Research Paper

Isotope signature of maize stem and leaf and investigation of transpiration and water transport

Youjie Wu a,b, Taisheng Du b,c*, Lixin Wang c

a College of Water Resources & Civil Engineering, Hunan Agricultural University, Changsha 410128, China
b Center for Agricultural Water Research in China, China Agricultural University, Beijing 100083, China
c Department of Earth Sciences, Indiana University-Purdue University Indianapolis (IUPUI), Indianapolis, IN 46202, USA

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A B S T R A C T

Stable isotope signature of plant water contains essential information on water transport pathway and plant transpiration, which has been shown to be a powerful tracer in plant physiological and ecological processes. However, stable isotopes fractionation in processes of plant water transport and the relationship between transpiration rate (E) and effective pathway length (L) and their possible mechanisms are still largely mysterious and confusing. Here, we tested stable isotope signature of maize stem and leaf based on anatomical measurements and modeling, and propose a deuterium deviation in leaf water (Δd) to understand variability leaf water isotope enrichment and transpiration. We found isotopes fractionation occurred in maize stems in arid area. Leaf transpiration rate was strongly affected by Δd. The data revealed L has a negative power relationship with E, with a single power function of $L = 284.77E^{-1.02}$, and the proportional deviation of leaf $^{18}$O enrichment $1 - \Delta_\delta^{18}O$ is negatively correlated with E under low E ($E < 2.0$ mmol m$^{-2}$ s$^{-1}$) and, a positively relationship under high E ($E > 2.0$ mmol m$^{-2}$ s$^{-1}$). Suggesting that a pivotal role of effective path length in driving variations in leaf transpiration rate. The deuterium deviation $\Delta_d$ may have great potential to serve as a new diagnostic tool for understanding pathways of water transport in plant. Care should be taken when examining source-water and estimating roots water uptake using the stable isotope method in arid areas, and further study is needed to be carried out and confirm the conclusions across a range of environmental conditions and species.

1. Introduction

Stable isotopes of $^2$H and $^{18}$O have been widely used in biophysical and biochemical systems to trace water including the processes of water recycling, plant water uptake and evapotranspiration partitioning (Aemisegger et al., 2014; Dawson and Ehleringer, 1991; Dubbert et al., 2017; Wang et al., 2014; Wu et al., 2018). Water isotopes in plant tissues may provide crucial information on pathways of water transport in plants (Barbour and Farquhar, 2004; Farquhar and Cernusak, 2005). It is important for many plant physiological and ecological applications, and necessary for understanding the mechanisms of plant transpiration and water transport (Barbour et al., 2017; Gan et al., 2003; Loucos et al., 2015).

Indeed, water of different pools in the agriculture-forestry ecosystem will have very distinctive isotopic compositions (Gerlein-Safdi et al., 2018), which can be effectively distinguished during the whole root water uptake process (Kaseke et al., 2017). Previous studies suggested that water uptake and transport from soil to the stem of most plants occurs without hydrogen and oxygen isotope fractionation, and the hydrogen and oxygen isotopic composition of transpiration ($\delta_{trans}$) are operationally defined as being equal to the xylem water of plant–stem under isotopic steady-state (ISS) (Dawson and Ehleringer, 1991; Flanagan et al., 1991b; Simonin et al., 2013; Wu et al., 2016; White et al., 1985). However, studies revealed that direct measurements of hydrogen and oxygen isotopic compositions of transpiration are relatively rare, and the few direct measurements of $\delta_{trans}$ exist suggest that $\delta_{trans}$ is often not equal to the water source $\delta_5$ (Harwood et al., 1998; Simonin et al., 2013; Wang et al., 2012; Yakir and Sternberg, 2000). Isotopes fractionation of hydrogen and oxygen in processes of plant water uptake and transport are still largely uncertain, in particular, for the halophytes and plants in arid areas.

While deuterium excess $\delta$-excess ($\delta$-excess = $\delta^2$H – $8\delta^{18}$O, (Dansgaard, 1964)) is widely used in atmospheric and meteorological science (Risi et al., 2013) and ice core data analysis (Laz et al., 2009), it is rarely...
used to interpret isotope fractionation processes in plant physiology. However, excess may contain additional information than the isotopologues taken separately because it combines both $^1H$ and $^{18}O$ isotopic signals (Gerlein-Safdi et al., 2018; Li et al., 2017; Voelker et al., 2014). It would have great potential to serve as a new diagnostic tool for understanding pathways of water transport in plant, if deuterium excess could be applied to plant tissues (Ehlertinger et al., 2010). In order to better understand the water transport pathway and plant transpiration mechanism related to isotope fractionation of stem and leaf water, the variation extent in $\delta$-excess of leaf water, and the relationships between plant transpiration rate $E$ and $\delta$-excess in leaf water should be considered.

Leaf water isotopic composition and isotope enrichment may contain information on the transport pathways within and beyond the plant-stem xylem (Barbour et al., 2017). Gradients in isotope enrichment of leaf water may vary with the different water movement pathways between leaf evaporating surface and the vein (Barbour and Farquhar, 2004). However, ambiguous results were found in subsequent test of this suggestion, even the gradients in enrichment are within the mesophyll was questioned (Barbour et al., 2017). The causal relationships between pathways water movement and leaf anatomical properties are difficult to establish due to the complexity of the strongly interrelated nature of leaf anatomical traits and outside-xylem water transport (Barbour et al., 2017; Sack et al., 2015). It will provide a tractable way forward when linking the specific hydraulic characteristics with the patterns of leaf isotopic enrichment, particularly with respect to water movement pathways from veins to the leaf sites of evaporation (Cernusak et al., 2016; Plavcová et al., 2018).

Stable isotopic enrichment leaf water ($\Delta_L$) will benefit many applications of selecting and establishing models to analyze this process under isotopic steady-state (ISS) and non–steady-state (NSS) and, adding innovative views into functioning in plant leaves and hydraulic design (Loucos et al., 2015). The isotope enrichment of bulk leaf water (whole leaf water) is often less than that predicted for leaf evaporation sites (Bögelein et al., 2017; Ferrio et al., 2012; Loucos et al., 2015; Song et al., 2015). The leaf water isotopic enrichment in evaporation sites $\Delta_L$ is predicted well by Craig–Gordon model, but that of bulk leaf water is often overestimated. Enriched water back-diffusion from evaporation site was limited by the advection of plant transpiration stream with less enriched water (Péclet effect) (Farquhar and Gan, 2003; Holloway–Phillips et al., 2016). In sampling across many different species vein-leaf saturated moisture contents and vein densities, Holloway-Phillips et al. (2016) demonstrated the significance of accounting for the relative ‘pool’ sizes of the mesophyll and vascular water to explain Péclet effect. To scale the Péclet effect to that relevant for sampling of bulk leaf water and obtain more information on transport pathways within vein-mesophyll system, both the tortuosity of the water associated with the veins and the pathways within the leaf must be considered. A Péclet effective pathway length ($L$), for convenience, was commonly used (Elsworth et al., 2013; Farquhar and Gan, 2003; Loucos et al., 2015; Ripullone et al., 2008; Song et al., 2013). However, the relationship between L and E and the possible mechanisms behind L-E dynamics are still uncertain, especially in the field crop in arid areas.

In this study, we report water stable oxygen and hydrogen isotope data for maize stem and leaf from arid areas of northwest China and determine the leaf water stable isotope enrichment $\Delta_L$, deuterium excess in leaf water (defined as deuterium deviations $\Delta_D$ in this study) and effective pathway length $L$. Our objectives are (1) to clarify the isotopes fractionation effect in processes of plant water absorption and transport, (2) to obtain data under ISS and NSS and, explore how transpiration rate vary with respect to leaf effective pathway length and deuterium deviations and, discuss possible mechanisms dynamics behind their relationships, and (3) to develop a better understanding of the transpiration mechanism and theoretical system of stable isotope to map a path for future work into understanding water transport pathways.

## 2. Isotope theory

Under the isotopic steady-state (ISS), the water leaving leaf surface has the same isotopic composition as stem water. Enrichment of leaf evaporative sites ($\Delta_D$) above source water under the ISS is calculated according to Craig–Gordon (C-G) model (Farquhar et al., 2007):

$$\Delta_D = (1 + \varepsilon_D)(1 + \varepsilon)(1 - h) + h(1 + \Delta_V) - 1 \quad (1)$$

where $h$ is the relative humidity, $\Delta_V$ is the enrichment of atmospheric vapor, $\varepsilon$ is the equilibrium fractionation, $\varepsilon = 1000(1-1/\alpha)$ (Cappa et al., 2003; Craig and Gordon, 1965), and $\alpha$ is the temperature-dependent equilibrium fractionation factor, which was calculated by:

$$\alpha^{(18}O = (1.137 \times 10^6/T^2 - 0.416 \times 10^7/T - 2.067)/1000 + 1 \quad (2)$$

$$\alpha(H) = (24.844 \times 10^6/T^2 - 76.248 \times 10^3/T + 52.612)/1000 + 1 \quad (3)$$

$$\varepsilon = \text{k}\text{inetic fractionation factor, taken as} \quad \text{Farquhar et al., 2007):}$$

$$\varepsilon_{b} = \frac{28.5\varepsilon_0 + 18.9\varepsilon_k}{\text{r}_{b} + \varepsilon_k} \quad (4)$$

$$\varepsilon_{s} = \frac{16\varepsilon_0 + 10\varepsilon_k}{\text{r}_{b} + \varepsilon_k} \quad (5)$$

where $\text{r}_{b}$ and $\text{r}_{s}$ are the stomatal resistance (equal to 217 s m$^{-2}$) and the resistance of the boundary layer (taken as 1.13 $10^8$ s m$^{-2}$) (Gerlein-Safdi et al., 2018; Hughes et al., 2014).

The isotopic enrichment ($\Delta_L$) of bulk leaf water in isotopic steady-state is conventionally modelled from the Péclet effect and the $\Delta_E$ as the following (Farquhar and Lloyd, 1993):

$$\Delta_L = \frac{\Delta_E(1 - e^{-\phi})}{\phi} \quad (6)$$

$\phi$ is the Péclet number and defined as (Farquhar and Lloyd, 1993):

$$\phi = \frac{EL}{CD} \quad (7)$$

where C (mol m$^{-3}$) is the water molar concentration (equal to 55.6 $10^3$ mol m$^{-3}$), and D (m$^2$ s$^{-1}$) is the diffusivity in liquid water ($D = 119 \times 10^{-9} \exp(-637/(T + 136.15))$ (Cuntz et al., 2007; Song et al., 2013); $E$ (mol m$^{-2}$ s$^{-1}$) is the transpiration rate; $L$ (m) is the effective path length of water movement from xylem to the leaf evaporation site.

ISS was assumed in the long-term midday campaign, such that the effective path length ($L$) can be calculated by fitting Eqs. (1), (6) and (7) to the measured $\Delta_L$ and $\Delta_V$.

At non-steady state (NSS), the enrichment of leaf water $\Delta_L$ can be predicted by a model (iteratively at a time step $t$) developed by Dongmann (1974) and Farquhar and Cernusak (2005).

$$\Delta_{L,t} = \Delta_L + (\Delta_{L,t-1} - \Delta_L)e^{\phi t} \quad (8)$$

The Péclet term $P$ is substituted by $(1-e^{-\phi})/\phi$, and the leaf water turnover times ($\tau$) can be described by (Farquhar and Cernusak, 2005):

$$\tau = (1-f) \frac{W}{g_{st}} \frac{a}{a} \quad (9)$$

where $\alpha^* = 1 + e^* a_k = 1 + a_k W$ (mol m$^{-2}$) is the leaf water content, $g_{st}$ (mol m$^{-2}$ s$^{-1}$) is the stomatal conductance, and $W$ (mol mol$^{-1}$) is the mole fraction of water vapor in the air in the intercellular spaces. The $f$ denotes Péclet term, can be defined as:

$$f = \frac{1 - e^{-\phi}}{\phi} \quad (10)$$

During the diurnal course, we used Eq. (8) to determine the effective
path length (L). L was iteratively solved at a certain time step \( t \) in this calculation. The approach of calculating \( L \) during NSS requires two measured values at both the current \( \Delta t \) and previous \( \Delta t - 1 \) time step (Simonin et al., 2013).

According to isotopic theory, the enrichment of different water samples (\( \Delta \)sample) relative to the source water can be linked back to the relative isotope ratios R:

\[
\Delta_{\text{sample}} = \frac{R_{\text{sample}}}{R_{\text{source}}} - 1
\]

isotope composition was expressed in per mil (‰) as:

\[
\delta_{\text{sample}} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the \( ^{18}O/^{16}O \) or \( ^{2}H/^{1}H \) ratios of the sample, water source and the Vienna Standard Mean Ocean Water (V-SMOW) standard, respectively.

Thus, we can obtain a relation between \( \Delta_{\text{sample}} \) and \( \delta_{\text{sample}} \):

\[
\delta_{\text{sample}} = \left( \left( \Delta_{\text{sample}} + 1 \right) R_{\text{source}} / R_{\text{standard}} - 1 \right) \times 1000
\]

By combining the expressions for \( \delta^{18}O \) and \( \delta^{2}H \) and Eqs. (1) and (13), we know the deuterium excess (\( \delta^{2}H \)) as a function of the relative humidity \( h \). For conceptual simplicity, we refer to deviations excess in plant leaf water (\( \Delta \)) with reference to the Global Meteoric Water Line (GMWL), since the intercept of GMWL is 10‰ caused by kinetic fractionation during the ocean free water evaporation. Thus, deuterium deviations in leaf will have more negative values, can be defined as:

\[
\Delta_{L} = \delta^{2}H - 88.5^{15}O - 10
\]

The transpiration rate \( E \) (mmol m\(^{-2}\) s\(^{-1}\)) can be estimated as (The vapor pressure inside the leaves is assumed to be saturation):

\[
E = g_{\text{m}} \frac{e_{a} - e_{s}}{P_{\text{atm}}}
\]

where \( P_{\text{atm}} \) is the atmospheric pressure (equal to 101.3 kPa), \( e_{a} \) and \( e_{s} \) (kPa) is the saturated vapor pressure calculated using leaf temperature and air temperature.

3. Materials and methods

3.1. Study location

The study was tested in a maize field (about 39 ha) during 2014–2015 at Shiyanghe Experimental Station for Water-saving in Agriculture and Ecology in the Shiyanghe river basin of north-west China (37°52′ N, 102°51′ E). The record in this weather station spanned a period from 2004 to 2018, with the average annual temperature of 8.3 °C, with annual precipitation of 164 mm. Water resource is scarce in this region with pan evaporation of 2000 mm. Groundwater varies in depth between 30 and 40 m. The soil in shallow (0–40 cm) and deeper layers (40–100 cm) are loamy and sandy, with mean field capacity of 0.28 cm\(^{-3}\) cm\(^{-3}\) and 0.26 cm\(^{-3}\) cm\(^{-3}\), respectively (Wu et al., 2018).

3.2. Sampling and measurements

Maize was planted in April and harvested in September. The plant density was about 66,000 plants per hectare. Furrow irrigation method was used and the irrigation scheduling was designed according to our previous studies (Wu et al., 2018). Fertilizer supply and other agronomic measures during the growing season was consistent with the local management.

3.2.1. Water isotopes

The sampling and measuring time was from 14 May to 7 August 2014, and from 19 May to 3 September 2015. The water samples of soil, maize stem, leaf and ambient atmospheric vapor were collected. The sample of plants and soil were randomly chosen within a 30 m diameter in the experimental field.

Based on anatomical measurements, maize stems and leaves were collected, and were cut into 5–10 cm segments (Fig. 1). The leaves of different canopy positions (lower, middle and upper) were chosen to cut into smaller fragments, and the veins and mesophyll were separated. At the middle growth stage of maize (DOY 154–206 in 2014, and DOY 160–208 in 2015), we chose two sunny and cloudy days for diurnal variation measurements. During the diurnal measurements, maize stems and leaves were collected at 2 h intervals during 00:00–24:00. Then plant samplings were immediately placed inside the zip bag and water was extracted using the vacuum extraction system (LI-2000, LICA, China). Ambient atmospheric water vapor (The mixture of transpiration, evaporation and atmospheric water vapor) was collected using a cold-trap multi-channel equipment (AWVCT04, LICA, China), following. Under such setup, 1.0–1.5 mL liquid water can be collected from the ambient atmospheric water vapor per hour. Soil water samples were collected from the depths of 0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–110 cm. Sampling time of ambient atmospheric water vapor and soil was the same as maize leaf and stem. All the samples were collected with three replications at least, and were stored in airtight container at 0–4 °C. Samples were analyzed for \( \delta^{2}H \) and \( \delta^{18}O \) (%) by Liquid-Water Isotope Analyzer (PICARRO L2130i, Picarro, USA). The analytical precision was < 2.0‰ for \( \delta^{2}H \) and < 0.1‰ for \( \delta^{18}O \).

3.2.2. Environmental and physiological measurements

Relative humidity, leaf stomatal conductance, and temperatures of leaf and air were measured throughout the maize growing season using LI-6400 portable photosynthesis systems (LI6400XT, LiCor Inc., Lincoln, NE, USA) equipped with a red-green-blue light source (LI6400-18; LiCor Inc.) (Qiu et al., 2019; Ocheltree et al., 2012). It should be pointed out that the measurements need to be considered in different canopy layers (lower, middle and upper), and in different site of one certain leaf (leaf base, leaf mid part and leaf apex). Measurements were recorded before 0.5–1 h of the maize stem and leaf sampling. Leaf water content (W, kg m\(^{-2}\)) per unit ground area was measured by weighing the fresh and dry leaves.

4. Results

4.1. Stable oxygen (\( ^{18}O \)) and hydrogen (\( ^{2}H \)) isotope composition

Descriptive statistics of water oxygen and hydrogen isotope composition (\( ^{18}O \) and \( ^{2}H \)) from all the sample types (soil, maize stems, leaves and air vapor) are presented in Table 1. It shows linear relationship between \( \delta^{2}H \) and \( \delta^{18}O \) of the water samples: \( \delta^{2}H = 6.0 \delta^{18}O - 6.9 \) (soil, \( R^{2} = 0.94 \)), \( \delta^{2}H = 6.1 \delta^{18}O - 8.3 \) (Stem, \( R^{2} = 0.97 \)), \( \delta^{2}H = 3.2 \delta^{18}O - 33.9 \) (Leaf, \( R^{2} = 0.91 \)) and \( \delta^{2}H = 5.9 \delta^{18}O - 10.5 \) (Vapor, \( R^{2} = 0.88 \)). The ‘leaf water line’ with lower intercept and slope values, reflected a strong evaporation effect on the surface of leaf water. Not surprisingly, leaves water were the most enriched with \( ^{18}O \) and \( ^{2}H \) ranging from −11.08 to 16.99‰ and from −69.14 to 20.47‰, with mean values of 3.26‰ and −23.12‰, respectively. To reduce redundancy, we focused on the discussion of oxygen isotope composition (\( ^{18}O \)) only in future sections since the distribution of \( ^{2}H \) and \( ^{18}O \) showed a good uniformity.

Significant differences were found in the isotopic compositions of different maize organs (stem, vein and mesophyll) (Fig. 1a). The \( ^{18}O \) of mesophyll was much higher than that of vein and stem. The distribution range of mesophyll \( ^{18}O \) was 7.70–16.99‰, with mean of 12.65‰. Maize stem water isotope composition was less than that of vein water, with \( ^{18}O \) ranging from −12.28‰ to −4.95‰, and mean of −9.78‰. But here in particular, differences were found in water isotope composition of upper stem and lower stem (stem base), the mean value of \( ^{18}O \) in upper stem (−10.21‰) was higher than lower stem (−8.76‰). Isotope composition of maize leaf water show similar distribution in
different canopy positions (Fig. 1b), ranging from −11.08 to 16.99‰ (bulk leaf water). However, δ18O data in a leaf suggest that there were significant differences, among the leaf base, leaf middle part and leaf apex (Fig. 1c). δ18O in the leaf apex was more enriched in comparison to the leaf base and leaf middle part ranging from 4.46‰ to 16.99‰, and mean of 10.59‰. As expected, isotopic enrichment within the leaf
increased from the base to the apex.

Diurnal variations of the isotope compositions of leaf ($\delta_{\text{leaf}}$), stem ($\delta_{\text{stem}}$) and soil ($\delta_{\text{soil}}$) water show that $\delta_{\text{stem}}$ and $\delta_{\text{soil}}$ had a similar distribution and variation both in sunny and cloudy day (Fig. 2a). Soil water isotope composition was a little higher than maize stem water, because of the strong evaporation of shallow soil water causing isotopic fractionation (Allison and Hughes, 1983; Braun et al., 2009). However, the $\delta_{\text{stem}}$ showed a crest value at noon of sunny day which higher than the soil water, indicating that isotopes fractionation occurred in processes of plant water absorption in study site. It shows an increase in $\delta_{\text{leaf}}$ from early morning to midday and then decreased until early morning the next day. The fluctuation range and the crest in cloudy day, however, were lower than that of sunny day. This is due to the strong solar radiation in sunny day resulting in stronger isotopes enrichment in leaves.

Fig. 2b showed that the leaf water isotope composition exhibited a negative correlation with relative humidity (RH), with an $R^2$ of 0.68, indicating that relative humidity was a crucial influential factor for leaf water isotope composition. But the $\delta_{\text{stem}}$ displayed a relatively stable value regardless of relative humidity.

### 4.1.1. Leaf water $^{18}$O enrichment $\Delta_L$ and deuterium deviation $\Delta_{\delta}$

Similar to the variations of $\delta_{\text{leaf}}$, there was an increase trend from early morning to midday and a decrease trend from midday to early morning the next day of the leaf water $^{18}$O enrichment $\Delta_L$ (Fig. 3). There were slight differences of $\Delta_L$ among maize leaf in upper, middle and lower layer. However, significant differences were found, among the $\Delta_L$ of leaf base, leaf mid-part and leaf apex (Fig. 3b), both in sunny and cloudy days.

Mean $^{18}$O enrichment $\Delta_L$ of leaf water and deuterium deviation $\Delta_{\delta}$ were showed in Fig. 4. Water extracted from the leaf apex and leaf blade was more enriched than the source water (stem water) by 15–24‰. Along the leaf blade, a progressive isotope enrichment was observed within vein water. Enrichment of vein water over source water was higher near the leaf apex with the midrib vein achieving of 16‰, and the upper vein achieving of 16‰. As the source water flows out of the vein and moves to the stomata, it enriches in $^{18}$O evaporately, and a slight enrichment of vein water was obtained due to the mixing of the isotopically distinct water back-diffusion (Helliker et al., 2000). Similarly, deuterium deviation $\Delta_{\delta}$ showed a significant difference between the vein and mesophyll. The $\Delta_L$ in the mesophyll (e.g. leaf blade and leaf apex) was 150–170.0‰ more negative than for the vein base (Fig. 4).

Our results can be used as new evidence that leaves isotopic enrichment is not occurring along the midrib but along the axis of the secondary veins. This is probably because of the high water flow and low hydraulic resistance in the midrib, and the large amount of unenriched water in the midrib diluting enriched water of back-diffusion (Gerlein-Safdi et al., 2017).

### 4.1.2. Leaf transpiration rate (E) and effective path length (L)

The deuterium deviation $\Delta_d$ in plant water pools reconstructed from the global meteoric water line GMWL clearly indicated that leaf transpiration rate E should be strongly affected by $\Delta_d$ (Fig. 5): lower $\Delta_d$ values (more negative) were associated with higher transpiration rates. The linear regression fitted to $\Delta_d$ and E was $E = -0.04\Delta_d + 0.36$ ($R^2 = 0.646, P < 0.05$), with small slope and intercept value might be the result of maize leaf physiological functioning.

The relationships between effective path length L (calculating L at ISS and NSS) and leaf transpiration rate E are showed in Fig. 6. L and E showed a significant inverse relationship (negative power), with a single power function of $L = 284.77E^{-1.02}$ ($R^2 = 0.546, P < 0.05$). In the dataset, L tended to remain within a narrow small values range when E was in the high values (E > 2 mmol m$^{-2}$ s$^{-1}$). L increased sharply when E reached the low range of values (E < 1 mmol m$^{-2}$ s$^{-1}$) and decrease towards zero.

When analyzing the leaf water $^{18}$O enrichment $\Delta_L$ and effective path length L, clear and significant (P < 0.05) negative relationship was observed between L and $\Delta_L$, with non-linear regression of $\Delta_L = -2.78\ln(L) + 29.93$ ($R^2 = 0.590$, Fig. 6b). This reflects the fact that average $\Delta_L$ decreases if the effective path length (L) is long or when transpiration rate (E) is high. The way that L and E changed in maize leaves relative to the available water source, minimized their differences in $\Delta_L$ (Eqs. (5) and (6)) (Ellsworth et al., 2013; Farquhar and Lloyd, 1993).

### 5. Discussion

#### 5.1. Isotopes fractionation in processes of plant water transport

Our results show that differences were found in water isotope composition of maize upper stem and lower stem (stem base), and the mean value of $\delta_{\text{stem}}$ was not consistent with that of water source (i.e. $\delta_{\text{soil}}$). Besides, a crest value at noon of sunny day was found in $\delta_{\text{stem}}$ which was higher than the soil water. Thus, we suggest that isotopes fractionation occurred in processes of water transport in maize stems in arid areas.

Stable isotope $^{18}$O in plant stem water had better response to the
variation of soil water (water source) (Dai et al., 2014; Wu et al., 2016), and the water isotope compositions are unchanged by plant roots uptake and during transport through the stem in most species (Dawson and Ehleringer, 1991; Meiβner et al., 2013; White et al., 1985). However, isotopes fractionation of $^{2}H$ were found in some wetland species, salt-tolerant (halophytic) plant and certain woody xerophytes (Ellsworth and Williams, 2007; Lin and da SL Sternberg, 1993; Zhao et al., 2016). Probably because in the halophytic and xerophytic plants, water with a large fraction taken up thought plant roots transverses cell membranes in the endodermis before entering the root xylem, and cause isotope fractionation of $^{2}H$ in root- stem xylem water (Ellsworth and Williams, 2007).

The region of our study is scarce in water resource with a mean annual evaporation of 2000 mm and annual precipitation of 164 mm. Maize leaves were enriched with isotopes in this arid environment (Figs. 3 and 4). Relationships between leaf isotope enrichment $\Delta L$ relative to water source (soil and stem) and the ratio of ambient to intercellular vapor pressure ($e_a/e_i$) for maize leaves are showed in Fig. 7. Both isotope enrichment relative to soil water source $\Delta L_{\text{Soil}}$ and stem water source $\Delta L_{\text{Stem}}$ exhibited a significant, negative correlation with $e_a/e_i$. The two regression lines should have coincided under the isotopic steady-state (ISS) (Fig. 7a), if there was no isotope fractionation during water transport in maize stem. However, both the slope and intercept of $\Delta L_{\text{Soil}}-e_a/e_i$ were significant differences from $\Delta L_{\text{Stem}}-e_a/e_i$, indicating the isotope fractionation had occurred during water uptake, and the magnitude of isotope fractionation should be closely related to the deviation of the intercept, and positively correlated with transpiration rates (Lin and da SL Sternberg, 1993; Song et al., 2015). In light of the results, care should be taken when examining source-water and estimating roots water uptake using the stable isotope method in halophytic and xerophytic species.

5.2. Leaf water isotopic signals of $\Delta L$ and potential applications of $\Delta d$

Maize leaves $^{18}O$ enrichment $\Delta L$ showed a significant difference between the vein and mesophyll, and had distinct diurnal variations in sunny and cloudy day. $\Delta L$ exhibited a significant negative correlation with $e_a/e_i$ in both ISS and NSS leaf groups, indicating $\Delta L$ is an important factor in applications of many plant physiological and ecological processes. Similar to leaf isotope enrichment, the deuterium deviation $\Delta d$ provided some key information for the study of water transport

![Fig. 3. Diurnal variations of the leaf water $^{18}O$ enrichment $\Delta L$ of (a) leaf water with different canopy positions, and (b) leaf water of different leaf positions.](image)

![Fig. 4. Distribution of maize leaf water $^{18}O$ enrichment $\Delta L$ (8) and deuterium deviation $\Delta d$ (8).](image)

![Fig. 5. The relationship between deuterium deviation $\Delta d$ (8) and leaf transpiration rate ($E$, mmol m$^{-2}$ s$^{-1}$).](image)
pathway and plant transpiration. Our data revealed that Δd in leaf apex was 150–170.0‰ more negative than for the vein base. This might be the result of several effects such as the changing leaf water volume, tapering of the leaf veins or leaf blade, and uneven transpiration rate (Ogée et al., 2007). There were significantly negative relationships between deuterium deviation Δd and transpiration rate E. That is, deuterium deviation Δd may have great potential to add a new diagnostic tool for understanding pathways of water transport in plant, and provide an effective quantifying method for explaining water transport pathway and plant transpiration.

Leaf water stable isotope enrichment significantly influences the isotope signatures of a number of atmospheric and biological processes (Barbour et al., 2017; Cernusak et al., 2016). Actually, most leaves is less affected by evaporative enrichment due to they have a large amount of water in their associated tissue and veins, which likely has its own Péclet effect (Farquhar and Gan, 2003). The isotopic enrichment ΔL of bulk leaf water is often less than that predicted for evaporation sites (ΔE) by Craig–Gordon model (Farquhar and Gan, 2003; Farquhar and Cernusak, 2005). A concentration gradient of enriched water between vein and stomata was created during the enrichment process in leaf, which results in the enriched water back-diffusion (Helliker et al., 2000; Cernusak et al., 2013; Gerlein-Safdi et al., 2017). But Ogée et al. (2007) suggest the isotopic gradient along the leaf slightly affected by the mesophyll liquid water longitudinal diffusion. In our study, ΔL of bulk leaf water was less than the measurement of mesophyll, and isotope of the leaf apex and leaf blade was more enriched than the vein base by 7–18‰ (Fig. 4). Thus, it is easy to understand why bulk leaf water ΔL (mean value) was low. Besides, significant differences of ΔL were found among leaf water with different canopy positions and between sunny and cloudy day (Fig. 3), suggesting that stronger evaporation in upper leaves caused by strong solar radiation enriched the 18O in leaf evaporation site. The leaf water isotope enrichment in evaporation sites should be closely related to transpiration rate (E) and the relative humidity (RH) (Holloway-Phillips et al., 2016; Kaushal and Ghosh, 2018; Lehmann et al., 2017; Song et al., 2013, 2015; Wang et al., 2012). Our results exhibited a significant, negative correlation between leaf transpiration rate (E) and deuterium deviation Δd (Fig. 5). Hence, Δd should be useful for understanding variability leaf water isotope enrichment and transpiration rate associated with RH and past climatic cycles. Similar study of deuterium deviation in plant leaves water came from Zhao et al. (2014), they found...
significantly positive relationships between deuterium excess in plant (d_leaf) and RH, and opposite diurnal variations for d_leaf during the sunny days. Deuterium excess (or deviation) in plant may provide direct evidence that dynamics of surface air moisture at continental locations can be significantly altered by plant transpiration, especially during sunny days (Zhao et al., 2014). Another study showed a strong linear relationship between 18-O excess in leaf and Δ 18O (Li et al., 2017), they believed this relationship should be typical for any evaporation process or any fractionation governed by kinetic effects.

5.3. The relationship between L and E and possible mechanisms

We estimated effective path length L of maize in the model framework of the Péclet effect. The data revealed L has a negative power relationship with E, with a single power function of L = 284.77E − 0.546 (R2 = 0.546), suggesting that a pivotal role of effective path length in driving variations in leaf transpiration rate.

Similar results were reported by Song et al. (2015), they showed that L was significantly related to E in cotton: L = 22.3E − 0.74(P < 0.01). In another study, Kahmen et al. (2010) reported that L was found to be positively related to 1/E, by investigating leaf isotope enrichment in 17 species. In agreement with current results, a similar relationship between L and E has been obtained in response to water status (Ferrio et al., 2010; Ripullone et al., 2008) and across plants leaf ontogeny (Barnard et al., 2010). On the contrary, several past results obtained under high E conditions on crop species showed that relatively constant (or small) L values in spite of variations in E (Barbour et al., 2000; Flanagan et al., 1991a; Ripullone et al., 2008). The mechanisms of such confused relationship, however, are still unclear. Some speculation come from previous researches, suggesting that effective path length L may reflect changes in the mesophyll hydraulic properties, which in turn are related with transpiration rates (Ferrio et al., 2012; Ripullone et al., 2008; Sack and Holbrook, 2006; Song et al., 2013; Zhou et al., 2011; Ocheltree et al., 2012).

The mechanism behind the L-E dynamics must analyze the relationship between 1 − Δ_i/Δ_e and E, and relate to the pathways of water movement through a leaf (Holloway-Phillips et al., 2016; Loucos et al., 2015; Song et al., 2013, 2015). Large values of L may be due to the lack of the predicted relationship between 1 − Δ 18O/Δ 18O and E inherent in the Péclet effect (Loucos et al., 2015). L must increase to extremely high values under low E, if the proportional deviation of leaf water and evaporative site water 18O enrichment is constant with E, because of the exponential formulation of the Péclet effect (Loucos et al., 2015; Ripullone et al., 2008; Song et al., 2015). The weak relationship between 1 − Δ 18O/Δ 18O and E can then be interpreted as a result of inverse (negative power) relationship between L and E (Ripullone et al., 2008; Song et al., 2015). Our results in Fig. 8 could be used to support this view, suggesting a negative relationship between 1 − Δ_i/Δ_e and E under low E (E < 2.0 mmol m − 2 s − 1) and, a positively relationship under high E (E > 2.0 mmol m − 2 s − 1). Similar results reported by Song et al. (2013): f(wv) (1 − Δ_i/E_Δ) being negatively related to E under low E across six species/sampling periods, but being somewhat positively related to E under high E conditions under either ISS or NSS conditions. However, some studies showed NSS dynamics can result in a negative relationship between 1 − Δ 18O/Δ 18O and E in low stomatal conductance species and large leaf water concentrations species (Cermusak and Kahmen, 2013; Ogée et al., 2007). Farquhar and Gan (2003) found that 1 − Δ i/E_Δ did not significantly correlate with E in castor bean, contradicting the pattern of positive relationship between 1 − Δ_i/Δ_e and E reported by an early research in the same species (Barbour et al., 2000). Given the observed lack of a statistically significant relationship between 1 − Δ_i/Δ_e and E, Song et al. (2013, 2015) reason that L should exhibit an inverse relationship with E in theory.

Actually, different effective path lengths L are associated with different water movement pathways and the symplastic resistance to flow. The overall effective path length L of the maize leaves in our study, was probably determined by the relative contribution of the pathways to the whole water flow. It is highly likely that the pathways of water movement will vary between species and will change dynamically with the change of E (Kim and Steudle, 2007; Morillon and Chrispeels, 2001; Song et al., 2013). However, further study is needed to confirm the conclusions across a range of environmental conditions and species, which requires a large range of data points of transpiration rates and isotope composition.

6. Conclusions

The data presented here show that maize stem water isotope composition was not consistent with that of water source, differs significantly across isotope composition of maize upper stem and lower stem (stem base). Furthermore, both the slope and intercept of Δ L-Stem-ε_i/ε_e were significant differences from Δ L-Stem-ε_i/ε_e, suggesting that isotopes fractionation occurred in processes of water transport in maize stems in arid areas. This finding is important because it greatly affects the accuracy of source-water calculation and roots water uptake estimation using the stable isotope method. We also found deuterium deviation Δ_i provided some key information for the study of water transport and plant transpiration, as it exhibited a significant negative correlation with ε_i/ε_e under both ISS and NSS conditions. Our data revealed effective path length L has a negative power relationship with E, with a single power function of L = 284.77E − 0.546 (R2 = 0.546), suggesting a pivotal role of effective path length in driving variations in leaf transpiration rate. However, further study about mechanisms behind L-E dynamics requires a large range of data points of transpiration rates and isotope composition across a range of environmental conditions and species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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