Variations in $\delta^{13}\text{C}$ of different plant organs: implications for post-photosynthetic fractionation

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Highlight

$\delta^{13}\text{C}$ of twig phloem water soluble organic materials varied no clear diel pattern.

In the leaf-twig-stem-root sequence, the greatest ^{13}C enrichment was recorded in stem phloem.

Abstract

Compared to photosynthetic fractionation, the mechanism of post-photosynthetic carbon isotope fractionation is not well understood. The aim of this study was to investigate post-photosynthetic fractionation in both above and below ground tissues and to evaluate potential hypotheses explaining differences in carbon isotope composition ($\delta^{13}\text{C}$) among different plant organs, which can provide valuable insights into plant physiology. The results revealed that there is no significant day-night difference in $\delta^{13}\text{C}$ of twig phloem water soluble organic materials (WSOM), which could be explained by the unrestricted exchange of triose-phosphates between the chloroplast and cytoplasm and a time lag for carbohydrate exportation. Further, we found that $\delta^{13}\text{C}$ of twig phloem WSOM is more sensitive to plant water status than leaf WSOM. Analysis of $\delta^{13}\text{C}$ in different plant organs showed that the greatest ^{13}C enrichment was recorded in stem phloem. Divergences in $\delta^{13}\text{C}$ of phloem WSOM among different plant organs were not likely to be explained by respiratory fractionation or time lag and were ascribed to transport of carbohydrates across organ boundaries and metabolic processes. Our study demonstrated that post-photosynthesis fractionation could not be ascribed to a single, unifying hypotheses; instead, it is the result of multiple processes.

Keywords: Carbon isotope composition, difference, phloem, plant organs,

30 post-photosynthesis fractionation, water soluble organic materials.

31 **Introduction**

32 ^{13}C fractionation during photosynthesis had been shown to produce divergence
 33 in carbon isotope composition ($\delta^{13}\text{C}$) between the atmosphere and plant organic
 34 material, leading to leaves that are ^{13}C enriched in comparison to the atmosphere
 35 (Seibt *et al.*, 2008; Hu *et al.*, 2010). After sugars are synthesized in the chloroplast,
 36 some of the new assimilates are then exported from leaves and transported in the
 37 phloem to different plant organs, such as twigs, stems, and roots (Mahboubi, 2014).
 38 To date, a large number of studies have reported that $\delta^{13}\text{C}$ of heterotrophic organs can
 39 be altered, resulting in distinct signatures in different plant organs because of carbon
 40 isotope fractionation following carboxylation (Badeck *et al.*, 2005; Eglin *et al.*, 2009;
 41 Zhao *et al.*, 2017). Post-photosynthetic carbon isotope fractionation has been
 42 associated with activities such as transport of metabolites, the process of respiration,
 43 and biosynthesis of organic materials (Damesin and Lelarge, 2003; Gessler *et al.*,
 44 2004; Brandes *et al.*, 2006; Bathellier *et al.* 2008). In contrast to photosynthetic
 45 carbon isotope fractionation, the mechanism of post-photosynthetic fractionation in
 46 different plant organs is not clear. Cernusak *et al.* (2009) proposed six hypotheses that
 47 may explain the divergence in $\delta^{13}\text{C}$ between source and sink tissues. In addition,
 48 Badeck *et al.* (2005) reviewed more than 80 studies and found that autotrophic tissues
 49 were generally ^{13}C -depleted in comparison to heterotrophic tissues. However, there
 50 are no definitive conclusions about carbon allocation among different organs owing to
 51 post-photosynthetic fractionation. Recently, post-photosynthetic fractionation has
 52 been extensively investigated, providing important insights into plant physiology.
 53 Further, the interpretation of post-photosynthetic fractionation allows the
 54 reconstruction of individual sinks and sources using $\delta^{13}\text{C}$ of CO_2 exchange flux at the
 55 ecosystem scale because $\delta^{13}\text{C}$ of respired CO_2 of different plant organs is closely
 56 related to that of the respiratory substrate (Bowling *et al.*, 2001; Gessler *et al.*, 2008;
 57 Wehr *et al.*, 2015).

58 Considerable uncertainty and complexity, including spatial variation,

complicates investigation of post-photosynthetic fractionation of tall adult trees with heterogeneous vertical structure under field conditions. To avoid spatial inconsistencies, we increased sample size and sampled leaf and twig material at different canopy heights. Most recent studies have interpreted post-photosynthetic fractionation based on total organic materials of both autotrophic and heterotrophic tissues (Badeck *et al.*, 2005; Cernusak *et al.*, 2009); however, this method is not very efficient when considering the process of carbon transport among different plant organs. Carbon isotopes from the fast-turnover carbohydrate pool (water-soluble organic materials, WSOM) in leaves are commonly used indicators of assimilation-weighted average C_i/C_a (the ratio of intercellular to atmospheric CO_2 concentration) integrated over a period of several hours to 1–2 days (Pons *et al.* 2009), which can be imprinted in phloem WSOM rapidly. Therefore, analysis of $\delta^{13}C$ of WSOM can provide valuable information about post-photosynthetic fractionation within plants. Recently, the development of phloem collection techniques has enabled ^{13}C signals of heterotrophic phloem WSOM to be precisely determined. In this study, we detected $\delta^{13}C$ of WSOM for both above and below ground organs, which may contribute to understanding the carbon exchange process between above and below ground organs and the mechanism of interaction between plants and the soil (Deng *et al.*, 2017).

The objectives of the study were to investigate post-photosynthetic fractionation in the leaf-twig-stem-root sequence, as carbohydrate is exported from leaves and transported in the phloem throughout the whole plant, and to evaluate the potential hypotheses that can explain divergence in $\delta^{13}C$ among different plant organs.

Materials and methods

Study site description

The research was conducted at the Jiufeng National Forest experimental station, located in Beijing in a rocky, mountainous area of north China (116°05' E, 40°03' N). The climate is a typical warm temperate, semi-humid, and semi-arid monsoon climate. Annual average temperature and precipitation are 11.6 °C and 650 mm, respectively,

88 and average evaporation ranges from 1,800 to 2,000 mm per year. Precipitation occurs
89 largely between July and September, which is much less than evaporation. The
90 growing season in this area lasts from April to October. The soil is sandy loam soil
91 with mean thickness of about 52.8 cm. Drought-tolerant conifers are the main tree
92 species.

93 The central area (about 1 km²) of the sampling site mainly consists of
94 50–60-year-old *Platycladus orientalis*, with an average tree height and breast diameter
95 of 8 m and 20 cm, respectively, and forest crown density is about 0.8. There are small
96 patches of shrubs and herbs, predominantly *Vitex negundo*, *Grewia biloba*, and
97 *Broussonetia papyrifera*.

98 **Sampling and $\delta^{13}\text{C}$ determination of different plant organs**

99 Sampling was performed on several sunny days in 2017. On 25 May, 17 July,
100 and 29 August, samples were collected every two hours from 1:00 to 24:00 for
101 analysis of diel variation in carbon isotopes of different plant organs. In addition, on
102 19 April, 8 August, 10 August, and 11 August, plant materials were collected every six
103 hours from 1:00 to 24:00 to calculate daily mean values. On each sampling day, three
104 mature *Platycladus orientalis* conifer leaves and small twigs were collected in each
105 canopy crown (including the upper, middle, and lower canopy), and three replicate
106 stem phloem and fine root samples were also collected. After sampling, all plant
107 materials were covered with foil and rapidly preserved in liquid nitrogen. All samples
108 were transported to the Key Laboratory of Soil and Water Conservation and
109 Desertification Combating of Ministry of Education of Beijing Forestry University for
110 carbon isotope composition ($\delta^{13}\text{C}$) analysis.

111 To obtain leaf water-soluble organic material (WSOM), the middle veins of the
112 leaves were removed. Next, 50 mg of freshly abraded leaf samples were well mixed
113 with 100 mg PVPP (polyvinylpyrrolidone) and double demineralized water up to 1.75
114 mL was added in a 2.0 mL centrifuge tube. These mixtures were incubated for 1 h at a
115 constant temperature of 5 °a. The small twig, stem, and root phloem WSOM were
116 collected according to the methods of Brandes *et al.* (2006). Similar to the collection

117 of leaf WSOM, 75 mg phloem samples were incubated in 1.75 mL double
118 demineralized water in a 2.0 mL centrifuge tube at room temperature for 24 h.
119 Subsequently, the leaf and phloem materials were boiled at 100 °C for 3 min and
120 centrifuged for 5 min (12000 g). Supernatants of WSOM (10 µL) were transferred
121 into tin capsules and oven-dried at 70 °C before $\delta^{13}\text{C}$ analysis. Finally, $\delta^{13}\text{C}$ of dry
122 residues were analyzed using a stable isotope ratio mass spectrometer (Finnigan
123 DELTAplusXP, Thermo Fisher Scientific, USA).

124 Meteorological measurements

125 A meteorological tower at the sampling site continuously output
126 micrometeorological data. Air temperature (T_a) and relative humidity (RH) sensors
127 (HOBO-U30, Onset, USA) were installed in the lower (5 m), middle (6.5 m), and
128 upper crown (8 m). Vapor pressure deficit (VPD) was calculated as
129 $VPD = 0.611 \times 10^{[17.502T_a(240.97+T_a)]} \times (1-RH)$.

130 Additionally, solar radiation (R_a) was measured at 8 m by a HOBO-U30 sensor
131 (Onset, USA). Further, soil water content (M_s) was determined using three sensors
132 (HOBO-U30, Onset, USA) which were installed 10–30 cm below the ground. All
133 micrometeorological data were recorded every 10 min.

134 Results

135 Measurements of meteorological conditions

136 Both T_a and VPD exhibited similar diel dome-shaped variation at each sampling
137 period, reaching maximum values around noon and minimum values during the night
138 (Fig. 1). However, RH had the opposite trend over time. Among canopy heights,
139 maximum T_a and VPD were generally recorded in the upper canopy and minimum
140 values were typically observed in the lower canopy. In contrast, RH tended to
141 decrease with increasing canopy height (Fig. 1).

142 M_s was almost constant over diel time scales within the three sampling days, but
143 it varied among the days (Fig. 2). M_s in the rainy season (on 17 July and 29 August)
144 was significantly higher than that in the dry season. R_a had a varying diel
145 dome-shaped pattern, reaching maximum values around noon, then decreasing to its

146 minimum value in the evening (Fig. 2).

147 $\delta^{13}\text{C}$ of different plant organs

148 The $\delta^{13}\text{C}$ of leaf and small twig phloem WSOM had no distinct diel pattern and
149 ranged from -27.82‰ to -26.32‰ and from -28.18‰ to -26.47‰, respectively (Fig.
150 3). However, at different canopy heights, we repeatedly observed clear intracanopy
151 gradients of $\delta^{13}\text{C}$ of leaf and twig phloem WSOM. The greatest $\delta^{13}\text{C}$ enrichment of
152 leaf and twig phloem WSOM was observed in the upper canopy, and $\delta^{13}\text{C}$ in the lower
153 canopy was more negative in comparison with those in the middle and upper canopy.

154 The $\delta^{13}\text{C}$ analysis of both autotrophic tissue (leaves) and heterotrophic tissue
155 phloem (small twigs, stems, and roots) WSOM is shown in Fig. 4. Although there
156 were no significant day-night differences in $\delta^{13}\text{C}$ of these plant organs ($p>0.05$), there
157 were clear differences among different plant organs. The greatest ^{13}C enrichment was
158 observed in stem exudates, which ranged between -26.21‰ and -24.48‰. $\delta^{13}\text{C}$ of leaf
159 WSOM was generally more positive than that of small twig phloem WSOM, with a
160 mean difference of 0.44 ± 0.36 ‰. On 25 May and 29 August, $\delta^{13}\text{C}$ of root phloem
161 WSOM was similar to that of leaf WSOM. However, on 17 July, root phloem WSOM
162 was slightly enriched in $\delta^{13}\text{C}$ compared to leaf WSOM.

163 Daily average values

164 Daily average values were analysed to describe day-to-day variation in $\delta^{13}\text{C}$ of
165 different plant organs. Fig. 5 shows that $\delta^{13}\text{C}$ of WSOM of all plant organs had the
166 most positive values on 25 May, and the second-highest values were recorded on 19
167 April, but the differences were not significant. The day-to-day variation of $\delta^{13}\text{C}$ of
168 small twig and stem phloem WSOM was similar to that of leaf WSOM. Further, daily
169 mean $\delta^{13}\text{C}$ values of root phloem WSOM tended to be more variable than those of
170 WSOM of other plant organs.

171 Correlation analysis

172 At smaller temporal scales (two-hour intervals), there were moderate correlations
173 between $\delta^{13}\text{C}$ of leaf WSOM and RH or VPD , with r of -0.62 and 0.63, respectively
174 ($p<0.01$) (Table 1). The results showed that neither $\delta^{13}\text{C}$ of WSOM of leaves nor that

of twig phloem had significant correlations with M_s . At larger temporal scales (daily mean values), there were significant correlations between M_s and $\delta^{13}\text{C}$ of leaf or twig WSOM (r equal to -0.83 and -0.91, respectively, $p < 0.01$), but the correlations between RH or VPD and $\delta^{13}\text{C}$ of leaf or twig WSOM were not significant (Table 2).

Discussion

^{13}C enrichment of above ground organs

Previous studies have demonstrated that chloroplasts are the place where carbon assimilation occurs (Seibt *et al.*, 2008; Bogelein *et al.*, 2012). During light periods, large amounts of glucose are synthesized in chloroplasts as a result of their high photosynthetic capacity. Some of this glucose is exported to the cytosol by triose phosphate translocators (TPTs) for sucrose biosynthesis and other metabolic activities, and excess glucose accumulates and converts into starch in the chloroplast. In contrast, during dark periods, photosynthesis is constrained without illumination, and starch in the chloroplast is remobilized to produce glucose and maltose, which are exported to the cytosol to be utilized in the biosynthesis of sucrose and the process of primary metabolism (Cho *et al.*, 2011; Mahboubi, 2014). During the course of aldolase-catalysed reactions in the chloroplast, the aldolase isotope effect bring about relatively ^{13}C enriched hexoses will be incorporated in starch while lighter trioses will be exported to the cytoplasm. Therefore, $\delta^{13}\text{C}$ in the chloroplast starch is more positive than that in the cytosolic WSOM in the daytime (Brandes *et al.*, 2006; Cernusak *et al.*, 2009). Instead, the breakdown of starch leads to sucrose being exported from leaves, which can carry the signature of the starch substrate. Previous studies determined that sucrose is the major carbohydrates that can be exported into phloem and transported within the whole plant (Gessler *et al.*, 2008; Mahboubi, 2014). Peuke *et al.* (2001), observed that about 90% of the carbohydrates transported in the phloem is made up of sucrose. This can result in distinct day-night differences in $\delta^{13}\text{C}$ of leaf and small twig phloem WSOM. In this study, $\delta^{13}\text{C}$ analysis of diel variation revealed that there are no significant day-night differences in $\delta^{13}\text{C}$ of the fast-turn-over carbon pool in leaves and in twig phloem (Fig.3). This finding is in

204 contrast to previous results showing that $\delta^{13}\text{C}$ in water-soluble leaf and twig
205 compounds are generally enriched during night or early morning and relatively
206 depleted during light periods. Our results may differ from these previous findings
207 because the exchange mechanism of triose-phosphates between the chloroplast and
208 cytoplasm may be species-specific. Tcherkez *et al.* (2004) proposed that
209 aldolase-catalysed activities in the cytoplasm and chloroplast would utilize the same
210 pool of triose-phosphates as substrates if the exchange was not restricted. In that case,
211 $\delta^{13}\text{C}$ of starch is quite similar to that of light-produced sucrose. Moreover, twig
212 phloem WSOM is a mixture of carbohydrates, with different metabolic histories,
213 derived from carbon exported from source tissues (leaves) during both light and dark
214 periods. According to Cernusak *et al.* (2009), if leaves and small twigs were sampled
215 at the same time during daylight, twig phloem could carry ^{13}C signals originating
216 from both day and night exports from source tissues (leaves). For example, Ruehr *et*
217 *al.* (2009) suggested that it takes less than 5 hours for carbohydrate exportation when
218 soil water content is high, while exportation increased to 4 days under drought
219 conditions. As a consequence, day-night differences in $\delta^{13}\text{C}$ of twig phloem WSOM
220 are strongly inhibited.

221 Although diel variation in $\delta^{13}\text{C}$ of WSOM of above ground organs was not clear,
222 intracanalopy gradients of $\delta^{13}\text{C}$ of leaf and twig phloem WSOM were evident (Fig.3).
223 Similar intracanalopy gradients have been found in Douglas-fir leaves and twig phloem
224 $\delta^{13}\text{C}$ (Bogelein *et al.*, 2012). This finding indicated that $\delta^{13}\text{C}$ of leaf and twig phloem
225 WSOM can be influenced by variation in micrometeorological conditions in different
226 canopy crowns. Surprisingly, we did not observe significant or even moderate ^{13}C
227 enrichment from leaves to small twigs at all canopy heights on the three sampling
228 days, except for 17:00–23:00 on 29 August (Fig. 4), which is similar to the findings of
229 Gessler *et al.* (2004) who did not observe enrichment. However, previous studies have
230 frequently reported that $\delta^{13}\text{C}$ of leaf WSOM is more negative than that of twig phloem
231 (Scartazza *et al.* 2004; Gessler *et al.* 2007; Eglin *et al.*, 2009; Bogelein *et al.*, 2012).
232 This observation could be explained by a time lag for twig phloem. The ^{13}C -enriched

233 materials are produced in leaves but are not transferred into the phloem, depending on
234 the relative allocation of new assimilates to ^{13}C -enriched (starch) or ^{13}C -depleted
235 (lipids, lignin) carbon (Gessler *et al.*, 2007), which might explain why WSOM in
236 leaves is slightly ^{13}C -enriched compared to that in twig phloem.

237 Furthermore, the results presented here indicated that significant ^{13}C enrichment
238 occurred when the water-soluble carbon was exported from twigs to stems ($p < 0.05$;
239 mean difference of $2.32 \pm 0.65\text{‰}$) (Fig.4). One hypotheses to explain the discrepancy
240 is ^{13}C fractionation associated with respiration. Recent reports claimed that $\delta^{13}\text{C}$ of
241 dark-respired CO_2 by woody tissues is more positive than that of respiratory
242 substrates (Damesin *et al.*, 2005; Gessler *et al.*, 2007; Maunoury *et al.*, 2007).
243 Brandes *et al.* (2006) also found a ^{13}C depletion of 2.3–5.2‰ in stem phloem WSOM
244 compared with emitted CO_2 . Another study in three herbs showed that CO_2 respired
245 by shoots was highly ^{13}C -enriched relative to the respiratory substrate (Klumpp *et al.*
246 2005). These studies consistently support that fractionation during respiration caused
247 ^{13}C depletion in stem substrates. Thus, fractionation during respiration poorly explains
248 phloem ^{13}C differences between twigs and stems. Another possible explanation for the
249 ^{13}C enrichment in stems compared with twigs is the long time residence time for
250 phloem carbohydrate transportation from twigs to stems. Assuming phloem transport
251 rate ranges between 0.5 and 1 h (Zimmermann and Braun, 1971), it would take 8–16 h
252 for phloem to be exported from a twig to the stem because the trees in this study are
253 about 8 m tall. Gessler *et al.* (2004) also determined that ^{13}C signals of stem base
254 phloem were significantly related to the average daily values of stomatal conductance
255 two days prior. However, the analysis of daily mean values showed that all twig
256 phloem is significantly ^{13}C -depleted compared with stem phloem (Fig.5). Therefore,
257 time lag does not explain the twig-stem difference. The third hypotheses is that ^{13}C
258 enrichment might be associated with phloem transport. Carbon transportation in the
259 stem does not cause ^{13}C fractionation because $\delta^{13}\text{C}$ of phloem sap does not change
260 significantly in the basipetal direction (Gessler *et al.*, 2008), but the partial retrieval of
261 carbon is responsible for the difference of $\delta^{13}\text{C}$ between twigs and stems. According

262 to the recently reviewed dynamic Münch mass flow model, 2/3 carbon is transported
263 back into the sieve tubes during the process of releasing carbon exported from twigs
264 to stems (Minchin and Thorpe, 1987; Van Bel, 2003). The $\delta^{13}\text{C}$ of retrieved carbon
265 could be changed. Moreover, allocation of carbon to lignin, carrying lighter ^{13}C
266 signals for wood formation, can partly explain the twig-stem difference.

267 **^{13}C depletion of below ground organs**

268 We observed root phloem has carbon isotopic composition similar to the sink
269 tissue (leaves), with mean difference of $0.57 \pm 0.41\%$. While compared with $\delta^{13}\text{C}$ of
270 stem phloem WSOM, there was significant ^{13}C -depletion in root phloem (with mean
271 difference of $1.85 \pm 0.64\%$) (Fig. 4). Similar results have been found in rice (Deng *et al.*
272 *et al.*, 2017). This effect might be ascribed to different dark respiratory mechanisms of
273 different plant organs. Current research found that root-respired CO_2 was ^{13}C depleted
274 in contrast to root carbon (Badeck *et al.*, 2005; Klumpp *et al.*, 2005; Bathellier *et al.*,
275 2009). This result indicated that dark respiratory processes will not cause significant
276 or even moderate ^{13}C -depletion in root phloem WSOM. Another possible explanation
277 is root carbohydrates could be recycled and transported in the acropetal direction
278 (Heizmann *et al.*, 2001). During the process of carbon transport from roots to stems or
279 shoots, $\delta^{13}\text{C}$ of root phloem WSOM might be altered. Moreover, some researchers
280 suggested that root carbohydrates could also leak into the soil in the form of root
281 exudates (Uchida *et al.*, 2010; Werth *et al.*, 2010). According to Uchida *et al.* (2010),
282 10% of carbon from atmospheric CO_2 is transferred into soil by plant photosynthesis
283 and carbon transportation. Deng *et al.* (2017) also found that 3.8%–10% soil organic
284 carbon was derived from root exudates during the rice flowering period, and the
285 percentage decreased along with the growth stages of the rice. Thus, the processes of
286 carbon transport from root into soil should not be neglected, so the ^{13}C signal of root
287 phloem WSOM could also be modified by the process. However, the latter two
288 hypotheses can be species-specific, resulting in different carbon fractionation patterns
289 in roots compared with the carbon source (stem phloem WSOM). For example, the
290 findings of Gessler *et al.* (2008) suggested that $\delta^{13}\text{C}$ of phloem sap organic matter in

stems at different sampling positions were similar to that in roots, and Badeck *et al.* (2005) analysed more than 80 publications and concluded that below ground organs were usually ^{13}C -enriched relative to above ground organs. We also call on a better description of the characteristics of these hypotheses and what percentage of each process is responsible for post-photosynthesis fractionation in different plant organs.

Day-to-day variation of $\delta^{13}\text{C}$ is tightly correlated with M_s

At diel time scales, there were moderate relationships between $\delta^{13}\text{C}$ of leaves and RH or VPD , while the relationship between $\delta^{13}\text{C}$ of leaves and M_s was less evident (Table 1). The results indicated that $\delta^{13}\text{C}$ of leaves is influenced by RH and VPD at short time scales and less dependent on M_s which changes very little compared with RH and VPD during a day-night cycle. In contrast, $\delta^{13}\text{C}$ of phloem WSOM in heterotrophic tissues was not strongly or even moderately dependent on these environmental factors as a result of a time lag. When diel variation is smoothed and daily mean values are calculated, we observed that both $\delta^{13}\text{C}$ of leaf and twig phloem WSOM were strongly dependent on M_s , and there was no dependence between other environmental factors and $\delta^{13}\text{C}$ of leaf or twig phloem WSOM (Table 2). One thing to note is that $\delta^{13}\text{C}$ of twig phloem WSOM is a better indicator of M_s than that of leaf WSOM. Merchant *et al.* (2010) also found that soluble leaf carbon, amino acid, and nutrient concentrations in the leaf were less predictive 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 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). Moreover, leaf WSOM is more heterogeneous consisting of various sugars, organic acids, and amino acids with different residence times (Brandes *et al.*, 2006; Gessler *et al.*, 2008), while twig phloem WSOM is relative simple and is primarily composed of sucrose. This difference might additionally lead to $\delta^{13}\text{C}$ of leaf phloem WSOM having relatively small responses to M_s compared with that of twig phloem WSOM. Thus, we propose that $\delta^{13}\text{C}$ of twig phloem WSOM could be used as proxy for plant water

320 status.

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326 **References**

- 327 **Badeck FW, Tcherkez G, Nogues S, Piel C, Ghashghaie J.** 2005.
328 Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a
329 widespread phenomenon. *Rapid Communications in Mass Spectrometry* **19**,
330 1381–1391.
- 331 **Bathellier C, Badeck FW, Couzi P, Harscoet S, Mauve C, Ghashghaie J.** 2008.
332 Divergence in $\delta^{13}\text{C}$ of dark respired CO_2 and bulk organic matter occurs during the
333 transition between heterotrophy and autotrophy in *Phaseolus vulgaris* plants. *New*
334 *Phytologist* **177**, 406–418.
- 335 **Bögelein R, Hassdenteufel M, Thomas FM, Werner W.** 2012. Comparison of leaf
336 gas exchange and stable isotope signature of water-soluble compounds along canopy
337 gradients of co-occurring Douglas-fir and European beech. *Plant Cell & Environment*
338 **357**, 1245–1257.
- 339 **Bowling DR, Tans PP, Monson RK.** 2001. Partitioning net ecosystem carbon
340 exchange with isotopic fluxes of CO_2 . *Global Change Biology* **7**, 127–145.
- 341 **Wehr R, Saleska SR.** 2015. An improved isotopic method for partitioning net
342 ecosystem–atmosphere CO_2 exchange. *Agricultural & Forest Meteorology* **214–215**,
343 515–531
- 344 **Brandes E, Kodama N, Whittaker K, Weston C, Rennenberg H, Keitel C, Adams**
345 **MA, Gessler A.** 2006. Short-term variation in the isotopic composition of organic
346 matter allocated from the leaves to the stem of *Pinus sylvestris*, Effects of
347 photosynthetic and post-photosynthetic carbon isotope fractionation. *Global Change*
348 *Biology* **1210**, 1922–1939.

349 **Cernusak LA, Tcherkez G, Keitel C, Cornwell WK, Santiago LS, Knohl A,**
350 **Barbour MM, Williams DG, Reich PB, Ellsworth DS, Dawson TE, Griffiths HG,**
351 **Farquhar GD, Wright IJ.** 2009. Why are non-photosynthetic tissues generally ^{13}C
352 enriched compared with leaves in C_3 plants? Review and synthesis of current
353 hypotheses. *Functional Plant Biology* **36**,199–213.

354 **Cho MH, Lim H, Shin DH, Jeon JS, Bhoo SH, Park YI, Hahn TR.** 2011. Role of
355 the plastidic glucose translocator in the export of starch degradation products from the
356 chloroplasts in *Arabidopsis thaliana*. *New Phytologist* **190**, 101–112.

357 **Damesin C, Barbaroux C, Berveiller D, Lelarge C, Chaves M, Maguas C, Maia R,**
358 **Pontailier JY.** 2005. The carbon isotope composition of CO_2 respired by trunks,
359 comparison of four sampling methods. *Rapid Communications in Mass Spectrometry*
360 **19** 369–374.

361 **Damesin C, Lelarge C.** 2003. Carbon isotope composition of current-year shoots
362 from *Fagus sylvatica* in relation to growth, respiration and use of reserves. *Plant Cell*
363 *& Environment* **26** 207–219.

364 **Deng YW, Tang C, Yuan HC, Ning Z, Zou GF, Wang JR, Tida GE, Wu JS.** 2016.
365 The $\delta^{13}\text{C}$ - CO_2 pulsing labeling method, distribution of rice photosynthetic carbon in
366 plant-soil systems during different rice growth stages. *Acta Ecologica Sinica* **37** 19
367 6466-6471

368 **Eglin T, Fresneau C, Lelargetrouverie C, Francois C, Damesin C.** 2009. Leaf and
369 twig $\delta^{13}\text{C}$ during growth in relation to biochemical composition and respired CO_2 .
370 *Tree Physiology* **29**, 777–788.

371 **Gessler A, Keitel C, Kodama N, Weston C, Winters AJ, Keith H, Grice K,**
372 **Leuning R, Farquhar GD.** 2007. $\delta^{13}\text{C}$ of organic matter transported from the leaves
373 to the roots in *Eucalyptus delegatensis*, short term variations and relation to respired
374 CO_2 . *Functional Plant Biology* **34**, 692–706

375 **Gessler A, Rennenberg H, Keitel C.** 2004. Stable isotope composition of organic
376 compounds transported in the phloem of European beech-evaluation of different
377 methods of phloem sap collection and assessment of gradients in carbon isotope

composition during leaf-to-stem transport. *Plant Biology* **6**, 1–9.

Gessler A, Tcherkez G, Peuke AD, Ghashghaie J, Farquhar GD. 2008. Experimental evidence for diel variations of the carbon isotope composition in leaf stem and phloem sap organic matter in *Ricinus communis*. *Plant Cell & Environment* **317**, 941–953.

Heizmann U, Kreuzwieser J, Schnitzler JP, Brüggemann N, Rennenberg H. 2001. Assimilate transport in the xylem sap of Pedunculate Oak *Quercus robur*. *Plant Biology* **3**, 132–138.

Hu J, Moore DJP, Riverosiregui DA, Burns SP, Monson RK. 2010. Modeling whole-tree carbon assimilation rate using observed transpiration rates and needle sugar carbon isotope ratios. *New Phytologist* **1854**, 1000–1015.

Klumpp K, Schauffele R, Lotscher M, Lattanzi FA, Feneis W, Schnyder H. 2005. C-isotope composition of CO₂ respired by shoots and roots, fractionation during dark respiration? *Plant, Cell & Environment* **28**, 241–250.

Maunoury F, Berveiller D, Lelarge C, Pontailier JY, Vanbostal L, Damesin C. 2007. Seasonal, daily and diurnal variations in the stable carbon isotope composition of carbon dioxide respired by tree trunks in a deciduous oak forest. *Oecologia* **151**, 268–279.

Merchant A, Peuke AD, Keitel C, Macfarlane C, Warren CR, Adams MA. 2010. Phloem sap and leaf $\delta^{13}\text{C}$, carbohydrates, and amino acid concentrations in *Eucalyptus globulus* change systematically according to flooding and water deficit treatment. *Journal of Experimental Botany* **61**, 1785–1793.

Mahboubi A. New insights into carbon transport and incorporation to wood. 2014. Ph D thesis, Acta Universitatis Agriculturae Sueciae.

Minchin PEH, Thorpe MR. 1987. Measurement of unloading and reloading of photo-assimilate within the stem of bean. *Journal of Experimental Botany* **38**, 211–220.

Peuke AD, Rokitta M, Zimmermann U, Schreiber L, Haase A. 2001. Simultaneous measurement of water flow velocity and solute transport in xylem and phloem of

407 adult plants of *Ricinus communis* over a daily time course by nuclear magnetic
408 resonance spectrometry. *Plant, Cell & Environment* **24**, 491–503.

409 **Pons TL, Brugnoli E.** 2009. Estimating mesophyll conductance to CO₂, methodology
410 potential errors and recommendations. *Journal of Experimental Botany* **608**,
411 2217–2234.

412 **Ruehr NK, Offermann CA, Gessler A, Winkler JB, Ferrio JP, Buchmann N,**
413 **Barnard RL.** 2009. Drought effects on allocation of recent carbon, from beech leaves
414 to soil CO₂ efflux. *New Phytologist* **184**, 950–961.

415 **Seibt U, Rajabi A, Griffiths H, Berry JA.** 2008. Carbon isotopes and water-use
416 efficiency, sense and sensitivity. *Oecologia* **155**, 441–454.

417 **Tcherkez G, Farquhar G, Badeck F.** 2004. Theoretical considerations about carbon
418 isotope distribution in glucose of C₃ plants. *Functional Plant Biology* **31**, 857–877.

419 **Uchida Y, Hunt JE, Barbour MM, Clough TJ, Kelliher FM , Sherlock RR.** 2010.
420 Soil properties and presence of plants affect the temperature sensitivity of carbon
421 dioxide production by soils. *Plant and Soil* **337**, 375–387 .

422 **Van Bel, AJE.** 2003. The phloem, a miracle of ingenuity. *Plant, Cell and*
423 *Environment* **26**, 125–149.

424 **Werth M, Kuzyakov Y.** 2010. ¹³C fractionation at the root-microorganisms-soil
425 interface, a review and outlook for partitioning studies. *Soil Biology and Biochemistry*
426 **42**, 1372–1384 .

427 **Zhao N, Meng P, He YB, Yu XX.** 2017. Interaction of CO₂ concentrations and water
428 stress in semiarid plants causes diverging response in instantaneous water use
429 efficiency and carbon isotope composition. *Biogeoscience* **14**, 3431–3444.

430 **Zimmermann MH, Braun CL.** 1971. Trees, Structure and Function. Berlin,
431 Springer.
432

433 Table 1. Correlation between environmental factors and $\delta^{13}\text{C}$ of WSOM of different
434 plant organs. ($n=12$, values are obtained at two-hour intervals at three sampling dates:
435 25 May, 17 July, and 29 August).

	Leaves	Twig-phloem	Stem-phloem	Root-phloem
T_a	-0.21	-0.06	0.47**	0.66**
RH	-0.63**	-0.33	-0.15	-0.06
VPD	0.633**	0.26	0.35	0.30
M_s	-0.56**	-0.55*	-0.44*	-0.53**
R_a	0.31	0.02	0.17	0.24

436 * $p<0.05$; ** $p<0.01$.

437

438 Table 2. Correlation between environmental factors and $\delta^{13}\text{C}$ of WSOM of different
439 plant organs. ($n=7$, daily mean values).

	Leaves	Twig-phloem	Stem-phloem	Root-phloem
T_a	-0.23	-0.33	-0.03	-0.21
RH	-0.56	-0.30	-0.49	-0.17
VPD	0.36	0.03	0.47	0.03
M_s	-0.83**	-0.91*	-0.65	-0.72
R_a	0.09	-0.08	0.17	-0.18

440 * $p<0.05$; ** $p<0.01$.

441

442

Figure 1. Diel variation in temperature (T_a), relative humidity (RH) and vapor pressure deficit (VPD) on three measurement days at different canopy heights.

447

448

449 Figure 2. Diel variation in soil moisture (M_s) and solar radiation (R_a) on three
450 measurement days at different canopy heights.

451

Figure 3. Intracavity gradients of $\delta^{13}\text{C}$ of leaf and twig phloem WSOM. Data are mean values $\pm$ SD.

Figure 4. $\delta^{13}\text{C}$ of WSOM of different plant organs; $\delta^{13}\text{C}$ of leaf and twig phloem WSOM are means of the upper, middle, and lower canopy values. Data are mean values $\pm$ SD.

462
 463 Figure 5. Day-to-day variation of $\delta^{13}\text{C}$ of different plant organs collected on 19 April,
 464 25 May, 17 July, 8 August, 10 August, 11 August, and 29 August. Error bars are the
 465 standard error of average values