Multiple isotopic models using various kinetic fractionation coefficients to estimate $\delta^{18}\text{O}$ of leaf water

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Highlight

For hourly and daily mean data sets, the employment of modified kinetic fractionation coefficients significantly improved model performance for $\delta^{18}\text{O}$ of bulk leaf water.

Abstract: Investigation of $\delta^{18}\text{O}$ of leaf water may improve our understanding of the evapotranspiration partitioning and material exchange between the inside and outside of leaves. In this study, $\delta^{18}\text{O}$ of bulk leaf water ($\delta_{\text{L,b}}$) was estimated by both isotopic–steady–state (ISS) and non–steady–state (NSS) assumptions considering the Péclet effect. Specifically, we carefully modified kinetic fractionation coefficients (α_k). The results showed that the Péclet effect is required to predict $\delta_{\text{L,b}}$. On the diel time scale, both NSS assumption + Péclet effect (NSS + P) and ISS assumption + Péclet effect (ISS + P) using modified α_k ($\alpha_{k\text{-modified}}$) for $\delta_{\text{L,b}}$ showed a good agreement with observed $\delta_{\text{L,b}}$ ($p > 0.05$). When using previously proposed α_k , however, both NSS + P and ISS + P were not reliable estimators of $\delta_{\text{L,b}}$ ($p < 0.05$). On a longer time scale (days), estimates of daily mean $\delta_{\text{L,b}}$ from ISS + P outperformed the estimates from NSS + P when using the same α_k values. Also, the employment of $\alpha_{k\text{-modified}}$ improved model performance in predicting daily mean $\delta_{\text{L,b}}$ compared to the use of previously proposed α_k . Clearly, special care must be taken concerning α_k when using isotopic models to estimate $\delta_{\text{L,b}}$.

Keywords: Assumption, isotopic model, kinetic fractionation coefficients, leaf water, non–steady–state, oxygen isotopes

Instruction

Oxygen isotope compositions of leaf water indicate material exchange between the inside and outside of leaves. For example, $\delta^{18}\text{O}$ of atmospheric CO_2 and atmospheric O_2 are partly dependent on the $\delta^{18}\text{O}$ of leaf water (Hoffmann *et al.*, 2004;

Luz and Barkan, 2011; Welp *et al.*, 2011). The oxygen isotope signal of leaf water can be incorporated in carbohydrates, which can be retained in cellulose (Sullivan *et al.*, 2006; Barbour, 2007; Gessler *et al.*, 2014). Therefore, the oxygen isotope signature of leaf water plays an important role in constraining models of the global carbon cycle and reconstructing past climates. In addition, the investigation of leaf water isotope composition has significant implications for hydrology. In recent years, leaf isotopic models have been developed and improved for the partitioning of evapotranspiration (Sun *et al.*, 2014; Wang *et al.*, 2015). However, these multiple uses require a thorough description and prediction of leaf water isotope compositions.

Isotope fractionation does not occur during water absorption and transport before transpiration, except in saline plants (Walker *et al.*, 2001), resulting in $\delta^{18}\text{O}$ values of water in non-transpiring plant organs (i.e. twigs) similar to those of the source water (δ_{source}). However, transpiration results in isotopic enrichment at evaporating sites within leaves (Cernusak *et al.*, 2016). According to Sun *et al.* (2014), H_2^{18}O is enriched at the evaporation sites of the stomata during water vapor diffusion from leaves to the ambient air as a result of isotope fractionation effects, namely temperature-controlled equilibrium isotope effects (equilibrium fractionation coefficient, α^+) and diffusion-controlled kinetic isotope effects (kinetic fractionation coefficient, α_k) (Luo *et al.*, 2013). The isotopically enriched water can then diffuse away from the evaporative sites into other parts of the leaf. Therefore, the $\delta^{18}\text{O}$ of leaf water becomes unevenly distributed, and the $\delta^{18}\text{O}$ of bulk leaf water ($\delta_{\text{L,b}}$) is depleted relative to the $\delta^{18}\text{O}$ of leaf water at the evaporative sites ($\delta_{\text{L,e}}$) (Farquhar and Cernusak, 2005; Farquhar *et al.*, 2007; Song *et al.*, 2013).

Gonfiantini *et al.* (1965) firstly discovered leaf water enrichment because of the evaporative process of transpiration. In the same year, Craig & Gordon (1965) proposed a model to describe the isotopic enrichment of open water bodies during evaporation, and this model can be modified to be applied in leaf water enrichment (Dongmann *et al.*, 1974). Recently, the traditional isotopic-steady-state (ISS) assumption (i.e., modified Craig-Gordon model), used to estimate $\delta_{\text{L,e}}$, has been challenged widely (Farquhar and Cernusak, 2005; Sun *et al.* 2014). The ISS

61 assumption requires that $\delta^{18}\text{O}$ of transpiration (δ_{T}) is equal to δ_{source} (Yepez *et al.*,
62 2003; Wang *et al.*, 2015). Indeed, this assumption can only be approximately satisfied
63 when transpiration is intensive (usually during midday) or over a long time scale
64 (Wen *et al.* 2008). Thus, a more complex non-steady-state (NSS) assumption,
65 considering heavy water fractionation during transpiration, has been introduced to
66 accurately predict diel variations in $\delta_{\text{L,e}}$ (Farquhar and Cernusak, 2005). Moreover,
67 studies have consistently suggested that the discrepancy between leaf water
68 enrichment at the evaporative sites beyond the source water ($\Delta_{\text{L,e}}$) and bulk leaf water
69 enrichment beyond the source water ($\Delta_{\text{L,b}}$) is due to the Péclet effect (P) (Farquhar *et*
70 *al.*, 2003; Farquhar and Cernusak, 2005; Song *et al.* 2013). This effect means that
71 unenriched source water in leaf veins, which is transferred to evaporative sites, is
72 opposed by a backwards diffusion of enriched water from the evaporative sites to the
73 leaf veins, which holds in both ISS and NSS and can be combined to predict leaf
74 water enrichment (Farquhar *et al.*, 2003; Farquhar and Cernusak, 2005). Various
75 models have been introduced; however, it remains unclear which one is the best to
76 describe leaf water enrichment over time under field conditions.

77 Furthermore, H_2 enrichment is predominately influenced by equilibrium isotope
78 effects, whereas ^{18}O enrichment is predominately influenced by the kinetic
79 fractionation coefficient (Cernusak *et al.*, 2016). Equilibrium isotope effects are
80 dependent on temperature and have been well described and understood, while kinetic
81 isotope effects are more complex. Kinetic fractionation occurs because the heavier
82 isotopes diffuse more slowly than the lighter ones, which include resistance associated
83 with water vapor transport and molecular diffusion (Dubbart *et al.*, 2013; Zhang *et al.*,
84 2018). The adequate determination of α_{k} is still subject of debate, and both values
85 previously assigned are still in use (Rothfuss *et al.*, 2012; Dubbart *et al.*, 2013).
86 According to Merlivat (1978), the molecular diffusion coefficient $D_{\text{v}}/D_{\text{v}}^{\text{i}}$, ratio of the
87 diffusivity of H_2^{18}O and H_2O in air equals 1.0285. However, recently, Cappa *et al.*
88 (2003) have claimed that this value needs to be revised and determined a new value of
89 1.032 on the basis of kinetic theory. Therefore, most studies have used an
90 approximate value of 1.032 ($\alpha_{\text{k-Cappa}}$) or 1.0285 ($\alpha_{\text{k-Merlivat}}$), although under field

conditions, α_k is variable (Rothfuss *et al.*, 2012). In contrast to expressing α_k by pure molecular diffusion, researchers associate α_k with the fractionation of pure molecular diffusion by an exponent nk , which is expressed as $\alpha_k = (D_v/D_v^i)^{nk}$ (Mathieu and Bariac, 1996; Stewart, 1975; Dubbert *et al.*, 2013). The exponent nk is dominated by the nature of the transport (Stewart 1975). For soil water evaporation, Dubbert *et al.* (2013) have linked nk with the soil water content, thereby reasonably well predicting the ^{18}O enrichment of soil evaporation water. However, studies on including various resistance influences on the kinetic effects for predicting leaf water enrichment are still scarce.

The estimation of $\delta^{18}\text{O}$ of leaf water largely depends on the leaf isotopic models and kinetic effects. In this study, $\delta_{L,b}$ values were simulated by ISS assumption with the Péclet effect (ISS+ P) and NSS assumption with the Péclet effect (NSS + P). Specifically, we carefully modified α_k ($\alpha_{k\text{-modified}}$) and tested different model performances when using $\alpha_{k\text{-modified}}$ and previously proposed α_k values ($\alpha_{k\text{-Cappa}}$ and $\alpha_{k\text{-Merlivat}}$). Our objectives were (1) to determine which model is useful to predict $\delta^{18}\text{O}$ of bulk leaf water and (2) to examine the validity of $\alpha_{k\text{-modified}}$ for the estimation of $\delta^{18}\text{O}$ of bulk leaf water.

Theoretical background

ISS assumption and kinetic fractionation coefficient

The $\delta^{18}\text{O}$ of leaf water at evaporative sites under ISS conditions, $\delta_{L,e\text{-ISS}}$, is commonly expressed in terms of kinetic fractionation factors ε_k (‰), equilibrium fractionation factors ε^+ (‰), $\delta^{18}\text{O}$ of xylem water δ_X (‰), $\delta^{18}\text{O}$ of water vapor outside the leaf δ_v (‰), and the relative humidity of air outside the leaf relative to leaf temperature h_v (%). This way, $\delta_{L,e\text{-ISS}}$ can be calculated as follows (Farquhar and Cernusak, 2005; Kahmen *et al.*, 2008):

$$\delta_{L,e\text{-ISS}} = \varepsilon_k + \varepsilon^+ + \delta_X + (\delta^{18}\text{O}_v - \varepsilon_k - \delta_X) \cdot h_v, \quad (1)$$

where $\varepsilon^+ = 2.644 - 3.206 \left(\frac{10^3}{T_L + 273.15} \right) + 1.534 \left(\frac{10^6}{(T_L + 273.15)^2} \right)$ changes as a function of leaf temperature T_L (°C) (Sun *et al.*, 2013), h_v is the ratio of water vapor pressure outside the leaf ($0.611 \times \exp(17.27 \times T_a / (T_a + 273.1)) \times h_a$, with T_a and h_a being air temperature

in °C and relative humidity in %, respectively) to saturated water vapor pressure in the leaf intercellular space ($0.611 \times \exp(17.27 \times T_L / (T_L + 273.15))$); ε_k is given by (Luo *et al.*, 2013):

$$\varepsilon_k = \frac{(\alpha_{ks} - 1)r_s + (\alpha_{kb} - 1)r_b}{r_s + r_b} \times 1000, \quad (2)$$

where r_s is stomatal resistance ($\text{m}^2 \cdot \text{s} \cdot \text{mol}^{-1}$), r_b is the boundary layer resistance ($\text{m}^2 \cdot \text{s} \cdot \text{mol}^{-1}$), α_{ks} is the kinetic fractionation coefficient associated with water vapor diffusion through the stoma ($\text{m}^2 \cdot \text{s} \cdot \text{mol}^{-1}$), α_{kb} is the kinetic fractionation coefficient associated with water vapor diffusion through the boundary layer. As r_b is far smaller than r_s , Equation (2) is rearranged as follows:

$$\varepsilon_k = (\alpha_k - 1) \times 1000. \quad (3)$$

Considering pure molecular diffusion, the α_k value is generally 1.032 (Cappa *et al.*, 2003) or 1.0285 (Merlivat, 1978); therefore, ε_k is equivalent to 32‰ ($\varepsilon_{k\text{-Cappa}}$) or 28‰ ($\varepsilon_{k\text{-Merlivat}}$). Deviating from the theory, Stewart (1975) presented α_k as follows:

$$\alpha_k = \left(\frac{D_v}{D_l} \right)^{nk}. \quad (4)$$

As field conditions generally vary between diffusive and laminar, for simulation isotope profiles of soil evaporating water, Mathieu and Bariac (1996) empirically expressed the contribution rate of turbulent resistance to total transport resistance in terms of soil water content. In addition, a recent study has suggested that the influence of aerodynamic resistance on kinetic effects needs to be considered (Lee *et al.*, 2009). This study did not take into account aerodynamic resistance as it is insignificant or extremely low in dense forests with relatively low wind speeds and high canopy height. Similar to the calculation of kinetic fractionation coefficients, which empirically linked the exponent nk with soil water content, we related the exponent nk to leaf surface water content θ_{surf} (%) as well as saturated θ_{sat} (%) and residual leaf water content θ_r (%) to describe the nature of α_k associated with transpiration. In this sense, the exponent nk , associated with transpiration, is defined as follows:

$$nk = \frac{(\theta_{\text{surf}} - \theta_r)n_a + (\theta_{\text{sat}} - \theta_{\text{surf}})n_s}{(\theta_{\text{sat}} - \theta_r)}, \quad (5)$$

where $n_a (=0.5)$ and $n_s (=1)$ are constants; θ_{surf} , θ_{sat} , and θ_r are expressed as follows:

$$\theta_{\text{surf}} = (W_F - W_D) / W_D \times 100\%, \quad (6)$$

$$\theta_{\text{sat}} = (W_T - W_D) / W_D \times 100\%, \quad (7)$$

$$\theta_r = (W_F - W_D) / W_F \times 100\%. \quad (8)$$

where W_F , W_T , and W_D are leaf fresh weight (g), turgid weight (g), and dry weight (g), respectively. In this study, θ_{surf} varied with little diel amplitudes (data not shown), so we considered θ_{surf} as constant on diel time scales to avoid an unnecessary complexity that does not significantly improve the model performance. Moreover, the differences in θ_{surf} among different days during the dry season or the wet season were not significant, whereas θ_{surf} differed between dry and wet season, with values of 107.7% for the dry season and 116.1% for the wet season. The values of θ_r were 51.8 and 53.7% for the dry season and the wet season, respectively, while θ_{sat} values for both seasons were 145%. Therefore, the calculated nk was 0.66 for the dry season and 0.70 for the wet season, and α_k was modified ($\alpha_{k\text{-modified}}$) as 1.0187 for the dry season and 1.0199 for the wet season.

162 The Péclet effect (P)

For leaves, researchers have described the Péclet effect in terms of leaf transpiration rate T_r ($\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), the molar density of water C ($= 55.56 \times 10^3 \text{ mol} \cdot \text{m}^{-3}$), the diffusion coefficient of H_2^{18}O in water D , and the effective path length for the effect L (m) (Sullivan *et al.*, 2007). Therefore, the Péclet effect (P) can be described as follows:

$$P = \frac{T_r L}{CD}, \quad (9)$$

where C ($= 55.56 \times 10^3 \text{ mol} \cdot \text{m}^{-3}$) is constant and D ($= 119 \times 10^{-9} \times \exp(-637/(136.15 + T_L))$) is temperature-dependent. The direct measurement of L is still difficult, but recent studies have shown that variations of L could be explained by variations in T_r (Zhou *et al.*, 2011; Ferrio *et al.*, 2012). Our study calculated L on the basis of L - T_r dynamics as proposed by Song *et al.* (2013), who carried out research on six field-grown broad-leaf and coniferous tree species and found that L values are significantly and negatively correlated with T_r ($L = 2.36 \times 10^{-5} \times T_r^{-1.20}$, $R^2 = 0.813$, $P < 0.0001$).

Under ISS conditions, bulk leaf water enrichment beyond the source water,

178 $\Delta_{L,b-iss} (= (\delta_{L,b-iss} - \delta_X)/(1 + \delta_X), \text{‰})$, can be modeled by leaf water enrichment at the
 179 evaporative sites beyond the source water, $\Delta_{L,e-iss} (= (\delta_{L,e-iss} - \delta_X)/(1 + \delta_X), \text{‰})$
 180 (Farquhar and Lloyd., 1993). In such a condition, $\Delta_{L,b-iss}$ is expressed as follows:

$$181 \quad \Delta_{L,b-iss} = \Delta_{L,e-iss} \times \frac{1 - e^{-P}}{P}, \quad (10)$$

182 i.e.,

$$183 \quad \delta_{L,b-iss} = \Delta_{L,b-iss} \cdot (1 + \delta_X) + \delta_X. \quad (11)$$

184 The NSS assumption

185 When stomatal conductance is low and/or when leaf water concentrations are
 186 high, NSS effects on the isotopic enrichment of leaf water should not be neglected
 187 (Cernusak *et al.*, 2016). Under NSS conditions, leaf water enrichment at the
 188 evaporative sites beyond the source water, $\Delta_{L,e-NSS}$ (‰), can be described by the
 189 improved model developed by Farquhar and Cernusak (2005) on the basis of
 190 Dongmann *et al.* (1974):

$$191 \quad \Delta_{L,e-NSS} = - \frac{\alpha^+ \alpha_k}{g w_i} \frac{d \left(W \cdot \frac{1 - e^{-P}}{P} \cdot \Delta_{L,e-NSS} \right)}{dt} + \Delta_{L,e-iss}, \quad (12)$$

192 where α^+ is $1 + \varepsilon^+$, g is total leaf conductance ($\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), $w_i (= W_a/h_v$, with W_a
 193 referring to the mole fraction of water vapor outside the leaf in $\text{mol} \cdot \text{mol}^{-1}$) is the mole
 194 fraction of water vapor in the leaf intercellular spaces ($\text{mol} \cdot \text{mol}^{-1}$), W is leaf water
 195 concentration ($\text{mol} \cdot \text{m}^{-2}$), and t is time (s). Note that $\Delta_{L,e-NSS}$ appears on both the right
 196 and the left side, making it difficult to calculate $\Delta_{L,e-NSS}$. To avoid iteratively
 197 calculating $\Delta_{L,e-NSS}$, Kahmen *et al.* (2008) advised a simpler approach to calculate
 198 $\Delta_{L,e-NSS}$. Over a relatively short time span, $\frac{d(W \cdot \Delta_{L,e-NSS})}{dt} = \frac{W \Delta_{L,e-NSS} - (W \Delta_{L,e-NSS})_{t-1}}{\Delta t}$,
 199 and Equation (12) changes as follows (Kahmen *et al.*, 2008):

$$200 \quad \Delta_{L,e-NSS} = \frac{\Delta_{L,e-iss} + W_{t-1} \cdot (\Delta_{L,e-iss})_{t-1} \cdot \left(\frac{1 - e^{-P}}{P} \right) \cdot \left(\frac{\alpha^+ \cdot \alpha_k}{g \cdot W_i \cdot \Delta t} \right)}{1 + W \cdot \left(\frac{1 - e^{-P}}{P} \right) \cdot \left(\frac{\alpha^+ \cdot \alpha_k}{g \cdot W_i \cdot \Delta t} \right)}, \quad (13)$$

$$201 \quad \text{i.e., } \delta_{L,e-NSS} = \Delta_{L,e-NSS} \cdot (1 + \delta_X) + \delta_X. \quad (14)$$

202 Assuming NSS conditions, bulk leaf water enrichment beyond the source water,
 203 $\Delta_{L,b-NSS}$, is expressed as follows (Farquhar and Cernusak, 2005):

$$\Delta_{L,b-NSS} = -\frac{\alpha^{+2}\alpha_{k2}}{gwi} \frac{d(W \cdot \frac{1-e^{-p}}{p} \Delta_{L,b-NSS})}{dt} + \Delta_{L,b-ISS}. \quad (15)$$

The calculation of $\Delta_{L,b-NSS}$ is similar to that of $\Delta_{L,e-NSS}$. Thus, Equation (15) is transformed in the following formula (Kahmen *et al.*, 2008):

$$\Delta_{L,b-NSS} = \frac{\Delta_{L,b-NSS} + W_{t-1} \cdot (\Delta_{L,b-ISS})_{t-1} \cdot (\frac{1-e^{-P}}{P}) \cdot (\frac{\alpha^{+2} \cdot \alpha_{k2}}{g \cdot W_i \cdot \Delta t})}{1 + W \cdot (\frac{1-e^{-P}}{P}) \cdot (\frac{\alpha^{+2} \cdot \alpha_{k2}}{g \cdot W_i \cdot \Delta t})}. \quad (16)$$

In addition,

$$\delta_{L,b-NSS} = \Delta_{L,b-NSS} \cdot (1 + \delta_X) + \delta_X. \quad (17)$$

Material and methods

Study site

The experiment was conducted in 2017 at the Jiufeng National Forest Experimental Station (116°05'E, N40°03'N). The station, at an elevation of 100 ~ 1,153 m above sea level, is situated in Beijing in a rocky mountainous area of north China. The climate is a typical continental monsoon climate characterized by four distinct seasons. The growing season in this area typically stretches from April to October. Mean annual precipitation is about 650 mm, with an uneven temporal distribution; 70 ~ 80% occur between July and September. Therefore, the period from April to June is the dry season, while the period from July to September is the rainy season. Mean annual potential evaporation is between 1,800 and 2,000 mm, which is significantly higher than precipitation. Mean annual temperature is 11.6°C, and mean monthly temperature ranges between -5 and 30°C, with maximum values in July and minimum values in November.

At the experimental station, the main tree species are drought-tolerant coniferous species. The central area (about 1 km²) of the sampling site, at an elevation of about 250 m, is dominated by 50 ~ 60 years old *Platycladus orientalis* trees with a similar growth status; average tree height is 8 m and average diameter at breast height 20 cm. The forest crown density is about 0.8.

Gas exchange and leaf and xylem water isotopic measurement

The experiment was carried out in 2017 at three typical sunny days in the dry season (May 24, May 30 and June 5,) and three typical sunny days in the wet season

(July 29, August 12 and August 17). Diel variations in leaf transpiration rate (T_r), stomatal conductance (g_s), and leaf temperature (T_L) were determined every hour from 0:00 to 24:00 using a portable photosynthesis system (LI-COR. USA, LI-6400). Gas exchange measurements were conducted on at least the top three intact mature leaves of the canopy from three *Platycladus orientalis* trees. Subsequently, these conifer leaves were sampled, covered with foil, and kept in liquid nitrogen tanks. In addition, annual branches from the upper canopy were collected from each of the three standard sample trees (three samples/tree), stored in 50-ml centrifuge tubes, tightly sealed, and stored in a portable refrigerator.

To determine bulk leaf water and xylem water, primary veins of the leaves were removed and the bark was removed from the branches. Subsequently, a cryogenic vacuum distillation system (Los Gatos Research Inc. USA, Li-2100) was used to extract leaf and xylem water. After extraction, $\delta^{18}\text{O}$ values of bulk leaf water ($\delta_{L,b-\text{observed}}$) and xylem water were analyzed using a liquid water isotope analyzer (Los Gatos Research Inc. USA, DLT-100) with a measurement precision of $\pm 0.1\text{‰}$.

Leaf water concentration and leaf water content measurement

On each sampling day, intact leaves from the upper canopy of each sampling tree were detached from twigs hourly from 0:00 to 24:00 and immediately weighed on an electronic scale to determine leaf fresh weight (FW_L). Image-processing software for Photoshop was used for leaf area (LA) measurement (Xiao *et al.* 2005). Thereafter, these leaves were soaked in deionized water for 24 h and then weighted again to determine leaf turgid weight (TW_L). Finally, all leaves were dried at 80°C for 48 h and weighted to obtain dry weight (DW_L); leaf water concentration (W) was calculated on the basis of leaf weight and leaf area as $W = (FW_L - DW_L)/LA$ (Kahmen *et al.* 2008).

Micrometeorological and atmospheric water vapor measurement

The sampling site was equipped with an 18-m high meteorological tower to continuously output micrometeorological data at 10-minute intervals. A temperature-humidity compound sensor (Onset. USA, HOBO-U30), installed at a height of 8 m, was used to measure air temperature (T_a) and relative humidity (h_a) at the upper canopy layer. In addition, a water vapor isotope analyzer (Los Gatos

Research Inc. USA, DLT-100), which is based on the Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS) approach, with a measurement precision of $\pm 0.2\text{‰}$ and a data output frequency of 1 Hz, was used to measure atmospheric water vapor concentration (W_a) and $\delta^{18}\text{O}$ (δ_v) at the upper canopy layer. Each measurement at the upper canopy lasted for 10 minutes. After measuring atmospheric water vapor three times, an extra 30 minutes were required to measure the standard water vapor concentration and $\delta^{18}\text{O}$ for calibration of the measured δ_v . In total, a full measurement cycle lasted for 1 hour.

Data analysis

Differences between observed and modeled $\delta_{L,b}$ as well as between observed $\delta_{L,b}$ and modeled $\delta_{L,e}$ were analyzed by one-way analysis of variance (ANOVA). Differences in modeled $\delta_{L,b}$ and $\delta_{L,e}$, calculated by assuming different model assumptions and using various α_k values, were also analyzed by one-way ANOVA. Results were considered statistically significant at $p < 0.05$. Deviations caused by modeled $\delta_{L,b}$ and $\delta_{L,e}$ from observed $\delta_{L,b}$ were absolute differences between observed and modeled $\delta_{L,b}$ as well as between observed $\delta_{L,b}$ and modeled $\delta_{L,e}$, respectively. The presented values are means $\pm$ standard deviations (SD).

Results

3.1 Environmental factors and atmospheric water vapor $\delta^{18}\text{O}$

On diel time scales, T_a represented an inverted-V shape, reaching maximum values during 12:00 ~ 16:00 and decreasing to minimum values at night, with diel amplitudes between 7.09 and 15.02 $^{\circ}\text{C}$ (Fig. 1). The h_a showed an opposite trend to that of T_a on diel time scales, with diel amplitudes between 23.56 and 39.44%. The average T_a and h_a values in the dry season were 23.30 $^{\circ}\text{C}$ and 45.50%, respective; while the average values in the wet season were 28.30 $^{\circ}\text{C}$ and 65.03%, respectively (Fig. 1).

However, W_a and δ_v varied with no clear diel patterns (Fig. 2). The W_a in the wet season (20.50 to 32.58 $\text{mmol}\cdot\text{mol}^{-1}$) was higher than that in the dry season (9.67 to 19.73 $\text{mmol}\cdot\text{mol}^{-1}$), while δ_v in the wet season (-19.27 to -13.11‰) was generally lower than that in the dry season (-17.73 to -10.00‰) (Fig. 2).

291

292 **Figure 1. Variations in air temperature (T_a) and relative humidity (h_a) during the six**

293 **experimental days.**

294

295 **Figure 2. Variations in atmospheric water vapor concentration (W_a) and $\delta^{18}\text{O}$ (δ_v) during the**

296 **six experimental days.**

3.2 Diel variations in T_r and g_s

Diel variations in leaf water enrichment were highly correlated with T_r and g_s . On diel time scales, T_r and g_s showed similar patterns of variability (Fig. 3). The greatest T_r and g_s values were recorded around midday (11:00 ~ 13:00), and their lowest values, approaching zero, were reached during the dark period. The T_r and g_s values in the dry season ranged from 0 to 0.15 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and from 0.01 to 1.67 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and were, in general, lower than the values in the wet season, which ranged from 0.01 to 0.20 and from 0.01 to 2.25 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. Midday depression of T_r and g_s was observed around 14:00 in the dry season, while no such phenomenon occurred in the wet season (Fig. 3).

Figure 3. Diel variability of leaf transpiration rate (T_r) and stomatal conductance (g_s) over the sampling days. Data represent mean values $\pm$ SD.

3.3 $\delta^{18}\text{O}$ of xylem water

The δ_X varied with no clear diel trend, and the diel amplitude value was between 0.43 and 2.73‰ (Fig. 4), indicating that the trees used identical or similar source water during the day. The δ_X values in the dry season varied from -7.80 to -4.38 ‰

and were thus lower than those in the wet season, which varied from -3.76 to -1.04 ‰ (Fig. 4).

Figure 4. Diel variation of $\delta^{18}\text{O}$ of xylem water (δ_x). The δ_x are represented as mean values $\pm$ SD.

3.4 $\delta^{18}\text{O}$ of bulk leaf water

The diel amplitude of observed $\delta_{L,b}$ ($\delta_{L,b\text{-observed}}$) ranged between 6.91 and 10.73‰ (Fig. 5), reaching maximum values during midday and minimum values in the early morning or evening. In the dry season, $\delta_{L,b\text{-observed}}$ ranged from 2.96 to 13.21‰. In contrast, $\delta_{L,b\text{-observed}}$ in the wet season ranged between -3.11 and 9.92‰.

Diel variations in modeled $\delta_{L,b}$ are also shown in Figure 5. Considering the Péclet effects, the $\delta_{L,b}$ were modeled assuming both ISS and NSS conditions and using different formulations for α_k values. Using $\alpha_{k\text{-modified}}$, both the ISS ($\delta_{L,b\text{-NSS,modified}}$, $-3.39 \sim 13.25$ ‰) and NSS assumptions for $\delta_{L,b}$ ($\delta_{L,b\text{-ISS,modified}}$, $-3.77 \sim 13.37$ ‰) showed a good agreement with $\delta_{L,b\text{-observed}}$ ($-3.11 \sim 13.21$ ‰). While using $\alpha_{k\text{-Merlivat}}$, modeled values of $\delta_{L,b}$ assuming the ISS ($\delta_{L,b\text{-ISS,Merlivat}}$, $-2.97 \sim 19.60$ ‰) and NSS conditions ($\delta_{L,b\text{-NSS,Merlivat}}$, $-2.61 \sim 19.58$ ‰) tended to overestimate $\delta_{L,b}$. In contrast, prediction of $\delta_{L,b}$ using $\alpha_{k2\text{-Cappa}}$, assuming both ISS ($\delta_{L,b\text{-ISS,Cappa}}$, $-2.60 \sim$

22.23‰) and NSS conditions ($\delta_{L,b-NSS,Cappa}$, $-2.24 \sim 22.21$ ‰), led to worst results than those one would calculate using $\alpha_{k-modified}$ or $\alpha_{k-Merlivat}$ (Fig. 5).

Figure 5. Diel variations in observed and modeled $\delta^{18}\text{O}$ of bulk leaf water ($\delta_{L,b}$). $\delta_{L,b-observed}$ is observed $\delta_{L,b}$; $\delta_{L,b-ISS,Cappa}$ and $\delta_{L,b-ISS,Merlivat}$ are simulated by ISS assumption (Eqn. 11) using $\alpha_{k2-Cappa}$ and $\alpha_{k2-Merlivat}$, respectively; $\delta_{L,b-ISS,modified}$ is simulated by ISS assumption (Eqn. 11) using $\alpha_{k2-modified}$; $\delta_{L,b-NSS,Cappa}$ and $\delta_{L,b-NSS,Merlivat}$ are simulated by NSS assumption (Eqn. 17) using $\alpha_{k2-Cappa}$ and $\alpha_{k2-Merlivat}$, respectively; $\delta_{L,b-NSS,modified}$ is simulated by NSS assumption (Eqn. 17) using $\alpha_{k2-modified}$. The modeled and observed $\delta_{L,b}$ are represented as mean values $\pm$ SD. Data are mean values $\pm$ SD.

3.5 $\delta^{18}\text{O}$ of leaf water at the evaporative sites

The diel variations in modeled $\delta_{L,e}$, assuming both ISS and NSS conditions and using different formulations to determine α_k values, were in accordance with the approximately dome-shaped diel patterns of modeled $\delta_{L,b}$ (Fig. 6). Values of $\delta_{L,e-ISS,Cappa}$ and $\delta_{L,e-NSS,Cappa}$ ranged from -2.99 to 24.27 ‰ and from -2.62 to 23.94 ‰, respectively, while $\delta_{L,e-ISS,Merlivat}$ and $\delta_{L,e-NSS,Merlivat}$ ranged from -2.55 to 27.39 ‰ and from -2.19 to 27.04 ‰, respectively. In contrast, $\delta_{L,e-ISS,modified}$ and $\delta_{L,e-NSS,modified}$ were

349 characterized at $-3.94 \sim 16.74\text{‰}$ and $-3.55 \sim 16.50\text{‰}$. Clearly, the modeled $\delta_{L,e}$
 350 values by using $\alpha_{k2\text{-Merlivat}}$ and $\alpha_{k2\text{-Cappa}}$ were significantly greater than those one
 351 would calculate using $\alpha_{k\text{-modified}}$ ($p < 0.05$; Fig. 6).

352
 353 **Figure 6. Diel variation in modeled $\delta^{18}\text{O}$ of leaf water at evaporative sites ($\delta_{L,e}$).** $\delta_{L,e\text{-ISS,Cappa}}$
 354 and $\delta_{L,e\text{-ISS,Merlivat}}$ are simulated by ISS assumption (Eqn. 1) using $\alpha_{k2\text{-Cappa}}$ and $\alpha_{k2\text{-Merlivat}}$,
 355 respectively; $\delta_{L,e\text{-ISS,modified}}$ is simulated by ISS assumption (Eqn. 1) using $\alpha_{k2\text{-modified}}$; $\delta_{L,e\text{-NSS,Cappa}}$
 356 and $\delta_{L,e\text{-NSS,Merlivat}}$ are simulated by NSS assumption (Eqn. 14) using $\alpha_{k2\text{-Cappa}}$ and $\alpha_{k2\text{-Merlivat}}$,
 357 respectively; $\delta_{L,e\text{-NSS,modified}}$ is simulated by ISS assumption (Eqn. 14) using $\alpha_{k2\text{-modified}}$. The
 358 modeled $\delta_{L,e}$ are represented as mean values $\pm$ SD.

359 Discussion

360 $\delta_{L,b}$ estimated by different assumptions and α_k values

361 In this study, δ_X in the wet season was significantly greater than that in the dry
 362 season (Fig. 4), indicating that *P. orientalis* used different water sources in different
 363 seasons. According to the findings of Liu *et al.* (2016), *P. orientalis* predominantly
 364 used water from natural springs during the dry season and uptake water from shallow
 365 layers during the wet season. However, on diel time scales, *P. orientalis* used similar

or identical water sources, and this could not explain the diel variation in $\delta_{L,b-\text{observed}}$. Our results showed that $\delta_{L,b-\text{observed}}$ presented a clear, dome-shaped diel pattern (Fig. 5). Midday maxima could be explained by ongoing residual leaf water enrichment from progressive loss of relatively ^{18}O -depleted evaporated water. In contrast, early morning or night minima could be ascribed to the addition of ^{18}O -depleted source water to leaves (Snyder *et al.*, 2010). Similar diel patterns have also been observed by Welp *et al.* (2008) and Cernusak *et al.* (2005).

On the diel time scale, NSS + P performed a little better in predicting $\delta_{L,b}$ over ISS + P when using $\alpha_{k-\text{modified}}$ (Fig. 7a). In contrast, ISS + P performed a little better in predicting $\delta_{L,b}$ over NSS + P when using the previously proposed α_k value. When using same α_k , differences in modeled $\delta_{L,b}$ assuming different isotopic conditions were not significant ($p > 0.05$); however, using different α_k values for modeled $\delta_{L,b}$ caused significant differences ($p < 0.05$). Using $\alpha_{k-\text{modified}}$, both ISS + P and NSS + P fit well to $\delta_{L,b-\text{observed}}$, with small deviations of $0.86 \pm 0.57\text{‰}$ and $0.66 \pm 0.44\text{‰}$, respectively. However, when using $\alpha_{k-\text{Merlivat}}$, ISS + P and NSS + P caused larger deviations of $2.91 \pm 1.63\text{‰}$ and $3.10 \pm 1.43\text{‰}$ from $\delta_{L,b-\text{observed}}$, respectively. The most significant deviations from $\delta_{L,b-\text{observed}}$ were caused by models using $\alpha_{k-\text{Cappa}}$ ($p < 0.05$), with deviations of 4.37 ± 2.07 (4.59 ± 1.88) ‰ for ISS+ P (NSS+ P) (Fig. 7a). It can thus be concluded that both ISS + P and NSS + P are useful for the estimation of $\delta_{L,b}$ at small temporal scales if α_k is well characterized.

On longer time scales (days), ISS + P performed better in predicting daily mean values of $\delta_{L,b}$ over NSS + P when using the same α_k (Fig. 7b). Using $\alpha_{k-\text{modified}}$, the ISS + P caused a negligible deviation of $0.27 \pm 0.23\text{‰}$ from daily mean values of $\delta_{L,b-\text{observed}}$, while NSS + P caused a larger deviation of $0.40 \pm 0.30\text{‰}$ from daily mean values of $\delta_{L,b-\text{observed}}$. In addition, using $\alpha_{k-\text{Merlivat}}$ and $\alpha_{k-\text{Cappa}}$, ISS + P also caused smaller deviations ($2.94 \pm 0.59\text{‰}$ and $4.41 \pm 0.85\text{‰}$, respectively) from daily mean values of $\delta_{L,b-\text{observed}}$ than those caused by NSS + P ($3.24 \pm 0.63\text{‰}$ and $4.73 \pm 0.89\text{‰}$, respectively) (Fig. 7b). This is most likely because the NSS assumption may introduce considerable complexities and uncertainties (Wang *et al.*, 2014; Cernusak *et al.*, 2016). Therefore, ISS + P is adequate and feasible on longer time scales.

Figure 7. Comparison of observed and modeled $\delta^{18}\text{O}$ of bulk leaf water ($\delta_{L,b}$). Fig. 7a represents an hourly data set, while Fig. 7b is based on a daily mean data set. $\delta_{L,b-ISS,Cappa}$ and $\delta_{L,b-ISS,Merlivat}$ are simulated by ISS assumption (Eqn. 11) using $\alpha_{k2-Cappa}$ and $\alpha_{k2-Merlivat}$, respectively; $\delta_{L,b-ISS,modified}$ is simulated by ISS assumption (Eqn. 11) using $\alpha_{k2-modified}$; $\delta_{L,b-NSS,Cappa}$ and $\delta_{L,b-NSS,Merlivat}$ are simulated by NSS assumption (Eqn. 16) using $\alpha_{k2-Cappa}$ and $\alpha_{k2-Merlivat}$, respectively; $\delta_{L,b-NSS,modified}$ is simulated by NSS assumption (Eqn. 16) using $\alpha_{k2-modified}$.

The Péclet effect

On diel time scales, the differences between $\delta_{L,b-observed}$ and $\delta_{L,e-ISS,modified}$ or $\delta_{L,e-NSS,modified}$ were significant ($p < 0.05$) (Fig. 7a), while the differences between $\delta_{L,b-observed}$ and $\delta_{L,b-ISS,modified}$ or $\delta_{L,b-NSS,modified}$ were not significant ($p > 0.05$) (Fig. 8a), indicating that the Péclet effect is required to predict plant leaf level $\delta_{L,b}$ at smaller temporal time scales. This is similar to the finding of Kahmen *et al.* (2008), who state that the Péclet effect is required to accurately predict leaf water enrichment in different Eucalyptus species on diurnal time scales. Ogee *et al.* (2007) also found that a spatially-explicit model was required to simulate leaf water $\delta^{18}\text{O}$ at a high temporal resolution. It should, however, be noted that $\delta_{L,e-ISS,modified}$ (or $\delta_{L,e-NSS,modified}$) rather matched $\delta_{L,b-observed}$ than $\delta_{L,b-ISS,Cappa}$ (or $\delta_{L,b-NSS,Cappa}$) and $\delta_{L,b-ISS,Merlivat}$ (or $\delta_{L,b-NSS,Merlivat}$) (Figs. 7a and 8a). Clearly, considering the Péclet effect but using previously proposed α_k values may not improve model performance for the estimation

416 of $\delta_{L,b}$.

417 On longer time scales (days), both ISS + P and NSS + P performed better in
418 predicting daily mean values of $\delta_{L,b}$ over ISS and NSS assumptions without the Péclet
419 effect when using the same α_k values (Fig. 8b), indicating that the Péclet effect is also
420 required to predict plant leaf level $\delta_{L,b}$ at larger temporal scales.

421
422 **Figure 8. Comparison of observed $\delta^{18}\text{O}$ of bulk leaf water ($\delta_{L,b}$) and modeled $\delta^{18}\text{O}$ of leaf**
423 **water at evaporative sites ($\delta_{L,e}$).** Fig. 8(a) represents an hourly data set, while Fig. 8(b) is based
424 on a daily mean data set. $\delta_{L,e}\text{-ISS,Cappa}$ and $\delta_{L,e}\text{-ISS,Merlivat}$ are simulated by ISS assumption (Eqn. 1)
425 using $\alpha_{k2}\text{-Cappa}$ and $\alpha_{k2}\text{-Merlivat}$, respectively; $\delta_{L,e}\text{-ISS,modified}$ is simulated by ISS assumption (Eqn. 1)
426 using $\alpha_{k2}\text{-modified}$; $\delta_{L,e}\text{-NSS,Cappa}$ and $\delta_{L,e}\text{-NSS,Merlivat}$ are simulated by NSS assumption (Eqn. 13) using
427 $\alpha_{k2}\text{-Cappa}$ and $\alpha_{k2}\text{-Merlivat}$, respectively; $\delta_{L,e}\text{-NSS,modified}$ is simulated by ISS assumption (Eqn. 13)
428 using $\alpha_{k2}\text{-modified}$.

429 Uncertainties associated with the estimation of $\delta_{L,b}$

430 Both ISS and NSS assumptions include imperfect parameterizations and
431 uncertain parameter values. Recent methodological advances in the Off-Axis
432 Integrated Cavity Output Spectroscopy (OA-ICOS) method have promoted the
433 development of the water vapor isotope analyzer, which is effective for the accurate
434 measurement of water vapor (Wang *et al.*, 2009). This allows us to directly determine
435 δ_V and δ_T (Wang *et al.*, 2012; Dubbert *et al.*, 2014, Song *et al.*, 2015), which could

greatly improve the accuracy of predictions of $\delta_{L,b}$. In this study, the high-frequency measurement of δ_V was expected to improve the accuracy of the estimation of $\delta_{L,b}$. However, the direct determination of δ_T , using a laser spectrometer with an integrated gas exchange chamber (Wang *et al.*, 2012), still bears uncertainties and limitations. The method developed by Wang *et al.* (2012) demands the water vapor in the chamber to be well mixed. Under such circumstance, the micro-climate conditions in the chamber are inevitably changed, that is to say, the measured flux under chamber conditions is more likely to turn into a turbulent one than under field conditions (Dubbert *et al.*, 2013), thus affecting the reliability of α_k and further impacting the measured results. This effect could be more pronounced in forests with high canopies and low wind speed (Pape *et al.*, 2009). In addition, the determination of δ_T , using the gas-exchange chamber approach, is characterized by a poor spatial representation. Therefore, δ_T was estimated by models rather than directly measured by the gas-exchange chamber approach. Nevertheless, a good agreement was found between observed and modeled $\delta_{L,b}$ for both ISS + P and NSS + P when using modified α_k . The parameter α_k is a key factor for determining model performance for the estimation of $\delta_{L,b}$. So far, there is no generally accepted calculation method for the accurate determination of α_k . In our study, we tried to relate α_k with leaf water content, which is useful to estimate $\delta_{L,b}$. However, how α_k varies among species and with environmental changes is still not clear. Therefore, a better description of the nature of α_k under field conditions is desperately needed.

In addition, the ISS and NSS assumptions used to calculate $\delta_{L,b}$ on the basis of the Craig-Gordon model, in which the parameter h_V is a controlling factor at smaller temporal scales (Lee *et al.*, 2007). Recent studies have suggested that high h_V could cause distortion of the computational solution (Lai *et al.*, 2006; Yang *et al.*, 2012). The denominator of the Craig-Gordon model is approaching zero when h_V exceeds 95%, and in such situations, outputs of models need to be excluded. In this study, h_V did not exceed 85% during the sampling period, which can improve the reliability of the Craig-Gordon model. Moreover, leaf water isotope enrichment is largely influenced by environmental and physiological parameters (Farquhar and Gan 2003;

Snyder *et al.*, 2010), and any variations in these parameters can be translated into $\delta_{L,b}$ fluctuation. Therefore, uncertainties in $\delta_{L,b}$ estimation might include measurement errors of environmental and physiological factors. In this study, environmental (i.e., T_a , h_a , C_v) and physiological parameters (g_s , T_r , W , T_L) were measured at a high frequency to improve the accuracy of $\delta_{L,b}$ prediction.

Conclusions

In this study, $\delta_{L,b}$ was estimated by multiple isotopic models using various α_k values. Modeled $\delta_{L,e}$ was significantly larger than modeled and observed $\delta_{L,b}$ ($\delta_{L,b-observed}$) at smaller and larger temporal scales, indicating that the Péclet effect is required to predict plant leaf level $\delta_{L,b}$. On the diel time scale, the NSS + P performed a little better in predicting $\delta_{L,b}$ over the ISS + P when using $\alpha_{k-modified}$, although both ISS + P and NSS + P using $\alpha_{k-modified}$ for $\delta_{L,b}$ showed a good fit with $\delta_{L,b-observed}$. However, both ISS + P and NSS + P using previously proposed α_k ($\alpha_{k-Cappa}$ and $\alpha_{k-Merlivat}$) have significantly overestimated $\delta_{L,b}$ values. On longer time scale (days), ISS + P was a better estimator of daily mean values of $\delta_{L,b}$ than NSS + P when using the same α_k values, as the NSS assumption is likely to introduce considerable complexities and uncertainties at larger temporal scales. Also, the employment of $\alpha_{k-modified}$ led to better results in predicting daily mean values of $\delta_{L,b}$ than one would calculate using $\alpha_{k-Cappa}$ and $\alpha_{k-Merlivat}$. Clearly, α_k is an important controlling factor for the estimation of $\delta_{L,b}$. To more accurately estimate $\delta_{L,b}$, we call on a better description of the nature of α_k .

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Author Contributions Statement

Yonge Zhang designed and performed the experiment. Yonge Zhang analysed the data and wrote the manuscript. Lihua Chen, Guodong Jia contributed significantly to data analysis, manuscript preparation and practice of experiment. Xinxiao Yu

496 revised the paper and finished the submission.

497 **References**

- 498 **Barbour MM.** 2007. Stable oxygen isotope composition of plant tissue, a review. *Functional*
499 *Plant Biology* **34**, 83–94.
- 500 **Cappa CD, Hendrichs MB, De Paolo DJ, Cohen RC.** 2003. Isotopic fractionation of water
501 during evaporation. *Journal of Geophysical Research* **108**, 4525.
- 502 **Cernusak LA, Farquhar GD, & Pate JS.** 2005. Environmental and physiological controls over
503 oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. *Tree*
504 *Physiology* **25**, 129–146.
- 505 **Cernusak LA, Barbour MM, Arndt SK, et al.** 2016. Stable isotopes in leaf water of terrestrial
506 plants. *Plant Cell & Environment* **39**, 1087–1102.
- 507 **Craig H, & Gordon LI.** 1965. Deuterium and oxygen-18 variations in the ocean and the marine
508 atmosphere. In Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and
509 Palaeotemperatures ed E. Tongiorgi., pp. 9–130. Lischi and Figli, Pisa.
- 510 **Dongmann G, Nurnberg HW, Förstel H, Wagener K.** 1974. On the enrichment of H₂¹⁸O in the
511 leaves of transpiring plants. *Radiation and Environmental Biophysics* **11**, 41–52.
- 512 **Dubbert M, Cuntz M, Piayda A, Werner C.** 2014. Oxygen isotope signatures of transpired
513 water vapor, the role of isotopic non–steady–state transpiration under natural conditions. *New*
514 *Phytologist* **203**, 1242–1252.
- 515 **Dubbert M, Cuntz M, Piayda A, Maguás C, Werner C.** 2013. Partitioning evapotranspiration–
516 Testing the Craig and Gordon model with field measurements of oxygen isotope ratios of
517 evaporative fluxes. *Journal of Hydrology* **496**, 142–153.
- 518 **Farquhar GD, & Gan KS.** 2003. On the progressive enrichment of the oxygen isotopic
519 composition of water along leaves. *Plant, Cell and Environment* **26**, 801–819.
- 520 **Farquhar GD, & Cernusak LA.** 2005. On the isotopic composition of leaf water in the
521 non–steady state. *Functional Plant Biology* **32**, 293–303.
- 522 **Farquhar GD, Cernusak LA, Barnes B.** 2007. Heavy water fractionation during transpiration.
523 *Plant Physiology* **143**, 11–18.
- 524 **Ferrio JP, Pou A, Florez–Sarasa I, Gessler A, Kodama N, Flexas J. & Ribas–Carbo, M.** 2012.
525 The Peclet effect on leaf water enrichment correlates with leaf hydraulic conductance and

mesophyll conductance for CO₂. *Plant Cell and Environment* **35**, 611–625.

Gessler A, Ferrio JP, Hommel R, Treydte K, Werner RA, & Monson RK. 2014. Stable isotopes in tree rings, towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood. *Tree Physiology* **34**, 796–818.

Gonantini R, Gratzu S, & Tongiorgi E. 1965. Oxygen isotope composition of water in leaves. In *Isotopes and Radiation in Soil-Plant Nutrition Studies*, pp. 405–410. IAEA, Vienna.

Hoffmann G, Cuntz M, Weber C, et al. 2004. A model of the Earth's Dole effect. *Global Biogeochemical Cycles* **18**, 1008.

Kahmen K, Simonin KP, Tu KP, Merchant A, Callister A, Siegwolf R, Dawson TE, Arndt SK. 2008. Effects of environmental parameters, leaf physiological properties and leaf water relations on leaf water delta ¹⁸O enrichment in different Eucalyptus species. *Plant Cell and Environment* **31**, 738–751.

Lai CT, Ehleringer JR, Bond BJ, Paw KTU. 2006. Contributions of evaporation, isotopic non-steady state transpiration and atmospheric mixing on the δ¹⁸O of water vapour in Pacific Northwest coniferous forests. *Plant Cell and Environment* **29**, 77–94.

Lee XH, Kim K, Smith R. 2007. Temporal variations of the ¹⁸O/¹⁶O signal of the whole-canopy transpiration in a temperate forest. *Global Biogeochemical Cycles* **21**, 3013.

Lee X, Griffis TJ, Baker JM, Billmark KA, Kim K, Welp LR. 2009. Canopy-scale kinetic fractionation of atmospheric carbon dioxide and water vapor isotopes. *Global Biogeochemical Cycles* **23**, 1002.

Liu ZQ, Yu XX, Jia GD, Jia JB, Lou YH, Lu WW. 2017. Contrasting water sources of evergreen and deciduous tree species in rocky mountain area of Beijing, China. *Catena* **150**, 108–115.

Luo L, Yu WS, Wan SM, Zhou P. 2013. Advances in the study of stable isotope composition of leaf water in plants. *Acta ecologica sinica* **33**, 1031–1041.

Luz B, Barkan E. 2011. The isotopic composition of atmospheric oxygen. *Global Biogeochemical Cycles* **25**, 3001.

Mathieu R, Bariac T. 1996. A numerical model for the simulation of stable isotope profiles in drying soils. *Journal of Geophysical Research Atmospheres* **101**, 12685–12696.

555 **Merlivat L.** 1978. Molecular diffusivities of H_2^{16}O , HD^{16}O , and H_2^{18}O in gases. *Journal of*
556 *Chemical Physics* **69**, 2864–2871.

557 **Ogée J, Cuntz M, Peylin P, Bariac T.** 2007. Non-steady-state, non-uniform transpiration rate and
558 leaf anatomy effects on the progressive stable isotope enrichment of leaf water along monocot
559 leaves. *Plant, Cell and Environment* **30**, 367–387.

560 **Pape L, Ammann C, Nyfeler–Brunner A, Spirig C, Hens K, Meixner FX** 2009. An automated
561 dynamic chamber system for surface exchange measurement of non–reactive and reactive trace
562 gases of grassland ecosystems. *Biogeosciences* **6**, 405–429.

563 **Rothfuss Y, Braud I, Moine LN, Biron P, Durand JL, Vauclin M, Bariac T.** 2012. Factors
564 controlling the isotopic partitioning between soil evaporation and plant transpiration, assessment
565 using a multi–objective calibration of Si SPAT–Isotope under controlled conditions. *Journal of*
566 *hydrology* **442**, 75–88.

567 **Snyder KA, Monnar R, Poulson SR, Hartsough P, Biondi F.** 2010. Diurnal variations of needle
568 water isotopic ratios in two pine species. *Trees* **24**, 585–595.

569 **Song X, Barbour MM, Farquhar GD, Vann DR, Helliker BR.** 2013. Transpiration rate relates
570 to within– and across–species variations in effective path length in a leaf water model of oxygen
571 isotope enrichment. *Plant Cell and Environment* **36**, 1338–1351.

572 **Song X, Loucos KE, Simonin KA, Farquhar GD, Barbour MM.** 2015. Measurements of
573 transpiration isotopologues and leaf water to assess enrichment models in cotton. *New Phytologist*
574 **206**, 637–646.

575 **Stewart MK.** 1975. Stable isotope fractionation due to evaporation and isotopic exchange of
576 falling water drops. Application to atmospheric processes and evaporation of lakes. *Journal of*
577 *Geophysical Research* **80**, 1133–1146.

578 **Sullivan PF, Welker JM.** 2007. Variation in leaf physiology of *Salix arctica* within and across
579 ecosystems in the High Arctic, test of a dual isotope $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$. conceptual model.
580 *Oecologia* **151**, 372–386.

581 **Sun SJ, Meng P, Zhang JS, Wan X, Zheng N, He CX.** 2014. Partitioning oak woodland
582 evapotranspiration in the rocky mountainous area of North China was disturbed by foreign vapor,
583 as estimated based on non–steady. *Agricultural and Forest Meteorology* **184**, 36–47.

584 **Walker, CD, Brunel, JP, Dighton J, Holland K, Leaney F, Mc Ewan K, Mensforth L,**

585 **Thorburn P, Walker C.** 2001. The use of stable isotopes of water for determining sources of
586 water for plant transpiration. In, Unkovich M, Pate J, McNeil A, Gibbs JD eds. Stable isotope
587 techniques in the study of biological processes and functioning of ecosystems. Kluwer Academic
588 Press, Dordrecht, pp 57–89.

589 **Wang L, Good SP, Caylor KK, Cernusak LA.** 2012. Direct quantification of leaf transpiration
590 isotopic composition. *Agricultural and Forest Meteorology* **154–155**, 127–135.

591 **Wang P, Yamanaka T, Li XY, Wei Z.** 2015. Partitioning evapotranspiration in a temperate
592 grassland ecosystem, Numerical modeling with isotopic tracers. *Agricultural and Forest*
593 *Meteorology* **208**, 16–31.

594 **Wang L, Caylor, KK, Dragoni, D.** 2009. On the calibration of continuous, high-precision $\delta^{18}\text{O}$
595 and $\delta^2\text{H}$ measurements using an off-axis integrated cavity output spectrometer. *Rapid Commun.*
596 *Mass Spectrom* **23**, 530–536.

597 **Welp LR, L X, Kim K, Griffis TJ, Billmark KA, Baker JM.** 2010. $\delta^{18}\text{O}$ of water vapour,
598 evapotranspiration and the sites of leaf water evaporation in a soybean canopy[J]. *Plant Cell &*
599 *Environment* **319**, 1214-1228.

600 **Welp LR, Keeling RF, Meijer HAJ, et al.** 2011. Interannual variability in the oxygen isotopes of
601 atmospheric CO₂ driven by El Nino. *Nature* **477**, 579–582.

602 **Wen XF, Zhang SC, Sun XM, Yu GR.** 2008. Recent advances in H₂O enrichment in leaf water.
603 *Journal of Plant Ecology* **32**, 961–966.

604 **Xiao Q, Ye WJ, Zhu Z, Chen Y, Zheng HL.** 2005. A simple non-destructive method to measure
605 leaf area using digital camera and Photoshop software. *Chinese Journal of Ecology* **24**, 711–714.

606 **Yang B, Xie FT, Wen XF, Sun XM, Wang JL.** 2012. Diurnal variations of soil evaporation $\delta^{18}\text{O}$
607 and factors affecting it in cropland in North China. *Chinese Journal of Plant Ecology* **366**,
608 539–549.

609 **Yepez EA, Williams DG, Scott RL, Lin G.** 2003. Partitioning overstory and understory
610 evapotranspiration in a semiarid savanna woodland from the isotopic composition of water vapor.
611 *Agricultural and Forest Meteorology* **119**, 53-68.

612 **Yepez EA, Huxman TE, Ignace DD, English NB, Weltzin JF, Castellanose AE, Williams DG.**
613 2005. Dynamics of transpiration and evaporation following a moisture pulse in semiarid grassland,

614 A chamber-based isotope method for partitioning flux components. *Agricultural and Forest*
615 *Meteorology* **132**, 359–376.

616 **Yu GR, Wang QF.** 2010. Ecophysiology of plant photosynthesis, Transpiration, and Water Use,
617 ed. Li Y, Chen SS, Science press, Beijing.

618 **Zhang Y, Yu X, Chen L, Jia GD.** 2018. Comparison of the partitioning of evapotranspiration –
619 numerical modeling with different isotopic models using various kinetic fractionation
620 coefficients[J]. *Plant & Soil* **14**, 1–22.

621 **Zhou Y, Grice K, Chikaraishi Y, Stuartwilliams H, Farquhar GD, Ohkouchi N.**
622 2011. Temperature effect on leaf water deuterium enrichment and isotopic fractionation during
623 leaf lipid biosynthesis, results from controlled growth of C₃ and C₄ land plants. *Phytochemistry* **72**,
624 207–213.