1 **Water stress and CO₂ concentration interactions affect carbon** 2 **isotope signatures of leaf and phloem organic matter**

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7 **Highlight**

8 The $\delta^{13}\text{C}$ of leaf and twig phloem WSOM exhibited the most positive values at
9 $\text{C}_{400} \times 35\% - 45\%$ FC.

10 Post-photosynthetic fractionation from leaf to twig was not be impacted by the
11 interacting effects.

12 **Abstract**

13 Investigation of $\delta^{13}\text{C}$ of leaf and twig phloem water-soluble organic material (WSOM)
14 is a promising approach for analysis of the effects of environmental factors on plant
15 performance. In this study, orthogonal treatments of three CO₂ concentrations (C_a) $\times$
16 five soil water contents (SWC) were conducted using *Platycladus orientalis* saplings
17 to investigate the interaction of water stress and CO₂ concentration on $\delta^{13}\text{C}$ of leaf and
18 twig phloem WSOM. Under the lowest SWC, the $\delta^{13}\text{C}$ of leaf and twig phloem
19 WSOM had the most positive values at any C_a and their values decreased as C_a
20 increased. However, at improved soil water conditions, the greatest values of $\delta^{13}\text{C}$ of
21 leaf and twig phloem WSOM were mostly observed at C_{600} . In addition, a more
22 significant relationship between SWC and $\delta^{13}\text{C}$ of twig phloem WSOM than that
23 between SWC and $\delta^{13}\text{C}$ of leaf WSOM demonstrated that $\delta^{13}\text{C}$ of twig phloem
24 WSOM is a more sensitive indicator of SWC. Twig phloem WSOM was generally
25 ¹³C-depleted compared with leaf WSOM for potential post-photosynthetic
26 fractionation, and the ¹³C discrimination from leaves to twig phloem was insensitive
27 to the interaction between SWC and C_a . Clearly, interacting effects play a more
28 important role in photosynthetic fractionation than in post-photosynthetic

29 fractionation.

30 Keywords: Carbon isotope composition, CO₂ concentration, discrimination,
31 interacting effect, soil water content, water-soluble organic matter.

32 **Introduction**

33 Over the past 160 years, atmospheric CO₂ (C_a) concentration have increased
34 from 280 μmol mol⁻¹ to almost 400 μmol mol⁻¹ and could surpass 700 μmol mol⁻¹
35 with an annual rate of increase of 0.4% (Zhao *et al.*, 2017). Concurrently, soil water
36 stress may be aggravated by a climate that is both warming and drying, especially in
37 regions where soil evaporation far exceeds precipitation. Within this context, it is
38 important to investigate the interaction between soil water content (SWC) and C_a on
39 plant performance and to predict plant fragmentation mechanisms under elevated C_a
40 and heightened drought conditions. Carbon isotopes from the fast-turnover
41 carbohydrate pool (water-soluble organic material, WSOM) in leaves are commonly
42 utilized as an indicator of the assimilation-weighted average ratio of C_i (foliar
43 intercellular CO₂ concentration) to C_a carboxylated over a period of time, from
44 several hours to 1–2 days (Pons *et al.*, 2009). In addition, the leaf exported carbon in
45 phloem carries the ¹³C signal of its source tissue. Therefore, the determination of
46 carbon isotope composition (δ¹³C) in a plant is a promising tool that is commonly
47 employed as an indicator of the effects of environmental factors on plant performance.

48 δ¹³C of WSOM is sensitive to subtle changes in environmental factors, including
49 SWC and C_a, as a result of variation in photosynthetic and physiological traits are
50 imprinted in δ¹³C of new assimilates (Robredo *et al.*, 2010; Adiredjo *et al.*, 2014; Luo
51 *et al.*, 2016). However, the mechanism of how changes in environmental factors
52 influence δ¹³C of twig phloem WSOM might be more complexity compared with that
53 of leaf WSOM because δ¹³C of leaf-exported carbon in phloem can be altered by
54 post-photosynthetic carbon isotope fractionation (Brandes *et al.*, 2006; Kodama *et al.*,
55 2011). Further, photosynthetic discrimination cannot completely explain the δ¹³C
56 variation in different plant organs (Damesin and Lelarge, 2003; Bowling *et al.*, 2008).
57 For example, Gessler *et al.* (2008) reported that 30% of the differences in δ¹³C

between leaf carbon and transported carbon can be explained by post-photosynthetic carbon isotope fractionation. In contrast to photosynthetic carbon isotope fractionation, post-photosynthetic carbon isotope fractionation has not been well defined or understood. Based on previous research, post-photosynthetic carbon isotope fractionation has been associated with activities such as transport of metabolites, the process of respiration, and biosynthesis of organic materials (Damesin and Lelarge, 2003; Gessler *et al.*, 2004; Brandes *et al.*, 2006; Bathellier *et al.* 2008).

The individual influence of SWC and C_a on $\delta^{13}C$ of leaf and twig phloem WSOM has been under investigation; however, scant research has explored how $\delta^{13}C$ of leaf and twig phloem WSOM responds to the interaction of SWC and C_a . Assessing variation in $\delta^{13}C$ of new assimilates in leaves and comparing $\delta^{13}C$ of leaf exported organic materials in the twig phloem are effective methods for describing both photosynthetic and post-photosynthetic carbon isotope fractionation, providing valuable insights into plant physiology and biogeochemistry (Badeck *et al.*, 2005). In this study, the interacting effects of SWC and C_a on $\delta^{13}C$ of water-soluble leaf and twig phloem material were investigated by orthogonal treatments of three C_a levels coupled with five SWC gradients (three $C_a \times$ five SWC). The objectives of this study were to describe the interaction between SWC and C_a on $\delta^{13}C$ of water-soluble leaf and twig phloem material, and to determine whether ^{13}C discrimination during the leaf-to-stem transition of carbohydrates was associated with the interacting effects of SWC and C_a .

Material and methods

Experimental design

Research was conducted at the Chinese Research Station of Forest Ecosystems in the capital circle, located in Jiufeng National Forest Park of northwest Beijing (116°05'E, N40°03'N). In May 2016, 45 local three-year-old *Platycladus orientalis* saplings were selected for transplantation into pots (one sapling per pot). The top diameter of each pot was 30 cm, base diameter was 22 cm, and height was 29 cm. Soil with a field capacity (FC) of 26.2% was collected from the local undisturbed forest.

The saplings had similar growth status, tree height (about 1.4 m), and root collar (about 28 mm). After two months of acclimation, the potted saplings were moved to growth chambers (FH-230, Taiwan Hipoint Corporation, Kaohsiung City, Taiwan) for orthogonal treatments.

The design scheme of the orthogonal treatments (three C_a levels coupled with five SWC gradients) is presented in Table 1. There were two chambers used in the study for orthogonal treatments. One chamber was connected to a CO_2 tank (Fig.1a), which can maintain C_a of $600 \mu\text{mol mol}^{-1}$ (C_{600}) and $800 \mu\text{mol mol}^{-1}$ (C_{800}). Another chamber was connected to an N_2 tank (Fig.1b), which was used to maintain an ambient atmospheric concentration of approximately $400 \mu\text{mol mol}^{-1}$ (C_{400}). The central controlling system of the chambers (FH-230) can consistently monitor and adjust C_a to their set values. Each chamber held five potted saplings and all five had a different SWC: the first was 35%–45% of FC (SWC between 9.17% and 11.79%), the second was 50%–60% of FC (SWC between 13.10% and 15.72%), the third was 60%–70% of FC (SWC between 15.72% and 18.34%), the fourth was 70%–80% of FC (SWC between 18.34% and 20.96%), and the fifth was 95%–100% of FC (SWC between 24.89% and 26.20%). Five saplings were cultivated at each SWC condition at each C_a . Saplings in each treatment were allowed to acclimate for two weeks, and each treatment was repeated three times. To recreate local field conditions, the temperature and relative humidity in the growth chambers were set as follows: from 07:00 to 19:00, the lighting system was activated (photosynthetic photon flux density ranged between 200 and $240 \mu\text{mol m}^{-2} \text{s}^{-1}$), temperature was $25 \pm 0.5^\circ\text{C}$, and relative humidity was 60%. From 19:00 to 9:00, all light was turned off, temperature was $18 \pm 0.5^\circ\text{C}$, and relative humidity was 80%.

Sampling and measurement

Foliar gas exchange measurement

Between 9:00 and 11:00, photosynthetic and physiological traits, including net photosynthetic rate (P_n), stomatal conductance (g_s), and foliar intercellular CO_2 concentration (C_i), were measured on fully expanded leaves using a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NE, US) after two weeks of

117 treatment. Measurements were made on three leaves per sapling. Light conditions in
118 the growth chamber remained stable, almost all leaves were exposed to a similar light
119 intensity, and there was no obvious intracanopy gradient; therefore, the measurement
120 of photosynthetic and physiological traits was only conducted in the upper parts of the
121 saplings.

122 Sampling and $\delta^{13}\text{C}$ of WSOM measurement

123 Following the measurements of gas exchange, the three measured leaves and
124 three small twigs attached the leaves per sapling were collected, wrapped in tinfoil,
125 and preserved in liquid nitrogen to prevent physiological activities.

126 To obtain leaf WSOM, 50 mg of freshly crushed leaf samples were incubated in
127 1.75 mL double demineralized water in a 2.0 mL centrifuge tube for one h at a
128 constant temperature of 5 °C. Subsequently, the leaf material was boiled at 100 °C for
129 3 min to precipitate proteins and centrifuged for 5 min (12000 g) to obtain the
130 supernatants for isotope analysis.

131 The twig phloem exudates were collected according to the method proposed by
132 Gessler *et al.* (2004). Similar to the collection of leaf WSOM, 75 mg twig phloem
133 samples were soaked in 1.75 mL double demineralized water in a 2.0 mL centrifuge
134 tube at room temperature for 24 h. According to Schneider *et al.* (1996) and Bogelein
135 *et al.* (2012), contamination of cellular constituents dissolving into the solution is
136 negligible during this time period. The exudates were then centrifuged for 5 min
137 (12000 g) to obtain supernatants.

138 The resulting supernatants of both leaf and twig material (10 μL) were placed in
139 tin capsules and oven dried at 70 °C. These capsules were transported to the Institute
140 of Botany of the Chinese Academy of Sciences for analysis. $\delta^{13}\text{C}$ in water-soluble leaf
141 and phloem material was analyzed using a stable isotope ratio mass spectrometer
142 (DELTA^{plus}XP, Thermo Finnigan), with a measurement precision of $\pm 0.1\text{‰}$.

143 On the plant sampling day, the atmosphere in the growth chambers was sampled
144 using airbags and an air pump before harvest (at least three replicates). These airbags
145 were also transported to the Institute of Botany of the Chinese Academy of Sciences

for analysis. $\delta^{13}\text{C}$ of atmospheric CO_2 was analysed using a stable isotope ratio mass spectrometer (DELTAplusXP, Thermo Finnigan), with a measurement precision of $\pm 0.1\text{‰}$.

Carbon isotope theory and calculation of mesophyll conductance

Carbon isotopic composition is generally expressed as an isotope ratio where delta (δ) is defined as:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000\text{‰} \quad (1)$$

where R_{standard} and R_{sample} are the $^{12}\text{C}/^{13}\text{C}$ ratios of the standard and the sample.

It is well known that ^{13}C discrimination occurs during the photosynthetic process, which results in different $\delta^{13}\text{C}$ values between the atmosphere and the plant. The measured photosynthetic ^{13}C discrimination between ambient air and leaf/twig is expressed as follows (Farquhar and Richards, 1984):

$$\Delta_{\text{C}_a\text{-leaf}} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_{\text{leaf}}) / (1 + \delta^{13}\text{C}_{\text{leaf}}) \quad (2)$$

$$\Delta_{\text{C}_a\text{-leaf}} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_{\text{leaf}}) / (1 + \delta^{13}\text{C}_{\text{leaf}}) \quad (3)$$

where $\delta^{13}\text{C}_a$, $\delta^{13}\text{C}_{\text{leaf}}$, and $\delta^{13}\text{C}_{\text{twig}}$ are $\delta^{13}\text{C}$ in ambient air, leaf WSOM, and twig WSOM, respectively.

In the most commonly used version of the Farquhar *et al.* (1982) simple model, the photosynthetic ^{13}C discrimination was linearly related to C_i/C_a . Thus, photosynthetic ^{13}C discrimination derived from gas exchange measurements (Δ_i) is calculated as:

$$\text{C}_i:\text{C}_a = (\Delta_i - a) / (b - a) \quad (4)$$

where a is the fractionation associated with CO_2 diffusion in air (4.4‰; Seibt *et al.*, 2008), and b is the fractionation relevant to reactions by Rubisco and PEP carboxylase (27‰; Hu *et al.*, 2010).

Indeed, CO_2 diffusion during photosynthesis includes three main steps: atmospheric CO_2 reaches the surface of the leaf, it then enters into sub-stomatic cavities through the stomata, and it finally arrives at the chloroplast stoma in which photosynthesis occurs (Flexas *et al.*, 2008). Therefore, photosynthetic ^{13}C discrimination is dependent on the chloroplastic CO_2 concentration (C_c), rather than

175 C_i . Recently, studies have demonstrated that mesophyll resistance to CO_2 diffusion
176 would cause a drawdown from C_i to C_c , which means mesophyll conductance (g_m)
177 should be carefully considered (Flexas *et al.*, 2006; Warren and Adams, 2006;
178 Borgelein *et al.*, 2012). The more comprehensive equation can be expressed as
179 (Farquhar *et al.*, 1982; Seibt *et al.*, 2008):

$$180 \Delta_{comp} = a_b \times \frac{C_a - C_b}{C_a} + a \times \frac{C_b - C_i}{C_a} + a_i \times \frac{C_i - C_c}{C_a} + b \times \frac{C_c}{C_a} + \left(\frac{e \times R_d / k + f \times \Gamma^*}{C_a} \right) \quad (5)$$

181 where C_b is the CO_2 concentration on the leaf surface; a_b is the fractionation
182 associated with atmospheric CO_2 diffusion at the boundary layer (2.9‰); a_i is the
183 fractionation for CO_2 diffusion and dissolution in the liquid phase (1.8‰); e and f are
184 the discrimination values for mitochondrial respiration (dark respiration, R_d) and
185 photorespiration, respectively; Γ^* is the CO_2 compensation point; and k is the
186 carboxylation efficiency. It is generally accepted that the fractionation caused by
187 boundary layer diffusion can be ignored and the equation can be simplified as (Shi *et*
188 *al.*, 2010):

$$189 \Delta_{comp} = a \times \frac{C_b - C_i}{C_a} + a_i \times \frac{C_i - C_c}{C_a} + b \times \frac{C_c}{C_a} + \left(\frac{e \times k \times R_d + f \times \Gamma^*}{C_a} \right) \quad (6)$$

190 Substituting Δ_i into equation 6 we obtain the following equation:

$$191 \Delta_i - \Delta_{comp} = (b - a_i) \times \frac{C_i - C_c}{C_a} + \left(\frac{e \times k \times R_d + f \times \Gamma^*}{C_a} \right) \quad (7)$$

192 According to Fick's first law, $P_n = g_s \times (C_a - C_i) = g_m \times (C_i - C_c)$ (Seibt *et al.*, 2008). To
193 calculate g_m , we substituted Fick's first law into equation (6) and the equation was
194 transformed into:

$$195 g_m = \frac{(b - a_i) \times \frac{P_n}{C_a}}{(\Delta_i - \Delta_{mea}) - \left(\frac{e \times k \times R_d + f \times \Gamma^*}{C_a} \right)} \quad (8)$$

196 Ignorance of dark respiration and photorespiration can avoid further uncertainties
197 and complexity, and including the two processes will not significantly improve model
198 outcomes. For this reason, equation (8) can be simplified as:

$$199 g_m = \frac{(b - a_i) \times \frac{P_n}{C_a}}{(\Delta_i - \Delta_{mea})} \quad (9)$$

200 Results

201 Gas exchange properties

202 The gas exchange properties tended to increase with an increase in SWC,
203 reaching a maximum at 70%–80% FC (Fig. 2). Under any given C_a , the most striking
204 increase in these gas exchange properties generally occurred when SWC increased
205 from 35%–45% FC to 50%–60% FC. However, when SWC peaked at 95%–100% FC,
206 these gas exchange properties began to decrease slightly (Fig. 2).

207 Elevation of C_a caused an increase in P_n and C_i under any given soil moisture
208 condition (Fig. 2c and 2d), while g_s decreased with the elevation of C_a (Fig. 2a).
209 Furthermore, g_m tended to decline as C_a increased except when SWC was 50%–60%,
210 which was similar to the pattern of change of g_s (Fig. 2b).

211 Correlation analysis revealed that P_n was significantly correlated with g_s , g_m ,
212 and C_i , with correlation coefficients (r) of 0.91, 0.90, and 0.72 ($p < 0.01$), respectively
213 (Table 2). In contrast, the relationship between P_n and C_i/C_a was not strong or even
214 moderate. Moreover, C_i and g_s or g_m were not significantly correlated, but both g_s
215 and g_m were highly correlated with C_i/C_a (r of 0.78 and 0.72, respectively; $p < 0.01$).
216 Additionally, g_m was closely correlated with g_s ($r = 0.94$; $p < 0.01$) (Table 2).

217 $\delta^{13}\text{C}$ of water-soluble leaf and twig phloem materials

218 $\delta^{13}\text{C}$ of leaf WSOM ranged between -28.18‰ and -25.18‰, while $\delta^{13}\text{C}$ of twig
219 phloem WSOM had a wider range of -28.79‰ and -23.78‰ (Fig. 3). The $\delta^{13}\text{C}$ of
220 both leaf and twig phloem WSOM was maximized under the lowest SWC (35%–45%
221 FC) at any given C_a . When SWC increased from 35%–45% FC to 50%–60% FC, $\delta^{13}\text{C}$
222 of water-soluble leaf and twig phloem materials had the most rapid decrease.
223 Reduction of $\delta^{13}\text{C}$ of water-soluble leaf and twig phloem materials typically leveled
224 off when SWC was higher than 60%–70% FC. Under non-severe water stress
225 conditions, $\delta^{13}\text{C}$ of both leaf and twig phloem WSOM were usually highest when C_a
226 was 600 $\mu\text{mol mol}^{-1}$ (Fig. 3). In contrast, elevation of C_a produced increases in $\delta^{13}\text{C}$ of
227 water-soluble leaf and twig phloem materials under the lowest SWC (35%–45% FC).

228 ^{13}C enrichment from ambient air to plant

229 $\Delta_{\text{Ca-leaf}}$ ranged between 8.63‰ and 14.76‰, reaching a maximum at 60%–70%
230 FC $\times C_{400}$ and a minimum at 35%–45% FC $\times C_{800}$ (Fig. 4a). In contrast, $\Delta_{\text{Ca-twig}}$ had a
231 greater range between 7.18‰ and 15.59‰, reaching a maximum at 95%–100% FC $\times$

C_{400} and a minimum at 35%–45% $FC \times C_{800}$ (Fig. 4b). Under any C_a , both $\Delta_{Ca-leaf}$ and $\Delta_{Ca-twig}$ tended to increase with increased SWC. Elevated C_a had an important influence on both $\Delta_{Ca-leaf}$ and $\Delta_{Ca-twig}$; $\Delta_{Ca-leaf}$ and $\Delta_{Ca-twig}$ decreased significantly when C_a increased from 400 $\mu\text{mol mol}^{-1}$ to 600 $\mu\text{mol mol}^{-1}$ ($p < 0.01$). However, there were no significant differences in $\Delta_{Ca-leaf}$ and $\Delta_{Ca-twig}$ when C_a increased from 600 $\mu\text{mol mol}^{-1}$ to 800 $\mu\text{mol mol}^{-1}$ ($p > 0.05$) (Fig. 4).

Correlation analyses

Analyses of correlation between gas exchange properties and $\delta^{13}\text{C}$ and isotope discrimination are presented in Table 3. There were significant correlations between g_s and g_m and $\delta^{13}\text{C}$ of leaf WSOM (r of -0.84 and -0.87, respectively; $p < 0.01$). The $\delta^{13}\text{C}$ of twig phloem WSOM was also highly correlated with g_s and g_m (r of -0.78 and -0.82, respectively; $p < 0.01$). Moreover, similar significant correlations between g_s and g_m and $\Delta_{Ca-leaf}$ (for both, $r = 0.69$, $p < 0.01$) as well as between g_s and g_m and $\Delta_{Ca-twig}$ (r of 0.66 and 0.67, respectively; $p < 0.01$) were observed. Table 3 also shows that C_i was moderately associated with $\delta^{13}\text{C}$ of leaf WSOM ($r = -0.62$, $p < 0.05$) but there was no significant or even moderate correlation between C_i and $\delta^{13}\text{C}$ of twig phloem WSOM ($r = -0.38$, $p > 0.05$). However, $\delta^{13}\text{C}$ of twig phloem WSOM was highly related with C_i/C_a ($r = -0.62$, $p < 0.05$). Additionally, C_i/C_a was strongly associated with $\Delta_{Ca-leaf}$ and $\Delta_{Ca-twig}$ (r of -0.92 and -0.88, respectively; $p < 0.01$).

Discussion

^{13}C fractionation from leaves to twig phloem

We observed no clear patterns of variation in ^{13}C fractionation from leaves to twig phloem in response to the interaction between SWC and C_a . In most cases, ^{13}C was depleted in the twig phloem WSOM in comparison to leaf WSOM (Fig. 5), indicating that $\delta^{13}\text{C}$ of fast turnover carbon can be altered as a result of post-photosynthetic fractionation. Such depletion was also reported by Gessler *et al.* (2004) for beech. However, Borgelein *et al.* (2012) found that there was ^{13}C enrichment in twig phloem exudates in comparison to leaf WSOM in the upper and middle beech canopy, but such enrichment was not detected in the lower shaded beech

261 canopy. Similar intra-canopy patterns of twig-to-leaf $\delta^{13}\text{C}$ differences were also
262 reported by Cernusak *et al.* (2009). Further, Eglin *et al.* (2009) observed that WSOM
263 had higher $\delta^{13}\text{C}$ values in twigs than in leaves within sunlit beech crowns.

264 It is well known that a large amount of glucose is synthesized in chloroplasts
265 when photosynthesis is active, part of which is exported to the cytosol by triose
266 phosphate translocators (TPTs) for sucrose biosynthesis and other metabolic activities;
267 excess glucose accumulates and is converted into starch in chloroplasts (Cho *et al.*,
268 2011; Mahboubi, 2014). During this process, the aldolase isotope effect leads to
269 relatively ^{13}C enriched hexoses being incorporated in starch while isotopically lighter
270 trioses will be exported to the cytoplasm. Therefore, $\delta^{13}\text{C}$ in the chloroplastic starch
271 was more positive than that in the cytosolic WSOM for plants with strong
272 photosynthetic capacity (Brandes *et al.*, 2006; Cernusak *et al.*, 2009). In contrast, the
273 remobilization of starch in chloroplasts as a result of lower photosynthetic rates leads
274 to sucrose exported from leaves carrying the signature of the starch substrate.
275 Previous studies determined that sucrose is the major carbohydrate that can be
276 exported into phloem and transported throughout the whole plant (Gessler *et al.*, 2008;
277 Mahboubi, 2014). Indeed, Peuke *et al.* (2001) observed that about 90% of the
278 carbohydrates transported in the phloem consists of sucrose. This might result in
279 differences in sunlit versus shaded crowns in ^{13}C fractionation patterns of leaves to
280 twig phloem. In this study, the daytime light intensity in the growth chambers ranged
281 from 200 and 240 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, which is a much smaller range than that of field
282 conditions (ranging from 40 to 1500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ with an average of about 350 μmol
283 $\text{m}^{-2} \text{ s}^{-1}$). Therefore, the $\delta^{13}\text{C}$ difference between leaf and twig phloem WSOM in this
284 study could be because of the absence of starch storage under low light intensity in
285 the growth chambers.

286 However, the potential post-photosynthetic carbon isotope fractionation
287 mechanism can be species-specific. For example, Borgelein *et al.* (2012) claimed that
288 twig phloem WSOM was ^{13}C enriched compared with leaf WSOM throughout the
289 whole canopy for Douglas fir. In a review of more than 80 studies, Badeck *et al.*
290 (2005) found that autotrophic tissues were generally ^{13}C -depleted compared with

heterotrophic tissues. According to Merchant *et al.* (2010) and Cernusak *et al.* (2009), different proportions of apoplastic and symplastic loading pathways could independently influence $\delta^{13}\text{C}$, introducing a potential species-independent factor in the fractionation of phloem isotope composition in contrast to the source tissue. In addition, twig phloem WSOM is a mixture of carbohydrates with different metabolic histories derived from carbon exported from the source tissue (leaves) during both light and dark periods. Cernusak *et al.* (2009) postulated that if leaves and small twigs were sampled at the same time during the day, twig phloem could carry ^{13}C signals originating from both day and night exports from source tissues. Specifically, Ruehr *et al.* (2009) suggested that carbohydrate exportation takes less than 5 hours when SWC is high but up to 4 days under drought conditions. This may cause the ^{13}C fractionation pattern from leaf to phloem to be more complicated. Previous studies also proposed that $\delta^{13}\text{C}$ differences between leaf and twig phloem WSOM could be associated with respiratory processes (Cernusak *et al.*, 2009; Merchant *et al.*, 2010). However, Eglin *et al.* (2009) observed that there were no significant differences in $\delta^{13}\text{C}$ of respired CO_2 between leaves and twigs. This finding indicated that divergence in $\delta^{13}\text{C}$ between the two organs is not likely to be explained by respiration fractionation. We suggest that post-photosynthesis fractionation may not be attributed to a single, unifying hypothesis; instead, it is the result of multiple processes. A better description of the characteristics of these hypotheses and what percentage of each processes is responsible for post-photosynthesis fractionation in different plant organs will improve our understanding of metabolism and plant physiology.

Variations in $\delta^{13}\text{C}$ of leaf and twig phloem WSOM

Variation in $\delta^{13}\text{C}$ of leaf WSOM has been widely used to reveal plant leaf-level responses to environmental conditions (Hasselquist *et al.* 2010; Tomas *et al.* 2012). In this study, the effect of the interaction between SWC and C_a on $\delta^{13}\text{C}$ of leaf WSOM was analysed. At any C_a , $\delta^{13}\text{C}$ of leaf WSOM was maximized under the lowest SWC (35%–45% FC). The effect of drought on stable carbon isotope composition at the leaf level of *Platycladus orientalis* saplings was similar to the effect on tolerant and

sensitive beech ecotypes (Peuke *et al.*, 2006). In addition, Nie *et al.* (2014) found that leaf $\delta^{13}\text{C}$ signals of nearly all selected species growing in subtropical China exhibited were more positive during the dry season than in the wet season. When SWC increased to more than 50% FC, $\delta^{13}\text{C}$ of leaf WSOM was maximized at C_{600} . The $\delta^{13}\text{C}$ of leaf WSOM didn't increase if C_a get too high. The interactive effect between SWC and C_a on $\delta^{13}\text{C}$ of twig phloem WSOM is similar to that on $\delta^{13}\text{C}$ of leaf WSOM. Further regression analysis showed that the correlation between SWC and $\delta^{13}\text{C}$ of twig phloem WSOM is significantly stronger than that between SWC and $\delta^{13}\text{C}$ of twig phloem WSOM (Fig. 6). This result indicates that $\delta^{13}\text{C}$ of twig phloem WSOM is a better predictor of SWC than that of leaf WSOM. Similar results have also be found by Merchant *et al.* (2010), who suggested that the soluble leaf carbon, amino acid, and nutrient concentrations in the leaf had lower sensitivity for the prediction of plant water status than $\delta^{13}\text{C}$ of twig phloem WSOM, although $\delta^{13}\text{C}$ of leaf sugars and soluble extracts mirrored the variation in $\delta^{13}\text{C}$ of twig phloem WSOM. A larger or smaller time lag for twig phloem carbon, which is a mixture of various carbohydrates with different metabolic histories, is insufficient to override the ^{13}C signal of newly synthesized carbohydrates (Merchant *et al.*, 2010). Further, the phloem loading pathways by which new assimilates are exported from the leaf could be influenced by environmental conditions (Turgeon *et al.*, 2001). This finding results from the phloem loading pathways being associated with membrane potential of mesophyll cells (Turgeon and Medville, 2004), and the pressure is sensitive to variations in the concentrations of sucrose and raffinose (van Bel *et al.*, 1996). Phloem sap is dominated by sucrose and raffinose (Tausz *et al.*, 2008), both of which are associated with plant water status (Merchant *et al.* 2010). Therefore, variations in SWC may alter the contributions of apoplastic and symplastic loading pathways, which affect $\delta^{13}\text{C}$ of twig phloem WSOM.

Correlations between carbon isotope discrimination and gas exchange properties

To date, numerous studies have confirmed the negative relationship between $\Delta_{\text{Ca-leaf}}$ and water use efficiency at the leaf level (Medrano *et al.*, 2009;

349 Cornejo-Oviedo *et al.*, 2017), as $\Delta_{\text{Ca-leaf}}$ is highly correlated with C_i/C_a (Farquhar *et al.*,
350 1982; Table 3). We found that peak $\Delta_{\text{Ca-leaf}}$ was reached at 60%–70% $\text{FC} \times C_{400}$, and
351 the minimum value was found at 35–45% $\text{FC} \times C_{800}$ (Fig. 4). This finding indicated
352 more conservative water use strategies under higher C_a and drought conditions. Under
353 elevated C_a , the inhibition of photosynthetic uptake caused by drought can be
354 alleviated, while transpiration capacity may remain constant or decrease (Bernacchi *et*
355 *al.*, 2006; Miranda Apodaca *et al.*, 2015), which caused $\Delta_{\text{Ca-leaf}}$ to decrease. In addition,
356 we found that $\Delta_{\text{Ca-twig}}$ is significantly correlated with C_i/C_a , suggesting that $\Delta_{\text{Ca-twig}}$ is a
357 sensitive predictor of plant water use efficiency.

358 Carbon isotope discrimination from ambient air to the plant is dependent on g_s as
359 C_i is regulated by g_s , and simultaneously, g_s is subject to the feedback of carbon
360 isotope discrimination. Early studies generally accepted that net photosynthesis
361 depends on the supply of CO_2 in the sub-stomata and ignored the important role that
362 g_m played (Lauer *et al.*, 1992). At that time, g_m was considered to be infinitely large,
363 which resulted in C_i being equal or close to C_c . However, more recent research has
364 shown that g_m is a species-specific and environmentally dependent factor, and
365 ignoring the potential difference between C_i and C_c could result in the erroneous
366 interpretation of ^{13}C discrimination. Therefore, caution must be exercised when
367 considering the mechanism of ^{13}C enrichment during photosynthetic CO_2 fixation
368 (Flexas *et al.*, 2006; Seibt *et al.*, 2008; Borgelein *et al.*, 2012). Correlation analysis
369 showed that g_s and g_m had similar positive effects on $\Delta_{\text{Ca-leaf}}$ and $\Delta_{\text{Ca-twig}}$ (Fig. 5). This
370 finding emphasizes the important influence of g_m on ^{13}C discrimination during
371 photosynthesis. The similar influence that g_s and g_m had on $\Delta_{\text{Ca-leaf}}$ has also been
372 reported by Shi *et al.* (2010). However, Warren (2004) claimed that mesophyll
373 resistance had far more influence than stomatal resistance on CO_2 diffusion during
374 photosynthesis for *Eucalyptus globulus*, so it is possible that the mechanism might be
375 species-specific.

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529

530 Table 1. Scheme of the orthogonal experiment used in this research. A represents C_a,
531 and B represents SWC. A₁, A₂, and A₃ are C₄₀₀, C₆₀₀, and C₈₀₀, respectively. And B₁,
532 B₂, B₃, B₄ and B₅ are different SWC levels (35%–45% FC, 50%–60% FC, 60%–70%
533 FC, 70%–80% FC, and 95%–100% FC, respectively). R represents the number of
534 replicates of each treatment.

C	SW	Replicate	C _a		
			A ₁	A ₂	A ₃
B ₁		R ₁	A ₁ B ₁ R ₁	A ₂ B ₁ R ₁	A ₃ B ₁ R ₁
		R ₂	A ₁ B ₁ R ₂	A ₂ B ₁ R ₂	A ₃ B ₁ R ₂
		R ₃	A ₁ B ₁ R ₃	A ₂ B ₁ R ₃	A ₃ B ₁ R ₃
B ₂		R ₁	A ₁ B ₂ R ₁	A ₂ B ₂ R ₁	A ₃ B ₂ R ₁
		R ₂	A ₁ B ₂ R ₂	A ₂ B ₂ R ₂	A ₃ B ₂ R ₂
		R ₃	A ₁ B ₂ R ₃	A ₂ B ₂ R ₃	A ₃ B ₂ R ₃
B ₃		R ₁	A ₁ B ₃ R ₁	A ₂ B ₃ R ₁	A ₃ B ₃ R ₁
		R ₂	A ₁ B ₃ R ₂	A ₂ B ₃ R ₂	A ₃ B ₃ R ₂
		R ₃	A ₁ B ₃ R ₃	A ₂ B ₃ R ₃	A ₃ B ₃ R ₃
B ₄		R ₁	A ₁ B ₄ R ₁	A ₂ B ₄ R ₁	A ₃ B ₄ R ₁
		R ₂	A ₁ B ₄ R ₂	A ₂ B ₄ R ₂	A ₃ B ₄ R ₂
		R ₃	A ₁ B ₄ R ₃	A ₂ B ₄ R ₃	A ₃ B ₄ R ₃
B ₅		R ₁	A ₁ B ₅ R ₁	A ₂ B ₅ R ₁	A ₃ B ₅ R ₁
		R ₂	A ₁ B ₅ R ₂	A ₂ B ₅ R ₂	A ₃ B ₅ R ₂
		R ₃	A ₁ B ₅ R ₃	A ₂ B ₅ R ₃	A ₃ B ₅ R ₃

535

536 Table 2. Correlation between gas exchange properties. n=15; * $p<0.05$, ** $p<0.01$.

	g_s	g_m	C_i	C_i / C_a	P_n
g_s	—	0.94**	0.42	0.78**	0.91**
g_m		—	0.41	0.72**	0.90**
C_i			—	—	0.72**
C_i / C_a				—	0.49
P_n					—

537

538 Table 3. Correlation between gas exchange properties and $\delta^{13}\text{C}$ and carbon isotope
539 discrimination. n=15; * $p<0.05$, ** $p<0.01$.

	$\delta^{13}\text{C}_{\text{leaf}}$	$\delta^{13}\text{C}_{\text{twig}}$	$\Delta_{\text{Ca-leaf}}$	$\Delta_{\text{Ca-twig}}$
g_s	-0.84**	-0.78**	0.69**	0.66**
g_m	-0.87**	-0.82**	0.69**	0.67**
C_i	-0.62*	-0.38	—	—
C_i/C_a	-0.48	-0.62*	-0.92**	-0.88**

540

Figure 1. Growth chambers used in the experiments. One growth chamber (a) was used to maintain C_a of 600 $\mu\text{mol mol}^{-1}$ and 800 $\mu\text{mol mol}^{-1}$, another growth chamber (b) was used to maintain C_a of 400 $\mu\text{mol mol}^{-1}$.

544

545 Figure 2. Gas exchange properties of *Platycladus orientalis* saplings under five SWC

546 $\times$ three C_a . Data are mean values $\pm$ SD.

547
548 Figure 3. δ¹³C of water-soluble leaf (a) and twig phloem material (b) of *Platycladus*
549 *orientalis* saplings under five SWC × three C_a. Data are mean values ± SD.

550

551 Figure 4. ^{13}C enrichment from ambient air to leaf (a) and twig (b) of *Platycladus*

552 *orientalis* saplings under five SWC $\times$ three C_a . Data are mean values $\pm$ SD.

553

554 Figure 5. The $\delta^{13}\text{C}$ of leaf WSOM versus $\delta^{13}\text{C}$ of twig phloem WSOM.

555

556 Figure 6. Relationship between SWC and $\delta^{13}\text{C}$ of leaf and twig phloem WSOM.