time series were then compared with our actual measured $\delta^{18}\text{O}$ chronology from *F. semicordata* and *S. tonkinensis* to test whether annual oxygen isotope cycles exist. Using the Sternberg (2009) model (Eq. (4)) and climate data to simulate tree ring cellulose $\delta^{18}\text{O}$ requires one important assumption: $\delta^{18}\text{O}$ of precipitation is equal to $\delta^{18}\text{O}$ of source water (xylem water).

Model parameters The equilibrium (ϵ^*) and kinetic fractionation factors (ϵ_k) are regarded as being relatively constant, and have been reported to be approximately 9‰ and 29‰, respectively (Allison *et al.*, 1985). The proportion of isotopic exchange between xylem water and carbohydrate oxygen (f_o) does not vary at different temperatures (Sternberg and Ellsworth, 2011), and we adopted a previously reported value of 0.42 as the f_o value for our calculations (Roden *et al.*, 2000; Sternberg and Ellsworth, 2011). Although the net biological isotopic fractionation factor between xylem water and exchanged oxygen (ϵ_o) is temperature-sensitive, ϵ_o changes little within a temperature range from 20 to 30°C (Sternberg and Ellsworth, 2011). Monthly temperature ranged from 18 to 26°C at our sampling site, and ϵ_o was 27‰, as has been observed in previous studies (Sternberg *et al.*, 1984; Sternberg and DeNiro, 1983; Yakir and DeNiro, 1990). Therefore, we used 27‰ as the ϵ_o value in the model. For the fraction of enriched water (α), values of 67% to 90% have been re-

Fig. 5. Radial variations in tree ring cellulose $\delta^{18}\text{O}$ in the three samples of *Styrax tonkinensis* and *Ficus semicordata* var. *semicordata*.

ported (Allison *et al.*, 1985; Flanagan *et al.*, 1991; Roden *et al.*, 2000). In our study, the sampling sites are in relatively wet mountainous regions where the Péclet effect does not seem to exert a strong influence on leaf water $\delta^{18}\text{O}$, and we assumed that α is equal to 80%.

Input data Based on Eq. (4), input data required are relative humidity and monthly precipitation $\delta^{18}\text{O}$. Although it is reasonable to assume that daytime mean relative humidity determines the leaf water $\delta^{18}\text{O}$, such data is not available in the study area. Therefore, we used daily mean relative humidity rather than daytime mean relative humidity as an input for the tree ring cellulose $\delta^{18}\text{O}$ model. Monthly relative humidity (Fig. 4a) was recorded by the Muang Xai weather station and Monthly oxygen isotope precipitation data (Fig. 4b) were derived from an Isotopes-incorporated Global Spectral Model (IsoGSM) (Yoshimura *et al.*, 2008). We averaged data from two sites that are the nearest grid points (20°N, 101.25°E; 20°N, 103.125°E) to the sampling sites.

RESULTS

$\delta^{18}\text{O}$ of *S. tonkinensis* and *F. semicordata*

The samples of *S. tonkinensis* show a distinct annual cyclicity in $\delta^{18}\text{O}$ values (Fig. 5a), with a mean $\delta^{18}\text{O}$ of 22.96‰. Minima and maxima in $\delta^{18}\text{O}$ can be distinguished with a mean amplitude of 8.7‰. There is a slightly decreasing trend of $\delta^{18}\text{O}$ maxima in the *S. tonkinensis* samples. The mean $\delta^{18}\text{O}$ values of two *F. semicordata* cores (Ka-018 and -019) are 24.22‰ and 24.42‰, with a mean amplitude of $\delta^{18}\text{O}$ cycles of 4.8‰

Fig. 6. Monthly variations for modeled tree ring cellulose $\delta^{18}\text{O}$.

and 5.1‰, respectively (Figs. 5b and c). No obvious long-term trend in $\delta^{18}\text{O}$ can be distinguished in the *F. semicordata* cores.

Modeled tree ring cellulose $\delta^{18}\text{O}$

Modeled tree ring cellulose $\delta^{18}\text{O}$ (Figs. 6 and 7) shows clear annual cycles, which are similar to those observed in the measured $\delta^{18}\text{O}$ of the *S. tonkinensis* and *F. semicordata* (Fig. 5). The peak of modeled tree ring $\delta^{18}\text{O}$ values in 1 year typically appears in February/March, with the modeled lowest value being in August/September (Fig. 6). Such annual cycles were also found in northern Thailand and Costa Rica, which has distinct dry and wet seasons (Anchukaitis *et al.*, 2008; Zhu *et al.*, 2012). Based on these cycles, measuring annual growth of trees without clear ring boundaries should be feasible.

To generate a continuous time series for measured tree ring cellulose $\delta^{18}\text{O}$, we developed a simple age model for the tree ring $\delta^{18}\text{O}$ by matching the shape of measured tree ring cellulose $\delta^{18}\text{O}$ with the modeled tree ring cellulose $\delta^{18}\text{O}$ time series, which has been used in previous studies (Anchukaitis *et al.*, 2008; Zhu *et al.*, 2012). We assigned the cellulose $\delta^{18}\text{O}$ maxima of each cycle of tree samples to the month with the highest value for modeled tree ring $\delta^{18}\text{O}$ in a year, and the cellulose $\delta^{18}\text{O}$ minima of each cycle to the month with the lowest modeled tree ring $\delta^{18}\text{O}$ value in a year. From these tie points, adjacent samples are linearly interpolated to monthly resolution. Age models were developed individually for each tree (Fig. 7). The mean inter-series correlation between the monthly $\delta^{18}\text{O}$ series for the three trees is 0.81, and the correlation between the two *F. semicordata* cores is 0.87. Annual radial growth increments of *S. tonkinensis* calculated from the age modeled $\delta^{18}\text{O}$ chronologies revealed a reduction in growth rate, which might be related to the juvenile effect (Fritts, 1976).

Fig. 7. Comparison between modeled (dashed line) and measured tree ring cellulose $\delta^{18}\text{O}$ (solid line).

The correlations between modeled monthly cellulose $\delta^{18}\text{O}$ and observed monthly $\delta^{18}\text{O}$ of Ka-d (*S. tonkinensis*), Ka-018, and Ka-019 (*F. semicordata*) are 0.79, 0.77, and 0.84, respectively. The mean of the modeled $\delta^{18}\text{O}$ is 23.99‰, which is close to the observed $\delta^{18}\text{O}$. However, the mean amplitude of the modeled $\delta^{18}\text{O}$ is 11.4‰, which is higher than that actually observed.

DISCUSSION

Annual oxygen isotope cycles

The cyclical variation of $\delta^{18}\text{O}$ in *S. tonkinensis* (Fig. 5a) suggests a forcing by seasonally varying environmental conditions. There are six clear $\delta^{18}\text{O}$ cycles, and the *S. tonkinensis* is 7 years old. Considering germination time, $\delta^{18}\text{O}$ cycles in *S. tonkinensis* (Fig. 5a) can be considered to represent annual cycles. $\delta^{18}\text{O}$ cycles of *F. semicordata* show similar features to those of *S. tonkinensis*, although the amplitude of the variations in *F. semicordata* is smaller than *S. tonkinensis*. Therefore, we also infer that the $\delta^{18}\text{O}$ cycles in *F. semicordata* are annual cycles. Modeled tree ring cellulose $\delta^{18}\text{O}$ also shows distinct annual cycles (Figs. 6 and 7). The formation of tree ring annual $\delta^{18}\text{O}$ cycles is thus dependent on the local climatic conditions (i.e., distinct wet/dry seasons). Based on tree ring cellulose oxygen isotope theory, precipitation $\delta^{18}\text{O}$ and relative humidity are the two main factors controlling tree ring cellulose $\delta^{18}\text{O}$. In the rainy season, low precipitation $\delta^{18}\text{O}$ is a function of both heavy rainfall (amount effect) and high relative humidity (weak evapotranspiration) and results in lower tree ring cellulose $\delta^{18}\text{O}$ (Eq. (4)), and vice versa in the dry season.

Given that the annual cycles include several apparent growth rings in *F. semicordata*, these apparent rings are clearly not annual in nature. Such growth rings that are not annual rings are also found in *Ficus sycomorus* in Kenya (Maingi, 2006) and in *Cercis siliquastrum* in Switzerland (Schweingruber, 2007). Based on the previous study, such recurrent patterns of intra-annual zones are usually genetically determined and modified by environmental factors (Schweingruber, 2007). Daily precipitation data from Muang Xai weather station show the periodic rainfall events during one year, which may lead to these growth rings within one year, because cambial growth was high during high-rainfall season for the trees in Venezuela (Worbes, 1999). To improve understanding on the growth rings of *F. semicordata*, dendrometer and wood anatomy research is needed.

These annual cycles in $\delta^{18}\text{O}$ make it possible to reconstruct an exact chronology and measure annual growth in trees without a clear ring structure. In addition, annual cycles in different species show similar features to some extent. For example, there are two $\delta^{18}\text{O}$ peaks, although one is small and is not an annual cycle, in the year 2010 (shown with gray shading in Fig. 5) for *S. tonkinensis* (Ka-d) and *F. semicordata* (Ka-019), which corresponds to two peaks of modeled tree ring $\delta^{18}\text{O}$ in 2010 and indicates that cross-dating by tree ring cellulose $\delta^{18}\text{O}$ is feasible. However, such two peaks pattern in year 2010 in sample Ka-018 is not distinct, although it exists. Therefore, building up a chronology only relying on one time series is challenging and may introduce uncertainties and, as such, several oxygen isotopes time series are required for cross-dating.

The mean $\delta^{18}\text{O}$ value in *S. tonkinensis* is ca. 1.3‰ lower than that in *F. semicordata*, whereas the annual amplitude of $\delta^{18}\text{O}$ values in *S. tonkinensis* is about 3.5‰ larger than that in *F. semicordata*. We can infer the following reasons for these differences. Firstly, the *S. tonkinensis* is relatively young and its shallow root system uptakes upper soil water (Vu Dinh Phuong, 1985), whereas the *F. semicordata* is older than the *S. tonkinensis* and its relatively deep root system uptakes soil water not only from upper soil water but also from the middle or lower part of the soil profile. Although the exact age of the *F. semicordata* is unknown, we infer that the age of the *F. semicordata* is more than 20 years, based on counting of $\delta^{18}\text{O}$ annual cycles. A $\delta^{18}\text{O}$ study of soil water in southwest China (Luo and Wang, 2008) indicated that the range of $\delta^{18}\text{O}$ of precipitation and $\delta^{18}\text{O}$ of soil water at depths of 50 and 100 cm are 0‰ to -10‰, -2‰ to -9.3‰, and -2.7‰ to -8.3‰, respectively. This decreasing trend in the amplitude of $\delta^{18}\text{O}$ soil water with depth is brought about by mixing of precipitation from different periods and events. Therefore, the annual amplitude of $\delta^{18}\text{O}$ values in *S. tonkinensis* with a relatively shallow

root system is higher than in *F. semicordata*. Secondly, shorter growing season of *F. semicordata* may lead to smaller amplitude for annual cycles of precipitation $\delta^{18}\text{O}$ that is related to tree ring $\delta^{18}\text{O}$. Thirdly, sampling resolution of *F. semicordata* is lower than of *S. tonkinensis*, which may also result in lower annual amplitude of *F. semicordata*. For the difference mean $\delta^{18}\text{O}$ value in *S. tonkinensis* and *F. semicordata*, leaf morphology influences the Péclet effect that is related to leaf water enrichment, and *F. semicordata* and *S. tonkinensis* have different leaf morphologies that may lead to the difference in the mean $\delta^{18}\text{O}$ values between the two species (Eq. (4)). Besides, the proportion of isotopic exchange between xylem water and carbohydrate oxygen (f_o) may vary according to the different species (Li *et al.*, 2011), which could also result in variable mean tree ring $\delta^{18}\text{O}$ values for different species (Eq. (4)).

Comparison between modeled and observed tree ring $\delta^{18}\text{O}$ values

The modeled cellulose $\delta^{18}\text{O}$ and observed $\delta^{18}\text{O}$ of *S. tonkinensis* and *F. semicordata* show similar variations (Fig. 7). The mean modeled $\delta^{18}\text{O}$ value is 23.99‰, which is similar to the observed $\delta^{18}\text{O}$ value. However, the mean amplitude of the modeled $\delta^{18}\text{O}$ is 11.4‰, which is higher than that observed. The main reason for this difference may be related to our assumption that the $\delta^{18}\text{O}$ of precipitation is equal to the $\delta^{18}\text{O}$ of the source water. In some cases, $\delta^{18}\text{O}$ values of soil water are much less variable than that of precipitation (Tang and Feng, 2001) as not only the precipitation in a current month, but also that in previous months, will control soil water $\delta^{18}\text{O}$. Given the long residence time of meteoric waters in soil result in temporal mixing of different seasonal water sources, this results in both smoothing and dampening of the amplitude of the annual signal. Therefore, mixing in the soil contributes to the smaller average amplitude of the soil water/tree ring cellulose $\delta^{18}\text{O}$ annual cycles. A previous tree ring cellulose $\delta^{18}\text{O}$ modeling study (Anchukaitis *et al.*, 2008) in Costa Rica also showed that the best match between overall variance in the simulated and actual $\delta^{18}\text{O}$ for experimentally planted trees was achieved using a soil–water mixing ratio of ca. 30:70 (i.e., 30% new precipitation mixing with 70% of the previous month's soil water). In addition, if trees absorb not only rainwater, but also underground water, the amplitude of the xylem water $\delta^{18}\text{O}$ annual cycle will be smaller than the amplitude of the precipitation $\delta^{18}\text{O}$ annual cycle.

The amplitude of modeled cellulose and observed $\delta^{18}\text{O}$ values (*S. tonkinensis*) during the first 3 years (Fig. 7a, gray shading) are very similar, this appears to support our assumption. We can infer that the roots of the young tree are shallow and absorb soil water in the upper part of the soil profile, and that the $\delta^{18}\text{O}$ of upper soil water is

mainly controlled by recent precipitation. However, the annual amplitude of modeled cellulose $\delta^{18}\text{O}$ is higher than the amplitude of observed $\delta^{18}\text{O}$ values in Ka-d (*S. tonkinensis*) after the tree is 3 years old, and as the roots become relatively deeper and uptake soil water from the deeper part of the soil profile. Future studies on $\delta^{18}\text{O}$ variations in profiles of soil water with different soil types should be carried out in order to improve the accuracy of tree ring cellulose $\delta^{18}\text{O}$ modeling.

CONCLUSIONS

We have measured radial variations in tree ring cellulose $\delta^{18}\text{O}$ for *S. tonkinensis* and *F. semicordata* and identified clear annual cycles. Tree ring cellulose $\delta^{18}\text{O}$ modeling results independently support our detection of $\delta^{18}\text{O}$ annual cycles. Therefore, tree ring cellulose $\delta^{18}\text{O}$ studies have great potential to detect the annual growth of tropical trees that do not exhibit distinct annual rings related to seasonal climate.

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