1 # Carbon flux to woody tissues in a beech/spruce forest during

2 summer and in response to chronic elevated $O_3$ exposure

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12 **Abstract**

13 The present study compares the dynamics in carbon (C) allocation of adult deciduous
14 beech (*Fagus sylvatica*) and evergreen spruce (*Picea abies*) during summer and in response to
15 seven-year-long exposure with twice-ambient ozone ($O_3$) concentrations ($2xO_3$). Focus was
16 on the respiratory turn-over and translocation of recent photosynthates at various positions
17 along the stems, coarse roots and soils. The hypotheses tested were that (1) $2xO_3$ decreases
18 the allocation of recent photosynthates to $CO_2$ efflux of stems and coarse roots of adult trees,
19 and that (2) according to their different $O_3$ sensitivities this effect is stronger in beech than in
20 spruce.

21 Labeling of whole tree canopies was applied by releasing $^{13}C$ depleted $CO_2$ ($\delta^{13}C$ of
22 -46.9 ‰) using a free-air stable carbon isotope approach. Canopy air $\delta^{13}C$ was reduced for
23 about 2.5 weeks by *c*. 8 ‰ in beech and 6 ‰ in spruce while the increase in $CO_2$
24 concentration was limited to about 110 µl $L^{-1}$ and 80 µl $L^{-1}$, respectively. At the end of the
25 labeling period, $\delta^{13}C$ of stem $CO_2$ efflux and phloem sugars was reduced to a similar extend
26 by *c*. 3-4 ‰ (beech) and *c*. 2-3 ‰ (spruce). The fraction of labeled C ($f_{E,new}$) in stem $CO_2$
27 efflux amounted to 0.3 to 0.4, indicating slow C turnover of the respiratory supply system in
28 both species.

29 Elevated $O_3$ slightly stimulated the allocation of recently fixed photosynthates to stem
30 and coarse root respiration in spruce (rejection of hypothesis I for spruce), but resulted in a
31 significant reduction in C flux in beech (acceptance of hypotheses I and II). The distinct
32 decrease in C allocation to beech stems indicates the potential of chronic $O_3$ stress to
33 substantially mitigate the C sink strength of trees on the long-term scale.

34 **1 Introduction**

35 Tropospheric ozone ($O_3$) is a major component of global climate change (IPCC, 2007),
36 mitigating the carbon (C) sink strength of forest trees and ecosystem productivity (Sitch et al.,
37 2007; Matyssek et al., 2010b). Along with increased emissions of anthropogenic precursors,
38 in particular nitrogen oxides, tropospheric $O_3$ concentrations are predicted to rise over Central
39 Europe and at the global scale (Fowler et al., 1999, 2008; Prather et al., 2001). Elevated $O_3$
40 concentrations are known to negatively affect the metabolism and growth of a wide range of
41 tree species, including deciduous European beech (*Fagus sylvatica*) and evergreen Norway
42 spruce (*Picea abies*; Matyssek et al., 2010a,b; Wieser et al. 2002; Nunn et al., 2006).
43 Photosynthetic decline, impaired phloem loading, and increased C demand for repair have all
44 been observed in response to ozone exposure. Detoxification may curtail the tree-internal
45 assimilate flux to stems, roots and soils in response to $O_3$ (Andersen, 2003; Matyssek and
46 Sandermann, 2003; Wieser and Matyssek, 2007).

47 Since the flux of current photosynthates is considered an important driver of woody
48 tissue and soil respiration in forests (Ryan et al., 1996, Högberg et al. 2001), limited C
49 availability caused by $O_3$ stress may affect the respiratory activity and growth of stems and
50 total belowground C allocation (Matyssek et al., 1992; Günthardt-Goerg et al., 1993; Coleman
51 et al., 1996; Spence et al., 1990). As a result, root biomass and sugar concentrations may be
52 reduced (Grulke et al. 1998, 2001). Highlighting the phototoxic potential of $O_3$ to Central-
53 European forests, Pretzsch et al. (2010) reported a 40 % decrease in stem growth of adult
54 beech upon eight years of twice-ambient $O_3$ exposure, whereas spruce showed no significant
55 growth response. Likewise, in phytotron experiments on juvenile beech, reduced allocation of
56 recent photosynthates to stems was identified as the mechanistic basis for reduced stem
57 growth in responses to $2xO_3$. (Kozovits et al., 2005a,b; Ritter et al., 2011).

58 Dynamics in C allocation of adult trees in response to chronically elevated $O_3$
59 concentrations are investigated and clarification is particularly needed for respiratory C fluxes

60 of woody tissues. Here, we compare the allocation of recent photosynthates to the respiratory
61 turn-over in stems, coarse roots and soils in adult beech and spruce in a naturally grown
62 forest.

63 In accordance with their contrasting $O_3$ sensitivity, we hypothesized that (1) $2xO_3$
64 decreases allocation of recent photosynthates to stem and coarse root $CO_2$ efflux of adult trees
65 and (2) that this effect is stronger in beech than in spruce. To this end, we took advantage of a
66 unique free-air $O_3$ fumigation experiment employed in a mixed forest with adult beech and
67 spruce trees (Matyssek et al., 2010). Stable carbon isotope labeling was performed on these
68 trees using the isoFACE exposure system (Grams et al., 2011). In view of hypothesis
69 evaluation, focus was on translocation of recent photosynthates and $CO_2$ efflux at various
70 positions along the stems and coarse roots.

71 **2 Material and methods**

72

73 **2.1 Experimental design**

74 The study was carried out during August/early September 2006 in a 60 to 70-year-old
75 mixed beech/spruce stand at "Kranzberger Forst" in southern Bavaria, near Freising,
76 Germany (elevation 485 m a.s.l., 48°25'N, 11°39'E; Pretzsch *et al.* 1998). Trees of European
77 beech (*Fagus sylvatica* [L.]) and Norway spruce (*Picea abies* [L.] Karst.), about 25 to 28 m
78 high, were exposed to either unchanged ambient (1x) or experimentally increased twice-
79 ambient (2x) $O_3$ concentrations. The $2xO_3$ regime had experimentally been enhanced since
80 2000, using a free-air $O_3$ exposure system (Werner and Fabian 2002, Karnosky et al. 2005. To
81 prevent risk of acute $O_3$ injury in the $2xO_3$ regime, maximum $O_3$ concentrations were
82 restricted to < 150 nL $L^{-1}$ (cf. Matyssek and Sandermann, 2003). The exclusion of untypically
83 high $O_3$ peaks resulted in a chronically enhanced 2x $O_3$ regime with a higher frequency of $O_3$
84 levels that currently occur sporadically at the site, by this, simulating the widely observed
85 trend of currently increasing $O_3$ background concentrations (Fowler et al. 2008; Sitch et al.
86 2007; Vingarzan 2004). The forest grew on luvisol derived from loess over tertiary sediments
87 with high nutrition and water supply. Long-term mean (1970-2000) annual air temperature
88 and rainfall were 7.8 °C and 786 mm, respectively (monitored by Deutscher Wetterdienst at
89 climate station "Weihenstephan", at 4 km distance from the research site; DWD Offenbach,
90 Germany; Matyssek et al., 2007). Scaffoldings and a canopy crane provided access to the tree
91 canopies.

92

93 **2.2 Climate conditions and stable carbon isotope labeling**

94 After a warm and dry period in July 2006 air temperature decreased during the
95 labeling experiments in August and September (Table 1, Fig. 1). Correspondingly, highest $O_3$
96 concentrations occurred during July, and AOT40 (i.e. accumulated $O_3$ concentrations above a

97 threshold of 40 nL $L^{-1}$) exceeded the critical level of 5 µL $O_3$ $L^{-1}$ h under the $1xO_3$ regime
98 already in May (LRTAP Mapping Manual 2004, Nunn et al., 2005a). $O_3$ concentrations in the
99 $2xO_3$ treatment were enhanced by a factor of 1.6 because of the maximum level of 150 µL $L^{-1}$
100 (see above). Continuous stable carbon isotope labeling was performed from August 18
101 through September 5 and August 26 through September 12 in beech and spruce, respectively,
102 using a free-air stable carbon isotope exposure system ("isoFACE", for details see Grams et
103 al., 2011). In brief, from 7:00 through 19:00 LT, $^{13}C$-depleted $CO_2$ ($\delta^{13}C$ of *c*. -46.9 ‰) was
104 homogenously released into the canopy of three study trees in each $O_3$ regime and species
105 (total of 12 trees) by means of micro-porous tubes. During label exposure, $O_3$ concentrations
106 (means ± SE) were 29.7 ± 6.9 ($1xO_3$) and 49.3 ± 11.9 nl $L^{-1}$ ($2xO_3$; Fig. 1a). Photosynthetic
107 photon flux density (PPFD) was moderate due to frequently overcast sky and occasional
108 precipitation (48 and 32 mm during beech and spruce labeling period, respectively, Fig. 1b).
109

110 **2.3 Isotope-ratio mass spectrometry (IRMS)**

111 Gas samples were analyzed for $\delta^{13}C$ within 48 hours by IRMS (GVI-Isoprime,
112 Elementar, Hanau, Germany) coupled to a gas autosampler (Gilson 221 XL, Gilson Inc.
113 Middleton, USA). Dried plant material was analyzed in a combined elemental analyzer
114 (EA3000, Euro Vector, Milan, Italy) and IRMS. Carbon isotope ratios are expressed in delta
115 notation ($\delta^{13}C$) using the Vienna PeeDee Belemnite (VPDB) as a standard. For gaseous and
116 solid samples, the iterated measurements of a laboratory working standard showed a precision
117 of $\delta^{13}C < 0.1$‰ (SD, n=10).
118

119 **2.4 Assessment of $CO_2$ concentration and $\delta^{13}C$ of canopy air**

120 $CO_2$ concentration ([$CO_2$]) and C isotope composition ($\delta^{13}C$) of canopy air were
121 monitored at two heights (i.e. at 1 and 5 m underneath the upper canopy edge, corresponding

122 to sun and shade leaves). Canopy air from all sampling positions was sucked through PVC
123 tubes by means of membrane pumps, analyzed for $CO_2$ concentration (infra-red gas analyzer
124 (IRGA), Binos 4b.1, Rosemount AG, Hanau) and sampled once a day (~12:00 LT) using a
125 100 mL syringe. Gas samples were flushed through 12 ml Exetainer vials and analyzed as
126 detailed above.

127 During labeling, $\delta^{13}C$ of canopy air was effectively decreased. Compared to the
128 unlabeled beech control, mean reductions in sun and shade crowns under $1xO_3$ were $8.1 \pm 0.2$
129 and $8.9 \pm 0.3$ ‰, respectively, and under $2xO_3$ $9.2 \pm 0.4$ and $8.4 \pm 0.5$ ‰, respectively, (Table
130 2 B). In spruce, mean reductions under $1xO_3$ were $6.0 \pm 0.6$ ‰ and $6.3 \pm 0.8$ ‰, respectively,
131 and under $2xO_3$, $7.5 \pm 0.9$ ‰ and $6.5 \pm 0.7$ ‰, respectively (Table 2 A). $CO_2$ concentration in
132 the canopy air of beech under both $O_3$ regimes was increased by about 110 µl $L^{-1}$ and in
133 spruce by about 80 µl $L^{-1}$ (Table 2 A). In both species, [$CO_2$] and $\delta^{13}C$ of canopy air were
134 each similar before and on the last day of labeling. Release of $CO_2$ and thus label application
135 in beech exceeded that of the spruce experiment. The increase in $CO_2$ concentration of the
136 canopy air did not affect the sap flow of labeled trees, suggesting unchanged stomatal
137 conductance at the leaf level (Grams et al. 2011). Hence, the rate of $CO_2$ uptake was assumed
138 to rise to some extent, while the increase in leaf internal to external $CO_2$ concentration was
139 estimated to be small (< 0.02). Therefore, changes in photosynthetic discrimination against
140 $^{13}C$ were calculated to stay below 0.4 ‰ (Grams et al., 2011).

141

142 **2.5 Assessment of stem and coarse root $CO_2$ efflux**

143 Stem and coarse root $CO_2$ efflux (*E*) of labeled and unlabeled control trees was
144 assessed by means of a computer-controlled open gas exchange system (for details see Grams
145 et al., 2011). Plexiglas chambers (Plexiglas®, Röhm GmbH, Darmstadt, Germany) were
146 attached at a lower and upper stem position and at one coarse root per tree (except for the
147 unlabeled control spruce tree). Chambers were darkened with aluminized polyester foil to

148 avoid refixation of efflux $CO_2$ by corticular photosynthesis. For assessment of $CO_2$ efflux,
149 chambers were connected through PVC tubing to an IRGA (Binos 4b, Emerson Process
150 Management, Weißling, Germany). Stem $CO_2$ efflux was based on the volume (V in m³) of
151 the stem sector behind the chamber (i.e. living tissue of bark and sapwood) and coarse root
152 $CO_2$ efflux on the totally enclosed coarse root volume, respectively (Desrochers et al., 2002;
153 Saveyn et al., 2008).
154

155 **2.6 $\delta^{13}C$ of stem and coarse root $CO_2$ efflux**

156 Data on $\delta^{13}C$ of $CO_2$ efflux ($\delta^{13}C_E$) sampled from stems and coarse roots are shown as
157 24h-means (± SE). Coarse root $\delta^{13}C_E$ was assessed once per day (between 10:00 and 13:00
158 LT) by means of a closed respiration system (for details see Grams et al., 2011). A total of six
159 12 ml Exetainer vials were subsequently flushed with chamber air of increasing $CO_2$
160 concentration and $\delta^{13}C_E$ of coarse roots was calculated according to the "Keeling Plot
161 approach" (Keeling, 1958, 1961). Air from stem respiration chambers was automatically
162 sampled in 12 ml Exetainer vials, which were flushed with sample gas for six minutes each, at
163 a flow rate of 0.15 L $min^{-1}$. A total of eight samples per day and chamber were assessed.
164 Isotopic signature of $CO_2$ efflux of the stem was calculated after Eq. 1 using a two end-
165 member mixing model.

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$$\delta^{13}C_E = \frac{([CO_2]_{sample} * \delta^{13}C_{sample}) - ([CO_2]_{reference} * \delta^{13}C_{reference})}{([CO_2]_{sample} - ([CO_2]_{reference})} \quad (‰) \qquad \text{Eq. (1)}$$

170 where,

171 $[CO_2]_{sample}$ = $CO_2$ concentration of sample gas from a stem respiration chamber (µl $L^{-1}$),
172 $[CO_2]_{reference}$ = $CO_2$ concentration of reference gas from an empty chamber (µl $L^{-1}$),
173 $\delta^{13}C_{sample}$ = $\delta^{13}C$ of sample gas from a stem respiration chamber (‰) and
174 $\delta^{13}C_{reference}$ = $\delta^{13}C$ of reference gas from an empty chamber (‰).

175

176 We considered that stem $CO_2$ efflux may not only consist of local tissue-respired $CO_2$,
177 but may be biased by xylem-transported $CO_2$ deriving from lower stem parts and/or root
178 respiration (Teskey et al., 2008). However, the absent correlation between xylem sap flow and
179 stem respiration rate or $\delta^{13}C_E$ (data not shown) suggests xylem-transported $CO_2$ to only
180 marginally interfere with sampled $CO_2$ or to originate from similar respiratory processes as
181 the locally respired $CO_2$ behind the stem chamber.

182

183 **2.7 Fraction of labeled C in stem respiration**

184 The fraction of labeled carbon ($f_{E,\ new}$) in $CO_2$ efflux (*E*) was calculated following
185 Lehmeier et al. (2008) and Gamnitzer et al. (2009):

186

187 $$f_{E,new} = (\delta^{13}C_{sample} - \delta^{13}C_{old}) / (\delta^{13}C_{new} - \delta^{13}C_{old}) \quad \text{Eq. (2)}$$

188

189 where, $\delta^{13}C_{old}$ represents the $\delta^{13}C$ of *E* before labeling and $\delta^{13}C_{new}$ the $\delta^{13}C$ of *E* of a tree
190 grown (theoretically) continuously with labeled $CO_2$. The labeling period of 18 to 19 days
191 was too short to fully achieve new isotopic equilibrium in *E* and therefore $\delta^{13}C_{new}$ was derived
192 from C isotope discrimination ($\Delta^{13}C$) before labeling, following Eqs. 3 and 4:

193

194 $$\Delta^{13}C = ([\delta^{13}C_{unlabeled\ air} - \delta^{13}C_{old}] / [1000 + \delta^{13}C_{old}]) * 1000\ (‰) \quad \text{Eq. (3)}$$

195 $$\delta^{13}C_{new} = ([\delta^{13}C_{labeled\ air} - \Delta^{13}C] / [1000 + \Delta^{13}C]) * 1000\ (‰) \quad \text{Eq. (4)}$$

196

197 where, $\delta^{13}C_{unlabeled\ air}$ and $\delta^{13}C_{labeled\ air}$ represent the $\delta^{13}C$ of canopy air before and during the
198 labeling, respectively.

199

200 Day-to-day variation in $\delta^{13}C_E$ may occur from variations in label incorporation and in

201 $\Delta^{13}C$ depending on weather conditions (Pate and Arthur 1998; Bowling et al. 2008). Thus,
202 $\delta^{13}C_E$ of the labeled trees were corrected for the day-to-day variations in $\Delta^{13}C$ (being rather
203 small, i.e. < 0.5 ‰) of the unlabeled control trees, which showed rather stable $\delta^{13}C_E$
204 throughout the experiment, i.e. 22.4 ± 0.1 and 21.4 ± 0.1 ‰ for the upper and lower stem
205 positions of beech, respectively, and 19.4 ± 0.1 ‰ for the lower stem position of spruce.

206

207 **2.8 Assessment of phloem sugars**

208 Phloem sap was sampled on day 0 and during the last labeling day from the lower
209 stem position following the method of Gessler et al. (2004). Small pieces of bark with
210 adherent phloem tissue (∅ 5 mm) were cored in the vicinity of the lower stem chamber and
211 incubated (5 h at 4 °C) in 15 mM sodium polyphosphate buffer (Sigma-Aldrich, Munich,
212 Germany). After centrifugation (12,500 rpm, 5 min), phloem sap was analyzed for water
213 soluble sugars (sum of sucrose, fructose, glucose, raffinose and pinitol; i.e. $C_{PS}$ in mg) by
214 means of HPLC (CARBOsep CHO-820 calcium column, Transgenomic, 219 Glasgow, UK).
215 Freeze-dried phloem sap was analyzed for stable carbon isotope ($\delta^{13}C_{sample}$ in ‰) and element
216 composition ($C_{sample}$ in mg), and $\delta^{13}C$ of phloem sugars ($\delta^{13}C_{PS}$ in ‰) was calculated
217 according to Eq. 5:

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$$\delta^{13}C_{PS} = \frac{\delta^{13}C_{sample} * C_{sample} - \delta^{13}C_{NPS} * C_{NPS}}{C_{PS}} \ (‰) \qquad \text{Eq. (5)}$$

222

223 with $\delta^{13}C_{NPS}$ representing $\delta^{13}C$ of non-sugar C (assuming $\delta^{13}C_{NPS}$ to correspond to $\delta^{13}C_{sample}$
224 before labeling, cf. Grams et al. 2011) and $C_{NPS}$ (in mg) denoting the non-sugar C content after
225 labeling (calculated as difference between $C_{sample}$ and $C_{PS}$) in the phloem sap.

226

227 **2.9 Sampling of leaves and fine roots**

228 Leaves and fine roots were sampled before and during the last labeling day. Leaves
229 were collected with different exposure to compass directions in sun and shade crowns.
230 Recently grown fine roots (≤ 2 mm diameter) were sampled from organic soil horizons (< 10
231 cm soil depth) and cleaned from soil with distilled water. Dried plant material (72 h at 65°C)
232 was fine-ground and weighed into tin capsules for $\delta^{13}C$ analysis.

233

234 **2.10 Assessment of soil respired $CO_2$**

235 Soil gas samples were collected as detailed by Andersen et al. (2010). In brief, specific
236 soil-gas sampling wells were placed belowground prior to tree labeling (distance from bole
237 base of about 0.2 to 0.5 m) at 8 cm and 15 cm depth. Teflon tubing was used to draw 5-8 mL
238 of soil gas from each sampler using a gas-tight syringe. Each beech and spruce tree served as
239 its own control by following the change in $\delta^{13}C$ of soil-respired $CO_2$ throughout 2.5 weeks of
240 labeling. In the case of beech, a total of four soil-gas sampling wells were additionally
241 installed at an unlabeled control plot. Gas samples were subsequently filled into 12 mL
242 Exetainer vials and analyzed for $\delta^{13}C$. Calculation of $\delta^{13}C$ of soil-respired $CO_2$ follows Eq. 1,
243 while $CO_2$ of ambient air above the soil served as reference. Note that soil $CO_2$ efflux was not
244 adjusted by -4.4‰ to account for the more rapid diffusion of $^{12}C$ compared to $^{13}C$ (Andersen
245 et al., 2010). $\delta^{13}C$ analysis of additional gas samples taken directly above the forest floor
246 indicated that $CO_2$ label was restricted to the crown and did not reach the forest soil (Grams et
247 al., 2011).

248

249 **2.11 Statistical analyses**

250 Statistical analysis was performed using the SPSS 16.0 software package (SPSS Inc.,
251 Chicago, USA). Individual study trees were regarded as experimental units, and beech and
252 spruce were analyzed separately. Data were statistically analyzed using General Linear Model

253 (GLM) approach and t-tests where appropriate. Statistical evaluation of the course in $\delta^{13}C_E$ of
254 stems and coarse roots and the fraction of labeled C in stem $CO_2$ efflux and coarse root $CO_2$
255 efflux of labeled trees was performed using repeated measures analysis of variance.
256 Differences at $p \leq 0.05$ were regarded as statistically significant, and at $p \leq 0.1$ as marginally,
257 and denoted by * and (*), respectively.

258 **3 Results**

259 **3.1 Stem and coarse root $CO_2$ efflux**

260 In general, both species displayed up to 4 times higher (beech) and up to 2 times
261 higher (spruce) $CO_2$ efflux rates at the upper compared to the lower stem position (Table 3),
262 whereas rates of coarse roots were 10 to 60 times higher than in stems. In beech, $2xO_3$
263 significantly diminished the $CO_2$ efflux rate of the upper stem (by *c*. - 60 %), but caused a
264 pronounced, but non-significant ($p$ = 0.065), increase in coarse roots (by *c*. + 65 %). In
265 spruce, $CO_2$ efflux rate of the upper stem position was significantly increased under $2xO_3$ (by
266 *c*. 90 %), whereas the effect was much smaller (*c*. 20%) and statistically not significant at the
267 lower stem position. However, long-term exposure to $2xO_3$ reduced the $CO_2$ efflux rate of
268 spruce coarse roots by *c*. 25 % (not statistically significant, $p$ = 0.157).

269

270 **3.2 $\delta^{13}$C in stem and coarse root $CO_2$ efflux**

271 Before labeling, daily means (± SE) of $\delta^{13}C_E$ in beech trees were -28.2 ± 0.1 and -27.9
272 ± 0.4 ‰ at the upper and lower stem position under $1xO_3$, respectively (Fig. 2). Exposure to
273 $2xO_3$ slightly increased values by about 0.4 ‰ (not statistically significant). In spruce, $\delta^{13}C_E$
274 of the upper and lower stems were -27.1 ± 0.1 and -26.6 ± 0.1 ‰, respectively. Here $2xO_3$
275 significantly reduced values by about 1.1 ‰. In both species, $\delta^{13}C_E$ of coarse roots were
276 similar to the values of the lower stems and responses to $2xO_3$ were consistent with stems.

277 While unlabeled control trees displayed minor day-to-day variations in $\delta^{13}C_E$ of the
278 various organs during labeling (SD < 0.3 ‰), labeled trees displayed decreasing values upon
279 label application (Fig. 2). In beech, $\delta^{13}C_E$ of the stems decreased from day 2 onwards under
280 both $O_3$ regimes (Fig. 2a), with a significantly more pronounced decline under $1xO_3$.
281 Likewise, coarse root $\delta^{13}C_E$ decreased from day 2 onwards (Fig. 2c), although this effect was
282 less prominent than in stems. Similar to beech, $\delta^{13}C_E$ of stems in spruce decreased from day 3

283 onwards under both $O_3$ regimes (Fig. 2b). Contrasting with beech, the decline was
284 significantly stronger under $2xO_3$ and more pronounced in the upper compared to the lower
285 stem position ($p < 0.05$, except for day 3). In coarse roots, the decline in $\delta^{13}C_E$ was somewhat
286 delayed, in particular under $1xO_3$ and somewhat stronger under $2xO_3$ ($p = 0.085$ at day 5, Fig.
287 2d).
288
289 **3.3 Fraction of labeled C in stem and coarse root $CO_2$ efflux**
290 In beech, the fraction of labeled carbon ($f_{E,new}$) in stem $CO_2$ efflux started to increase
291 during labeling day 2 and was significantly lower in $2xO_3$ compared to $1xO_3$ from day 3
292 onwards (Fig. 3a). At the end of the labeling period (day 19), $f_{E,new}$ had approached maximum
293 levels of 0.40 ± 0.01 under $1xO_3$, whereas under $2xO_3$ only 0.33 ± 0.06 and 0.26 ± 0.06 at the
294 upper and lower stem position, respectively, were reached. Lowest $f_{E,new}$ was observed for
295 coarse roots (maximum of 0.2), being significantly reduced under $2xO_3$ from day 5 onwards
296 (Fig. 3c). In spruce, $f_{E,new}$ of stem $CO_2$ efflux started to increase on labeling day 2, reaching
297 maximum levels of 0.37 ± 0.03 (upper stem) and 0.25 ± 0.05 (lower stem) under $1xO_3$, and
298 0.39 ± 0.06 and 0.30 ± 0.02, respectively, under $2xO_3$ at the end of the labeling period (day
299 18, Fig. 3b). Increase of $f_{E,new}$ in spruce coarse roots started somewhat delayed (day 3) but
300 reached levels similar to those of the lower stem position (Fig. 3d). Contrasting with beech,
301 $2xO_3$ did not result in a consistently reduced $f_{E,new}$ in stems and coarse roots.
302
303 **3.4 $\delta^{13}C$ in leaves, phloem sugars, fine roots and soil respired $CO_2$ before labeling**
304 Before labeling, no apparent differences in $\delta^{13}C$ caused by the long-term $2xO_3$
305 exposure were found in the foliage, phloem sap of the stem, fine roots and soil respired $CO_2$
306 in either species (Table 4). In general, $\delta^{13}C$ in the sun leaves was significantly increased by *c.*

307 3 ‰ (beech) and 2 ‰ (spruce) compared with shade leaves each. The $\delta^{13}C$ of soil-respired
308 $CO_2$ underneath beech of about -24 ‰ was not affected by the $O_3$ treatment. In comparison
309 with beech, all samples from spruce were enriched in $^{13}C$ by 1 to 2 ‰ ($p \leq 0.05$). In spruce,
310 $\delta^{13}C$ of soil respired $CO_2$ was reduced by about 1.2 ‰ under $2xO_3$ and increased by about 1.0
311 ‰ at a soil depth of 15 cm compared to 8 cm.
312

313 **3.5 Shift in $\delta^{13}C$ of $CO_2$ efflux and organic material by the end of labeling**

314 During the 2.5 week labeling period, the $\delta^{13}C$ of stem and root $CO_2$ efflux, soil-
315 respired $CO_2$ and organic samples (phloem sugars, leaves and fine roots) in the unlabeled
316 control trees of both species was only marginally affected (< 0.5 ‰, Fig. 4). In labeled beech,
317 the drop in $\delta^{13}C_E$ at the end of label application in the upper stem position was unaffected by
318 $O_3$ (3.5 ± 0.2 ‰ in both $O_3$ treatments), but less pronounced at the lower stem position under
319 $2xO_3$ (3.3 ± 0.1 ‰ and 2.3 ± 0.5 ‰ under 1x and $2xO_3$, respectively) (Fig 4b,c). Phloem
320 sugars sampled from the lower stem position displayed similar shifts in $\delta^{13}C$ of 4.0 ± 1.4 ‰
321 and 3.5 ± 0.6 ‰ under 1x and $2xO_3$, respectively. In consistency with the reduced label
322 strength in spruce canopy air (about 6.0 ‰ compared to 8.2 ‰ in beech), the drop in stem
323 $\delta^{13}C_E$ of spruce was lower than in beech (Fig 4e,f). Conversely to beech, the drop was
324 somewhat increased by $2xO_3$: upper and lower stem position of 2.4 ± 0.2 ‰ and 1.8 ± 0.3 ‰
325 under $1xO_3$, respectively, and 2.8 ± 0.2 ‰ and 2.1 ± 0.2 ‰ under $2xO_3$, respectively. Again, a
326 similar shift was observed in phloem sugars (3.2 ± 0.3 ‰ and 2.5 ± 0.2 ‰ under 1x and 2x
327 $O_3$, respectively). Corresponding changes of $\delta^{13}C$ in leaf bulk material were much smaller
328 (about 1.5 ‰).

329 Upon labeling, belowground allocation of recent photosynthates was not affected by
330 the $O_3$ treatment and, in general, was reduced compared to stem $CO_2$ efflux and phloem
331 sugars. The decline upon labeling in $\delta^{13}C_E$ of coarse roots was 1.8 ± 0.1 ‰ and 1.4 ± 0.1 ‰ in

332 beech and 1.7 ± 0.9 ‰ and 2.1 ± 0.8 ‰ in spruce under 1x and $2xO_3$, respectively. Under

333 beech, changes in $\delta^{13}C$ of soil-respired $CO_2$ were similar to coarse roots $\delta^{13}C_E$ (about 1.5 to

334 2.5 ‰), whereas soil $CO_2$ under spruce remained unchanged. (Fig. 4e,f). Similar to leaf bulk

335 material, $\delta^{13}C$ of fine roots displayed smaller changes than sampled $CO_2$ efflux and was in the

336 range of 0.5 ‰, irrespective of the $O_3$ treatment.

337 **4 Discussion**

338 Our study compares the flux of recent photosynthates to the $CO_2$ efflux of stems and
339 coarse roots in adult deciduous beech and evergreen spruce during summer and in response to
340 seven-year long $2xO_3$ treatment. The hypothesis I that long-term exposure to elevated $O_3$
341 reduces the flux of recently fixed C to $CO_2$ efflux of stems and coarse roots was accepted for
342 beech but rejected in the case of spruce, which is in accordance with their contrasting $O_3$
343 sensitivities (support for hypothesis II).

344 Long-term exposure to $2xO_3$ for seven years did not significantly affect the $\delta^{13}C$ of
345 beech and spruce leaves or sugars transported in the phloem sap during late summer (Tab. 4,
346 cf. Grams et al., 2007, Gessler et al., 2009). Nevertheless, $\delta^{13}C$ of beech sun leaves displayed
347 a tendency similar to that reported by Kitao et al. (2009) in that $2xO_3$ increased $\delta^{13}C$ of leaf
348 dry matter caused by $O_3$-induced stomatal closure. Likewise, spruce displayed some
349 photosynthetic and stomatal limitation under $2xO_3$ although varying from year to year (Nunn
350 et al., 2005b, 2006). In general, $\delta^{13}C$ of leaf and fine root biomass was about 2 ‰ higher in
351 spruce compared to beech, likely resulting from higher leaf-level water-use efficiency in the
352 evergreen conifer compared to deciduous trees (Matyssek, 1986; Garten and Taylor, 1992;
353 Diefendorf et al., 2010).

354 In both beech and spruce, labeled photosynthates were detected in the upper and lower
355 stem $CO_2$ efflux from day 3 onwards (Fig. 2 and 3). The fraction of labeled C ($f_{E,new}$) in the
356 $CO_2$ efflux of beech stems was significantly reduced under $2xO_3$ (support of hypothesis I),
357 indicating a higher dependency on C stores of the respiratory supply under $2xO_3$ (cf. Ritter et
358 al. 2011). Such a response may be caused by (1) a direct adverse effect of $O_3$ on beech
359 photosynthesis and thus reduced label uptake, although reductions were typically small (Nunn
360 et al., 2005b; 2006), or (2) a changed C allocation pattern by e.g. an $O_3$-inhibited assimilate
361 transport from the leaves. As a consequence the respiratory activity of stem tissues may be
362 restricted (Matyssek et al., 2002) and C stores in stems and roots may decrease towards the

363 end of the growing season (Mc Laughlin et al., 1982). Consequently, re-growth and bud
364 development in spring may become limited (Matyssek and Sandermann, 2003). The
365 significantly decreased flux of recent photosynthates to beech stems represents the
366 mechanistic basis for the observed loss in stem productivity of 40% under long-term exposure
367 of $2xO_3$ (Pretzsch et al., 2010). In consistency with model predictions (Sitch et al., 2007), this
368 indicates the potential of chronic $O_3$ stress to substantially mitigate the C sink strength of trees
369 (Matyssek et al., 2010b). Contrasting with beech, exposure to $2xO_3$ in tendency increased the
370 fraction of labeled C ($f_{E,new}$) in stem $CO_2$ efflux of spruce, rejecting hypothesis I for spruce. At
371 the same time, the rate of stem $CO_2$ efflux was significantly increased under $2xO_3$. Such a
372 stimulation following $O_3$ exposure has been reported in several studies on herbaceous plants
373 (Grantz and Shrestha, 2006; Reiling and Davison, 1992) and is known to sustain repair- and
374 detoxification processes (Matyssek et al., 1995; Rennenberg et al., 1996). The slightly
375 increased C allocation to such processes in spruce may relate to its overall lower $O_3$
376 sensitivity compared to beech (Kozovits et al. 2005a,b; Matyssek et al., 2010b; Pretzsch et al.
377 2010). Whereas under 2x $O_3$ allocation of C to reserves in beech stems may be restricted
378 (Ritter et al., 2011; Kuptz et al. 2011a) putatively reducing C supply for stem growth in the
379 following year.

380 We do not expect the observed $O_3$ effects to be counteracted by the short-term increase
381 in $CO_2$ concentration during labeling as $CO_2$ x $O_3$ interactions in beech are typically related to
382 reductions in stomatal aperture (Grams et al. 1999, Grams and Matyssek 1999) that were
383 absent during labelling (Grams et al. 2011). Moreover, structural adjustments of beech in
384 response to the long-term exposure (i.e. 7 years) to the 2x $O_3$ regime are unlikely to be
385 ameliorated by short-term (i.e. 2.5 weeks) increases in $CO_2$ concentration by about 100 $\mu L\ L^{-}$
386 $^{1}$.

387 Reduction of $\delta^{13}C$ in canopy air for 2.5 weeks by about 8 and 6 ‰ resulted in a drop
388 of stem $\delta^{13}C_E$ in beech of 3-4 ‰ and in spruce by 2-3 ‰, respectively (Fig. 4b-f).

389 Correspondingly, $f_{E,new}$ of stem $CO_2$ efflux amounted to about 0.3 to 0.4 in both species. In
390 parallel, $\delta^{13}C$ of labeled phloem sugars was reduced to a similar extent by about 4 and 3 ‰ in
391 beech and spruce, respectively, suggesting respiration of phloem sugars to be the main C
392 source for stem $CO_2$ efflux (Kuptz et al. 2011a). Unlabeled C in phloem sugars after 2.5
393 weeks of continuous labeling may derive from "old C" atoms in C skeletons of currently
394 synthesized sucrose as a consequence of slow turnover of precursor molecules or from
395 remobilized C stores (Gessler et al., 2008; Tcherkez et al., 2003). We note that $CO_2$ efflux
396 sampled from stems (and roots) may be affected by xylem-transported $CO_2$ deriving from
397 lower stem regions and/or root respiration (Teskey et al., 2008). We did not find a correlation
398 between sap flow and both rates of stem $CO_2$ efflux and stem $\delta^{13}C_E$ in our study (cf. Grams et
399 al., 2011, Kuptz et al., 2011a,b). Hence, contribution of xylem transported $CO_2$ to sampled
400 $CO_2$ efflux may be small or originate from similar respiratory processes as at the sampled
401 stem position. In fact, the contribution from soil $CO_2$ to stem $CO_2$ efflux was recently
402 concluded to be rather small (Gebhardt, 2008; Aubrey and Teskey, 2009; Ubierna et al.,
403 2009). However, contribution of respiratory $CO_2$ from lower parts of the stem or roots to
404 sampled $CO_2$ efflux can not be ruled out completely and the extent of this putative influence
405 remains obscure.

406 In consistency with the findings on $\delta^{13}C_E$ in stems, $2xO_3$ distinctly reduced $f_{E,new}$ of
407 coarse root efflux of beech, supporting hypothesis I. The decrease in coarse root $\delta^{13}C_E$ during
408 the labeling in summer was about 1-2 ‰ smaller than in stems, indicating a lower dependence
409 of root $CO_2$ efflux on current photosynthates (Wingate et al., 2008; Bathellier et al., 2009;
410 Kuptz et al., 2011a). However, soil-respired $CO_2$, which includes large contributions by root-
411 respired $CO_2$ of unlabeled neighboring trees and heterotrophic soil respiration (Högberg et al.,
412 2001; Andersen et al., 2005, 2010), was reduced in $\delta^{13}C$ by 1.5 to 3 ‰. Hence, beech fine
413 roots and associated microbes appear to be a relatively strong sink for recently fixed C during
414 summer (Högberg et al., 2001; Plain et al., 2009). Slightly pronounced shifts in soil-respired

415 $CO_2$ under $2xO_3$ fit well with previously reported increases in fine-root turn-over of beech
416 under long-term $O_3$ exposure (Nikolova et al., 2010). Similar to C flux in spruce stems,
417 elevated $O_3$ did not reduce the allocation of recent photosynthates to coarse root $CO_2$ efflux
418 during summer (cf. Andersen et al., 2010). However, the C label was hardly detectable in the
419 soil-respired $CO_2$ around the trees (Andersen et al., 2010), which may indicate favored
420 allocation of labeled C to storage and/or structural pools in the fine roots during summer (cf.
421 Kuptz et al. 2011a), resulting in a drop of $\delta^{13}C$ in the fine root tissue during labeling (Fig.
422 4e,f).

423 In conclusion, the transfer of recently fixed C from beech and spruce crowns to stem
424 and coarse root $CO_2$ efflux within 2 to 3 days displays tight coupling with canopy
425 photosynthesis during summer. Our labeling approach for tracking of individual, isotopically
426 labeled sugar molecules through tall beech and spruce trees should not be confused with the
427 faster propagation of phloem pressure-concentration waves (Kuzyakov and Garvrichkova,
428 2010, Mencuccini and Hölttä, 2010). Chronic exposure to $2xO_3$ reduced allocation of
429 photosynthates to the stem and coarse roots of beech and spruce in contrasting ways. The
430 conifer spruce significantly increased the flux of photosynthates to stems (rejection of
431 hypothesis I for spruce), whereas this flux was restricted in stems and coarse roots of
432 deciduous beech (acceptance of hypotheses I and II). The observed patterns in translocation of
433 recent photosynthates are interpreted as a mechanistic basis for observed reductions in beech
434 stem growth, highlighting the potential of chronic $O_3$ stress to substantially mitigate the C
435 sink strength of trees.

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## Tables

Table 1 Weather conditions and $O_3$ levels at the study site "Kranzberger Forst" during the growing season of 2006. Monthly sum of precipitation and average of daytime photosynthetic photon flux density (PPFD), relative air humidity (RH), air temperature ($T_{air}$), vapor pressure deficit (VPD) and soil moisture (± SE, n = 30 to 31). Ozone levels as monthly means ± SE (n = 30 to 31), AOT40 (i.e. accumulated $O_3$ concentrations above a threshold of 40 nL $L^{-1}$) and SUM0 (i.e. daily sum of hourly $O_3$ concentrations).

| 2006 | May | June | July | Aug | Sep | Oct |
|---|---|---|---|---|---|---|
| PPFD [µmol $m^{-2}$ $s^{-1}$] | 458.6±29.7 | 565.3±28.7 | 601.1±23.7 | 345.6±20.4 | 363.4±23.1 | 217.7±12.4 |
| RH [%] | 69.8±2.2 | 68.5±2.0 | 66.2±2.3 | 80.7±1.2 | 77.7±1.4 | 80.7±0.6 |
| $T_{air}$ [°C] | 12.8±0.5 | 16.9±0.9 | 21.4±0.4 | 14.5±0.4 | 16.2±0.4 | 11.5±0.5 |
| Rainfall [mm] | 82.4±0.7 | 92.1±1.3 | 29.0±0.4 | 113.8±0.9 | 12.6±0.3 | 35.6±0.8 |
| VPD [hPa] | 5.1±0.5 | 7.1±0.7 | 10.2±0.8 | 3.5±0.4 | 4.7±0.4 | 2.8±0.2 |
| Soil moisture [vol %] at | | | | | | |
| 5 cm depth | 30.7±0.2 | 28.4±0.5 | 22.5±0.8 | 21.4±0.4 | 17.5±0.2 | 17.1±0.1 |
| 30 cm depth | 34.1±0.2 | 32.3±0.4 | 27.9±0.4 | 26.1±0.1 | 24.7±0.1 | 25.4±0.1 |
| 70-140 cm depth | 29.6±0.2 | 27.9±0.2 | 25.0±0.3 | 22.9±0.1 | 21.5±0.1 | 21.7±0.1 |
| $1xO_3$ concentration [nl $L^{-1}$] | 47.5±2.8 | 45.3±1.8 | 53.0±1.7 | 29.5±1.5 | 26.0±1.6 | 15.5±1.4 |
| $2xO_3$ concentration [nl $L^{-1}$] | 67.0±3.3 | 72.6±3.7 | 86.2±3.6 | 47.9±2.3 | 44.1±2.9 | 23.5±2.2 |
| AOT40 $1xO_3$ [µL $L^{-1}$ h] | 5.7 | 4.7 | 7.4 | 0.8 | 0.6 | 0.0 |
| AOT40 $2xO_3$ [µL $L^{-1}$ h] | 13.0 | 17.1 | 23.2 | 6.7 | 5.1 | 1.0 |
| SUM0 $1xO_3$ [µL $L^{-1}$ h] | 33.0 | 30.1 | 36.8 | 21.6 | 18.6 | 8.7 |
| SUM0 $2xO_3$ [µL $L^{-1}$ h] | 47.7 | 52.2 | 64.1 | 35.6 | 31.7 | 13.4 |

Table 2 (A) $CO_2$ concentration (µl $L^{-1}$) and (B) $\delta^{13}C$ (‰) in canopy air of labeled beech and spruce trees under 1x and $2xO_3$ and one unlabeled control tree for each species. Data are presented for sun and shade crowns as means ± SE before (n = 12 h), during (n = 18 to 19 days) and after (n = 12 hours) label exposure.

| | Unlabeled Control | | Labeled beech | | | | Labeled spruce | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $1xO_3$ | | $2xO_3$ | | $1xO_3$ | | $2xO_3$ | |
| | Sun | Shade | Sun | Shade | Sun | Shade | Sun | Shade | Sun | Shade |
| A [$CO_2$] (µl $L^{-1}$) | | | | | | | | | | |
| Before | 384 ± 2 | 380 ± 2 | 383 ± 3 | 379 ± 4 | 384 ± 8 | 379 ± 1 | 382 ± 6 | 381 ± 9 | 382 ± 21 | 383 ± 14 |
| During | 384 ± 1 | 385 ± 1 | 488 ± 5 | 505 ± 9 | 508 ± 6 | 498 ± 7 | 455 ± 3 | 460 ± 5 | 473 ± 4 | 465 ± 8 |
| After | 385 ± 7 | 384 ± 7 | 380 ± 2 | 382 ± 2 | 380 ± 5 | 383 ± 7 | 383 ± 8 | 381 ± 10 | 381 ± 7 | 385 ± 3 |
| B $\delta^{13}C$ (‰) | | | | | | | | | | |
| Before | -8.2 ± 0.1 | -8.2 ± 0.1 | -8.6 ± 0.3 | -8.1 ± 0.2 | -8.4 ± 0.3 | -8.1 ± 0.5 | -8.5 ± 0.6 | -8.3 ± 1.2 | -8.4 ± 1.0 | -8.3 ± 1.1 |
| During | -8.6 ± 0.1 | -8.6 ± 0.1 | -16.7 ± 0.3 | -17.5 ± 0.5 | -17.8 ± 0.4 | -17.0 ± 0.4 | -14.6 ± 0.2 | -14.9 ± 0.3 | -16.0 ± 0.3 | -15.1 ± 0.5 |
| After | -8.7 ± 0.2 | -8.2 ± 0.2 | -8.2 ± 0.1 | -8.5 ± 0.3 | -8.2 ± 0.5 | -8.5 ± 0.4 | -8.5 ± 0.5 | -8.3 ± 0.4 | -8.3 ± 0.8 | -8.4 ± 0.2 |

Table 3 Stem and coarse root $CO_2$ efflux (µmol $m^{-3}$ $s^{-1}$) of beech and spruce during the 2.5 weeks of labeling. Data are shown as means ± SE (n = 3 trees). Within one species, lowercase letters denote significant differences among upper and lower stems ($^{a}$, $^{b}$) and lower stems and coarse roots ($^{c}$, $^{d}$), respectively ($p \leq 0.05$). Asterisks denote significant differences between $O_3$ regimes ($p \leq 0.05$). Statistical evaluation was performed using the t-test for paired comparisons.

| | Beech | | Spruce | |
|---|---|---|---|---|
| | $1xO_3$ | $2xO_3$ | $1xO_3$ | $2xO_3$ |
| Upper Stem | $14.1 \pm 2.7^{a}$ | $5.5 \pm 1.1^{a}$ * | $12.8 \pm 0.6^{a}$ | $24.6 \pm 1.6^{a}$ * |
| Lower Stem | $3.8 \pm 1.8^{b, c}$ | $4.9 \pm 1.9^{a, c}$ | $11.9 \pm 0.9^{a, c}$ | $14.7 \pm 4.0^{b, c}$ |
| Coarse root | $166.3 \pm 62.0^{d}$ | $272.2 \pm 71.2^{d}$ | $554.6 \pm 94.1^{d}$ | $412.0 \pm 108.3^{d}$ |

Table 4 $\delta^{13}C$ (‰) of sun and shade leaves, phloem sugars, fine roots and soil respired $CO_2$ of beech and spruce before labeling. Data are shown as means ± SE (n = 3 trees)(± SE). Lowercase letters denote significant differences between crown levels and soil depths ($p \leq 0.05$). Statistical evaluation was performed using the t-test for paired comparisons. [1]Data taken from Andersen et al. (2010).

| | Beech | | Spruce | |
|---|---|---|---|---|
| | $1xO_3$ | $2xO_3$ | $1xO_3$ | $2xO_3$ |
| Phloem sugars | -29.1 ± 0.3 | -29.5 ± 0.3 | -27.0 ± 0.4 | -27.5 ± 0.5 |
| Leaves | | | | |
| Sun | -28.3 ± 0.1[a] | -28.0 ± 0.3[a] | -26.4 ± 0.5[a] | -27.3 ± 0.2[a] |
| Shade | -31.3 ± 0.3[b] | -31.6 ± 0.3[b] | -28.6 ± 0.4[b] | -29.6 ± 0.6[b] |
| Fine roots[1] | -28.6 ± 0.2 | -28.4 ± 0.2 | -26.4 ± 0.3 | -26.5 ± 0.2 |
| Soil-respired $CO_2$[1] | | | | |
| at 8 cm depth | -24.4 ± 0.2 | -24.0 ± 0.6 | -23.1 ± 0.3[a] | -24.2 ± 0.5 |
| at 15 cm depth | -24.5 ± 0.2 | -23.8 ± 0.2 | -22.0 ± 0.4[b] | -23.3 ± 0.4 |

**Figure captions**

Fig. 1 Ozone concentrations and weather conditions during label exposure. (a) 1x (open circles) and $2xO_3$ (closed circles). (b) Daily sums of photosynthetic photon flux density (PPFD) given as means of daylight hours ± SE (hatched bars), daily means of air temperature (± SE, triangles) and sums of rainfall (black bars).

Fig. 2 Course in $\delta^{13}C_E$ of stems (triangles: upper stem, circles: lower stem) and coarse roots (diamonds) of labeled beech (a, c) and spruce (b, d) under 1x (white) and $2xO_3$ (black) (daily means ± SE, n = 3 trees) during labeling. Consideration was given to the initial difference in $\delta^{13}C_E$ by using data of day 0 as covariate. Dashed line indicates the initiation of the label application. Significant differences between $O_3$ regimes and stem positions at $p \leq 0.05$ are indicated by * and °, respectively. Marginal significance at $p \leq 0.10$ is denoted by (*). Statistical evaluation was performed using repeated measures analysis of variance.

Fig. 3 Fraction of labeled C in stem $CO_2$ efflux (triangles: upper stem, circles: lower stem) and coarse root $CO_2$ efflux (diamonds) of labeled beech (a, c) and spruce (b, d) under 1x (white) and $2xO_3$ (black) (daily means ± SE, n = 3 trees). Dashed line indicates the initiation of the label application. Significant difference between $O_3$ regimes at $p \leq 0.05$ is denoted by *. Marginal significance at $p \leq 0.10$ is denoted by (*). Statistical evaluation was performed using repeated measures analysis of variance.

Fig. 4 Shift in $\delta^{13}C$ of canopy air, upper and lower stem $CO_2$ efflux, soil respired $CO_2$ at 8 and 15 cm soil depth, phloem sugars, sun and shade leaves as well as fine roots of beech (a-c) and spruce (d-f) after 2.5 weeks of labeling. Data are shown as means (± SE) for three labeled trees under 1x and $2xO_3$, respectively. In addition, data from one unlabeled control beech and spruce tree are included to confirm no effect of weather conditions on $\delta^{13}C$ during experimentation. Overall, the t-test for paired comparisons indicated no significant differences in $\delta^{13}C$ shift between $O_3$ regimes within $CO_2$ and solid samples of labeled beech and spruce.