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Carbon flux to woody tissues in a beech/spruce forest during summer and in response to chronic elevated O_3 exposure

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Abstract

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

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

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

1 Introduction

Tropospheric ozone (O_3) is a major component of global climate change (IPCC, 2007), mitigating the carbon (C) sink strength of forest trees and ecosystem productivity (Sitch et al., 2007; Matyssek et al., 2010). Along with increased emissions of anthropogenic

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precursors, in particular nitrogen oxides, tropospheric O_3 concentrations are predicted to rise over Central Europe and at the global scale (Fowler et al., 1999, 2008; Prather et al., 2001). Elevated O_3 concentrations are known to negatively affect the metabolism and growth of a wide range of tree species, including deciduous European beech (*Fagus sylvatica*) and evergreen Norway spruce (*Picea abies*; Karnosky et al., 2007; Matyssek et al., 2010; Wieser et al., 2002; Nunn et al., 2006). Photosynthetic decline, impaired phloem loading, and increased C demand for repair have all been observed in response to ozone exposure. Detoxification may curtail the tree-internal assimilate flux to stems, roots and soils in response to O_3 (Andersen, 2003; Matyssek and Sandermann, 2003; Wieser and Matyssek, 2007).

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

Dynamics in C allocation of adult trees in response to chronically elevated O_3 concentrations are investigated and clarification is particularly needed for respiratory C fluxes of woody tissues. Here, we compare the allocation of recent photosynthates to the respiratory turn-over in stems, coarse roots and soils in adult beech and spruce in a naturally grown forest. We noted that CO_2 efflux sampled from stem and root positions may be affected by xylem-transported CO_2 deriving from lower stem regions and/or root respiration (Teskey et al., 2008). The contribution from soil CO_2 to stem

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CO_2 efflux was recently concluded to be rather small (Gebhardt, 2008; Aubrey and Teskey, 2009; Ubierna et al., 2009).

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

2 Material and methods

2.1 Experimental design

The study was carried out during August/early September 2006 in a 60 to 70-year-old mixed beech/spruce stand at "Kranzberger Forst" in Southern Bavaria, near Freising, Germany (elevation 485 m a.s.l., 48°25′ N, 11°39′ E; Pretzsch et al., 1998). Trees of European beech (*Fagus sylvatica* [L.]) and Norway spruce (*Picea abies* [L.] Karst.), about 25 to 28 m high, were exposed to either ambient (1×) or twice-ambient (2×) ozone (O_3) concentrations. The $2 \times O_3$ regime had experimentally been enhanced since 2000, using a free-air O_3 exposure system (Nunn et al., 2002; Werner and Fabian, 2002). To prevent risk of acute O_3 injury in the $2 \times O_3$ regime, maximum O_3 concentrations were restricted to $< 150\,nL\,L^{-1}$. The forest grew on luvisol derived from loess over tertiary sediments with high nutrition and water supply. Long-term mean (1970–2000) annual air temperature and rainfall were 7.8 °C and 786 mm, respectively (monitored by Deutscher Wetterdienst at climate station "Weihenstephan", at 4 km distance from the research site; DWD Offenbach, Germany; Matyssek et al., 2007a). Scaffoldings and a canopy crane provided access to the tree canopies (Häberle et al., 2004).

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2.2 Climate conditions and stable carbon isotope labeling

After a warm and dry period in July 2006 air temperature decreased during the labeling experiments in August and September (Table 1, Fig. 1). Correspondingly, highest O_3 concentrations occurred during July, and AOT40 exceeded the critical level of 5 µL O_3 L^{-1} h under the 1 × O_3 regime already in May (LRTAP Mapping Manual, 2004; Nunn et al., 2005a). O_3 concentrations in the 2 × O_3 treatment were enhanced by a factor of 1.6 because of the maximum level of 150 µL L^{-1} (see above). Continuous stable carbon isotope labeling was performed from 18 August through 5 September and 26 August through 12 September in beech and spruce, respectively, using a free-air stable carbon isotope exposure system ("isoFACE", for details see Grams et al., 2011). In brief, from 07:00 through 19:00 LT, ^{13}C-depleted CO_2 ($\delta^{13}C$ of ca. −46.9‰) was homogenously released into the canopy of three study trees in each O_3 regime and species (total of 12 trees) by means of micro-porous tubes. During label exposure, O_3 concentrations (means ± SE) were 29.7 ± 6.9 (1 × O_3) and 49.3 ± 11.9 nL L^{-1} (2 × O_3; Fig. 1a). Photosynthetic photon flux density (PPFD) was moderate due to frequently overcast sky and occasional precipitation (48 and 32 mm during beech and spruce labeling period, respectively, Fig. 1b).

2.3 Isotope-ratio mass spectrometry (IRMS)

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

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

CO_2 concentration ($[CO_2]$) and C isotope composition ($\delta^{13}C$) of canopy air were monitored at two heights (i.e. at 1 and 5 m underneath the upper canopy edge, corresponding to sun and shade leaves). Canopy air from all sampling positions was sucked through PVC tubes by means of membrane pumps, analyzed for CO_2 concentration (infra-red gas analyzer (IRGA), Binos 4b.1, Rosemount AG, Hanau) and sampled once a day (~12:00 LT) using a 100 mL syringe. Gas samples were flushed through 12 mL exetainer vials and analyzed as detailed above.

During labeling, $\delta^{13}C$ of canopy air was effectively decreased. Compared to the unlabeled beech control, mean reductions in sun and shade crowns under $1 \times O_3$ were 8.1 ± 0.2 and 8.9 ± 0.3‰, respectively, and under $2 \times O_3$ 9.2 ± 0.4 and 8.4 ± 0.5‰, respectively (Table 2b). In spruce, mean reductions under $1 \times O_3$ was 6.0 ± 0.6‰ and 6.3 ± 0.8‰, respectively, and under $2 \times O_3$ 7.5 ± 0.9‰ and 6.5 ± 0.7‰, respectively (Table 2a). CO_2 concentration in the canopy air of beech was under both O_3 regimes increased by about 110 µL L^{-1}, and in spruce by about 80 µL L^{-1} (Table 2a). In both species, $[CO_2]$ and $\delta^{13}C$ of canopy air were similar each before and on the last day of labeling. Release of CO_2 and thus label application in beech exceeded that of the spruce experiment. The increase in CO_2 concentration of the canopy air did not affect the sap flow of labeled trees, suggesting unchanged stomatal conductance at the leaf level (Grams et al., 2011). Increase in leaf internal to external CO_2 concentration was assumed to be small (< 0.02) and therefore, changes in photosynthetic discrimination against ^{13}C were calculated to stay below 0.4‰ (Grams et al., 2011).

2.5 Assessment of stem and coarse root CO_2 efflux

Stem and coarse root CO_2 efflux (E) of labeled and unlabeled control trees was assessed by means of a computer-controlled open gas exchange system (for details see Grams et al., 2011). Plexiglas chambers (Plexiglas®, Röhm GmbH, Darmstadt,

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Germany) were attached at a lower and upper stem position and at one coarse root per tree (except for the unlabeled control spruce tree). Chambers were covered with aluminized polyester foil to avoid refixation of efflux CO_2 by corticular photosynthesis. For assessment of CO_2 efflux, chambers were connected through PVC tubing to an IRGA (Binos 4b, Emerson Process Management, Weißling, Germany). Stem CO_2 efflux was based on the volume (V in m^3) of the stem sector behind the chamber (i.e. living tissue of bark and sapwood) and coarse root CO_2 efflux on the totally enclosed coarse root volume, respectively (Desrochers et al., 2002; Saveyn et al., 2008).

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

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

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

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

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We considered that stem CO_2 efflux may not only consist of local tissue-respired CO_2, but may be biased by xylem-transported CO_2 deriving from lower stem parts and/or root respiration (Teskey et al., 2008). The absent correlation between xylem sap flow and stem respiration rate or $\delta^{13}C_E$ suggests limited interference of xylem-transported CO_2 with stem CO_2 efflux (data not shown).

2.7 Fraction of labeled C in stem respiration

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

$$f_{E,\,new} = \left(\delta^{13}C_{sample} - \delta^{13}C_{old}\right) / \left(\delta^{13}C_{new} - \delta^{13}C_{old}\right) \quad (2)$$

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

$$\Delta^{13}C = \left(\left[\delta^{13}C_{unlabeled\,air} - \delta^{13}C_{old}\right] / \left[1000 + \delta^{13}C_{old}\right]\right) * 1000(‰) \quad (3)$$

$$\delta^{13}C_{new} = \left(\left[\delta^{13}C_{labeled\,air} - \Delta^{13}C\right] / \left[1000 + \Delta^{13}C\right]\right) * 1000(‰) \quad (4)$$

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

Day-to-day variation in $\delta^{13}C_E$ may occur from variations in label incorporation and in $\Delta^{13}C$ depending on weather conditions (Pate and Arthur, 1998; Bowling et al., 2008). Thus, $\delta^{13}C_E$ of the labeled trees were corrected for the day-to-day variations in $\Delta^{13}C$ (being rather small, i.e. < 0.5‰) of the unlabeled control trees, which showed rather stable $\delta^{13}C_E$ throughout the experiment, i.e. 22.4 ± 0.1 and 21.4 ± 0.1‰ for the upper

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and lower stem positions of beech, respectively, and 19.4 ± 0.1‰ for the lower stem position of spruce.

2.8 Assessment of phloem sugars

Phloem sap was sampled on day 0 and during the last labeling day from the lower stem position following the method of Gessler et al. (2004). Small pieces of bark with adherent phloem tissue (⌀ 5 mm) were cored in the vicinity of the lower stem chamber and incubated (5 h at 4 °C) in 15 mM sodium polyphosphate buffer (Sigma-Aldrich, Munich, Germany). After centrifugation (12.500 rpm, 5 min), phloem sap was analyzed for water soluble sugars (sum of sucrose, fructose, glucose, raffinose and pinitol; i.e. C_{PS} in mg) by means of HPLC (CARBOsep CHO-820 calcium column, Transgenomic, 219 Glasgow, UK). Freeze-dried phloem sap was analyzed for stable carbon isotope ($\delta^{13}C_{sample}$ in ‰) and element composition (C_{sample} in mg), and $\delta^{13}C$ of phloem sugars ($\delta^{13}C_{PS}$ in ‰) was calculated according to Eq. (5):

$$\delta^{13}C_{PS} = \frac{\delta^{13}C_{sample} * C_{sample} - \delta^{13}C_{NPS} * C_{NPS}}{C_{PS}} (‰) \quad (5)$$

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

2.9 Sampling of leaves and fine roots

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

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2.10 Assessment of soil respired CO_2

Soil gas samples were collected as detailed by Andersen et al. (2010). In brief, specific soil-gas sampling wells were placed belowground prior to tree labeling (distance from bole 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 of soil gas from each sampler using a gas-tight syringe. Each beech and spruce tree served as its own control by following the change in $\delta^{13}C$ of soil-respired CO_2 throughout 2.5 weeks of labeling. In the case of beech, a total of four soil-gas sampling wells were additionally installed at an unlabeled control plot. Gas samples were subsequently filled into 12 mL exetainer vials and analyzed for $\delta^{13}C$. Calculation of $\delta^{13}C$ of soil-respired CO_2 follows Eq. (1), while CO_2 of ambient air above the soil served as reference. Note that soil CO_2 efflux was not adjusted by −4.4‰ to account for the more rapid diffusion of ^{12}C compared to ^{13}C (Andersen et al., 2010). $\delta^{13}C$ analysis of additional gas samples taken directly above the forest floor indicated that CO_2 label was restricted to the crown and did not reach the forest soil (Grams et al., 2011).

2.11 Statistical analyses

Statistical analysis was performed using the SPSS 16.0 software package (SPSS Inc., Chicago, USA). Individual study trees were regarded as experimental units, and beech and spruce were analyzed separately. Data were statistically analyzed using General Linear Model (GLM) approach and t-tests where appropriate. Differences at $p \leq 0.05$ were regarded as statistically significant, and at $p \leq 0.1$ as marginally, and denoted by * and (*), respectively.

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3 Results

3.1 Stem and coarse root CO_2 efflux

In general, both species displayed 1 to 4 times higher (beech) and 1 to 2 times higher (spruce) CO_2 efflux rates at the upper compared to the lower stem position (Table 3), whereas rates of coarse roots were 10 to 60 time higher than in stems. In beech, $2 \times O_3$ significantly diminished the CO_2 efflux rate of the upper stem (by ca. −60%), but caused a pronounced increase in coarse roots (by ca. +65%). In spruce, CO_2 efflux rate of the upper and lower stem position was increased by a factor of 1.9 and 1.2, respectively, under $2 \times O_3$. However, $2 \times O_3$ reduced the coarse root CO_2 efflux rate of spruce strongly by ca. −25%.

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

Before labeling, daily means (± SE) of $\delta^{13}C_E$ in beech trees was -28.2 ± 0.1 and -27.9 ± 0.4‰ at the upper and lower stem position under $1 \times O_3$, respectively (Fig. 2). Exposure to $2 \times O_3$ increased values by about 0.4‰. In spruce, $\delta^{13}C_E$ of the upper and lower stems were -27.1 ± 0.1 and -26.6 ± 0.1‰, respectively. Here $2 \times O_3$ reduced values by about 1.1‰. In both species, $\delta^{13}C_E$ of coarse roots were similar to the values of the lower stems and responses to $2 \times O_3$ were consistent with stems.

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

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somewhat delayed, in particular under $1 \times O_3$ and slightly stronger under $2 \times O_3$ (day 5, Fig. 2d).

3.3 Fraction of labeled C in stem and coarse root CO_2 efflux

In beech, the fraction of labeled carbon ($f_{E,\,new}$) in stem CO_2 efflux started to increase during labeling day 2 and was significantly lower in $2 \times O_3$ compared to $1 \times O_3$ from day 3 onwards (Fig. 3a). At the end of the labeling period (day 19), $f_{E,\,new}$ had approached maximum levels of 0.40 ± 0.01 under $1 \times O_3$, whereas under $2 \times O_3$ only 0.33 ± 0.06 and 0.26 ± 0.06 at the upper and lower stem position, respectively, was reached. Lowest $f_{E,\,new}$ was observed for coarse roots (maximum of 0.2), being significantly reduced under $2 \times O_3$ from day 5 onwards (Fig. 3c). In spruce, $f_{E,\,new}$ of stem CO_2 efflux started to increase on labeling day 2, reaching maximum levels of 0.37 ± 0.03 (upper stem) and 0.25 ± 0.05 (lower stem) under $1 \times O_3$, and 0.39 ± 0.06 and 0.30 ± 0.02, respectively, under $2 \times O_3$ at the end of the labeling period (day 18, Fig. 3b). Increase of $f_{E,\,new}$ in spruce coarse roots started somewhat delayed (day 3) but reached levels similar to those of the lower stem position (Fig. 3d). Contrasting with beech, $2 \times O_3$ did not result in a consistently reduced $f_{E,\,new}$ in stems and coarse roots.

3.4 $\delta^{13}C$ in leaves, phloem sugars, fine roots and soil respired CO_2 before labeling

Before labeling, no apparent differences in $\delta^{13}C$ caused by the long-term $2 \times O_3$ exposure were found in the foliage, phloem sap of the stem, fine roots and soil respired CO_2 in either species (Table 4). In general, $\delta^{13}C$ in the sun leaves was significantly increased by ca. 3‰ (beech) and 2‰ (spruce) compared with shade leaves each. The $\delta^{13}C$ of soil-respired CO_2 underneath beech of about −24‰ was not affected by the O_3 treatment. In comparison with beech, all samples from spruce were enriched in ^{13}C by 1 to 2‰ ($p \leq 0.05$). In spruce, $\delta^{13}C$ of soil respired CO_2 was reduced by about 1.2‰ under $2 \times O_3$ and increased by about 1.0‰ at a soil depth of 15 cm compared to 8 cm.

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

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

Upon labeling, belowground allocation of recent photosynthates was not affected by the O_3 treatment and, in general, was reduced compared to stem CO_2 efflux and phloem sugars. The decline upon labeling in $\delta^{13}C_E$ of coarse roots was 1.8 ± 0.1‰ and 1.4 ± 0.1‰ in beech and 1.7 ± 0.9‰ and 2.1 ± 0.8‰ in spruce under 1× and 2 × O_3, respectively. Under beech, changes in $\delta^{13}C$ of soil-respired CO_2 were similar to coarse roots $\delta^{13}C_E$ (about 1.5 to 2.5‰), whereas soil CO_2 under spruce remained unchanged. (Fig. 4e,f). Similar to leaf bulk material, $\delta^{13}C$ of fine roots displayed smaller changes than sampled CO_2 efflux and was in the range of 0.5‰, irrespective of the O_3 treatment.

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4 Discussion

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

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

In both beech and spruce, labeled photosynthates were detected in the upper and lower stem CO_2 efflux from day 3 onwards (Figs. 2 and 3). The fraction of labeled C ($f_{E,\,new}$) in the CO_2 efflux of beech stems was significantly reduced under $2 \times O_3$ (support of hypothesis I), indicating a higher dependency on C stores of the respiratory supply under $2 \times O_3$. Such a response may be caused by O_3-inhibited assimilate transport from the leaves, restricting the respiratory activity of stem tissues (Matyssek et al., 2002) and decreasing C stores in stems and roots towards the end of the growing season (Mc Laughlin et al., 1982). Consequently, re-growth and bud development in spring may become limited (Matyssek and Sandermann, 2003). The significantly decreased flux of recent photosynthates to beech stems represents the mechanistic basis for the observed loss in stem productivity of 40% under long-term exposure of

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$2 \times O_3$ (cf. Pretzsch et al., 2010). In consistency with model predictions (cf. Sitch et al., 2007), this indicates the potential of chronic O_3 stress to substantially mitigate the C sink strength of trees (Matyssek et al., 2010). Contrasting with beech, exposure to $2 \times O_3$ increased the fraction of labeled C ($f_{E,\,new}$) in stem CO_2 efflux of spruce, rejecting hypothesis I for spruce. Accordingly, the rate of stem CO_2 efflux was significantly increased under $2 \times O_3$. Such a stimulation following O_3 exposure has been reported in several studies on herbaceous plants (Grantz and Shrestha, 2006; Reiling and Davison, 1992) and is known to sustain repair- and detoxification processes (Matyssek et al., 1995; Rennenberg et al., 1996).

Reduction of $\delta^{13}C$ in canopy air for 2.5 weeks by about 8 and 6‰ resulted in a drop of stem $\delta^{13}C_E$ in beech of 3–4‰ and in spruce by 2–3‰, respectively (Fig. 4b–f). Correspondingly, $f_{E,\,new}$ of stem CO_2 efflux amounted to about 0.3 to 0.4 in both species. In parallel, $\delta^{13}C$ of phloem sugar was reduced to a similar extent by about 4 and 3‰ in beech and spruce, respectively, suggesting phloem sugars to be the main C source for stem CO_2 efflux. Unlabeled C in phloem sugars may derive from "old C" atoms in C skeletons of currently synthesized sucrose as a consequence of slow turnover of precursor molecules or from remobilized C stores (Gessler et al., 2008; Tcherkez et al., 2003). This suggests xylem-transported CO_2 to contribute only to a smaller extent to stem CO_2 efflux in our study species. This conclusion is supported by the lack of correlation between sap flow and both rate of stem CO_2 efflux and stem $\delta^{13}C_E$ in our study (cf. Grams et al., 2011; Kuptz et al., 2011a,b). However, contribution of CO_2 respired in lower parts of the stem or roots to sampled CO_2 efflux can not be ruled out completely and the extent of this putative influence remains obscure (cf. Teskey et al., 2008).

In consistency with the findings on $\delta^{13}C_E$ in stems, $2 \times O_3$ distinctly reduced $f_{E,\,new}$ of coarse root efflux of beech, supporting hypothesis I. The decrease in coarse root $\delta^{13}C_E$ during the labeling in summer was about 1–2‰ smaller than in stems, indicating a lower dependence of root CO_2 efflux on current photosynthates (Wingate, 2008; Bathellier et al., 2009; Kuptz et al., 2011a). However, soil-respired CO_2, which includes large contributions by root-respired CO_2 of unlabeled neighboring trees and

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heterotrophic soil respiration (Högberg et al., 2001; Andersen et al., 2005, 2010), was reduced in $\delta^{13}C$ by 1.5 to 3 ‰. Hence, beech fine roots and associated microbes appear to be a relatively strong sink for recently fixed C during summer (Högberg et al., 2001; Plain et al., 2009). Slightly pronounced shifts in soil-respired CO_2 under $2 \times O_3$ fits well with previously reported increased fine-root turn-over of beech under long-term O_3 exposure (Nikolova et al., 2010).

Similar to C flux in spruce stems, elevated O_3 did not reduce the allocation of recent photosynthates to coarse root CO_2 efflux (cf. Andersen et al., 2010). However, the C label was hardly detectable in the soil-respired CO_2 around the trees (Andersen et al., 2010), which may indicate favored allocation of labeled C to storage and/or structural pools in the fine roots, resulting in a drop of $\delta^{13}C$ in the fine root tissue during labeling (Fig. 4e, f).

In conclusion, the transfer of recently fixed C from beech and spruce crowns to stem and coarse root CO_2 efflux within 2 to 3 days displays tight coupling with canopy photosynthesis during summer. Chronic exposure to $2 \times O_3$ reduced allocation of photosynthates to the stem and coarse roots of beech and spruce in contrasting ways. The conifer spruce significantly increased the flux of photosynthates to stems (rejection of hypothesis I for spruce), whereas this flux was restricted in stems and coarse roots of deciduous beech (acceptance of hypotheses I and II). The observed patterns in translocation of recent photosynthates are interpreted as a mechanistic basis for observed reductions in beech stem growth, highlighting the potential of chronic O_3 stress to substantially mitigate the C sink strength of trees.

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administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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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 ppb) and SUM0 (i.e. daily sum of hourly O_3 concentrations).

2006	May	June	July	August	September	October
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
$1 \times O_3$ 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
$2 \times O_3$ 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 $1 \times O_3$ [µL L^{-1} h]	5.7	4.7	7.4	0.8	0.6	0.0
AOT40 $2 \times O_3$ [µL L^{-1} h]	13.0	17.1	23.2	6.7	5.1	1.0
SUM0 $1 \times O_3$ [µL L^{-1} h]	33.0	30.1	36.8	21.6	18.6	8.7
SUM0 $2 \times O_3$ [µL L^{-1} h]	47.7	52.2	64.1	35.6	31.7	13.4

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Table 2. (A) CO_2 concentration ($\mu L\,L^{-1}$) and (B) $\delta^{13}C$ (‰) in canopy air of labeled beech and spruce trees under 1× and 2 × O_3 and one unlabeled control tree 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 ($n = 12$ h) label exposure.

	Unlabeled control		Labeled beech				Labeled spruce			
			1 × O_3		2 × O_3		1 × O_3		2 × O_3	
	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade
A [CO_2] ($\mu L\,L^{-1}$)										
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

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Table 3. Rates of stem and coarse root CO_2 efflux ($\mu mol\, m^{-3}\, s^{-1}$) of beech and spruce during the 2.5 weeks of labeling. Data are shown as means (± SE). Within one species, lowercase letters denote significant differences among stem positions and coarse roots, and asterisks between O_3 regimes, respectively ($p \leq 0.05$). Statistical evaluation was performed using the t-test for paired comparisons.

	Beech		Spruce	
	$1 \times O_3$	$2 \times O_3$	$1 \times O_3$	$2 \times O_3$
Upper stem	14.1 ± 2.7^{a}	$5.5 \pm 1.1^{a*}$	12.8 ± 0.6^{a}	$24.6 \pm 1.6^{a*}$
Lower stem	3.8 ± 1.8^{b}	4.9 ± 1.9^{a}	11.9 ± 0.9^{a}	14.7 ± 4.0^{b}
Coarse root	166.3 ± 62.0^{c}	272.2 ± 71.2^{b}	554.6 ± 94.1^{b}	412.0 ± 108.3^{c}

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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). 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.

	Beech		Spruce	
	$1 \times O_3$	$2 \times O_3$	$1 \times O_3$	$2 \times O_3$
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*	−28.6 ± 0.2	−28.4 ± 0.2	−26.4 ± 0.3	−26.5 ± 0.2
Soil-respired CO_2*				
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

* Data taken from Andersen et al. (2010).

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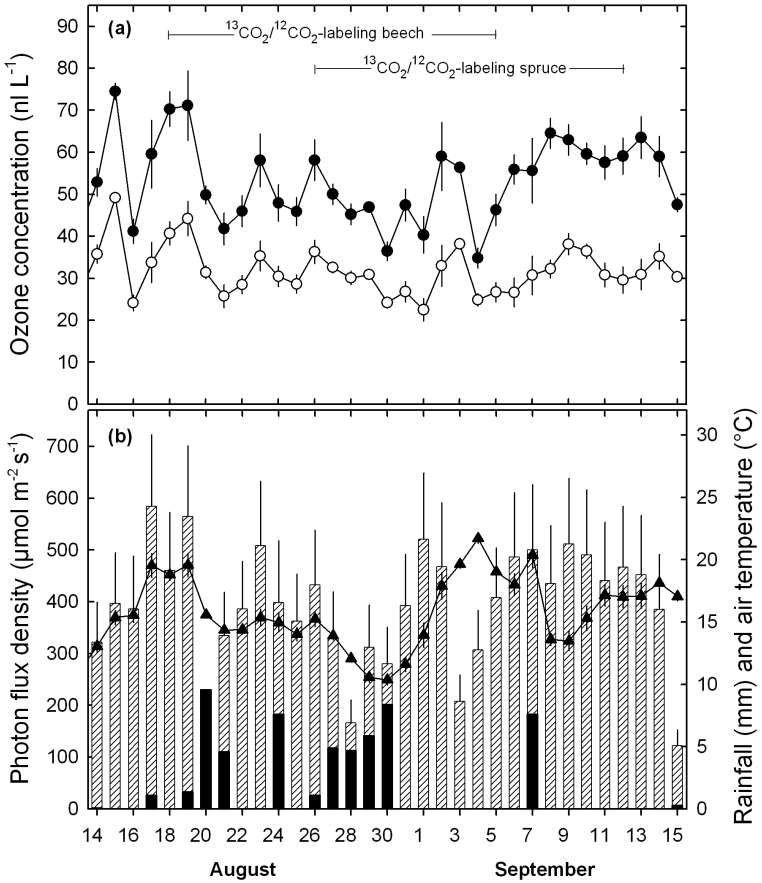

Fig. 1. Ozone concentrations and weather conditions during label exposure. **(a)** 1× (open circles) and 2 × O_3 (closed circles). **(b)** Daily sums of photosynthetic photon flux density (PPFD) given as means of daylight hours ± SE (hatched bars), daily means of air temperature (± SE, triangles) and sums of rainfall (black bars).

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

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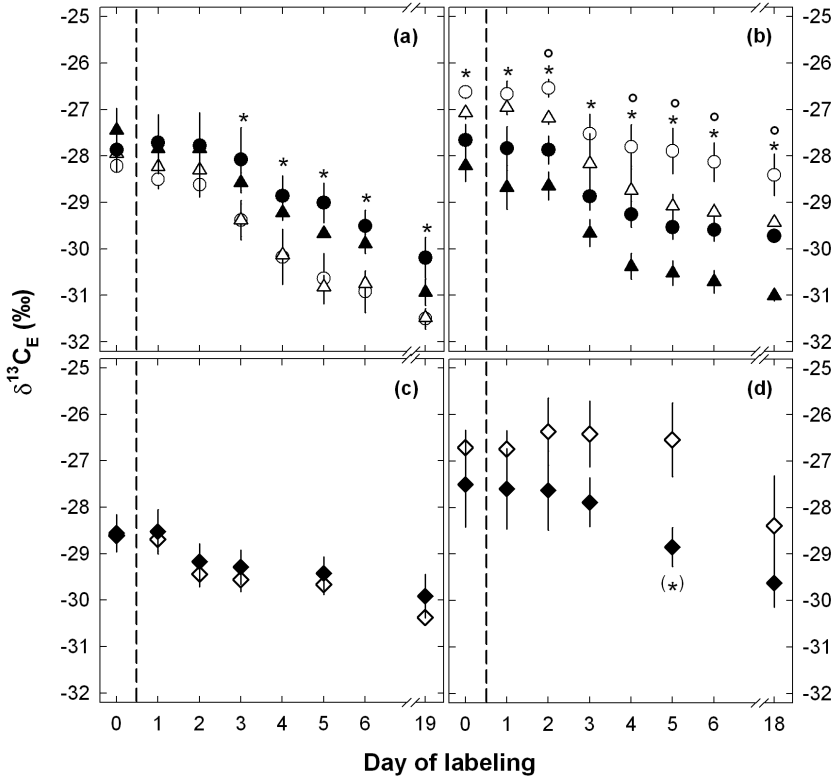

Fig. 2. Course in $\delta^{13}C_E$ of stems (triangles: upper stem, circles: lower stem) and coarse roots (diamonds) of labeled beech **(a, c)** and spruce **(b, d)** under 1× (white) and 2 × O_3 (black) (daily means ± SE, $n = 3$ trees) during labeling. Consideration was given to the initial difference 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.

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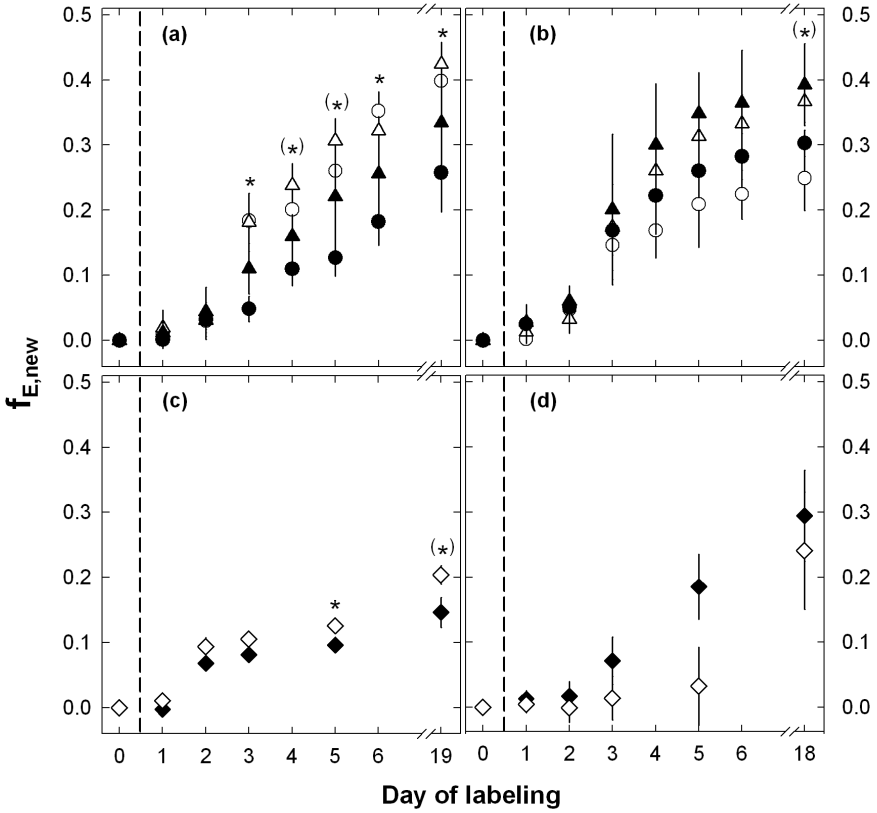

Fig. 3. Fraction of labeled C in stem CO_2 efflux (triangles: upper stem, circles: lower stem) and coarse root CO_2 efflux (diamonds) of labeled beech **(a, c)** and spruce **(b, d)** under 1× (white) and 2 × O_3 (black) (daily means ± SE, $n = 3$ trees). Dashed line indicates the initiation of the label 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.

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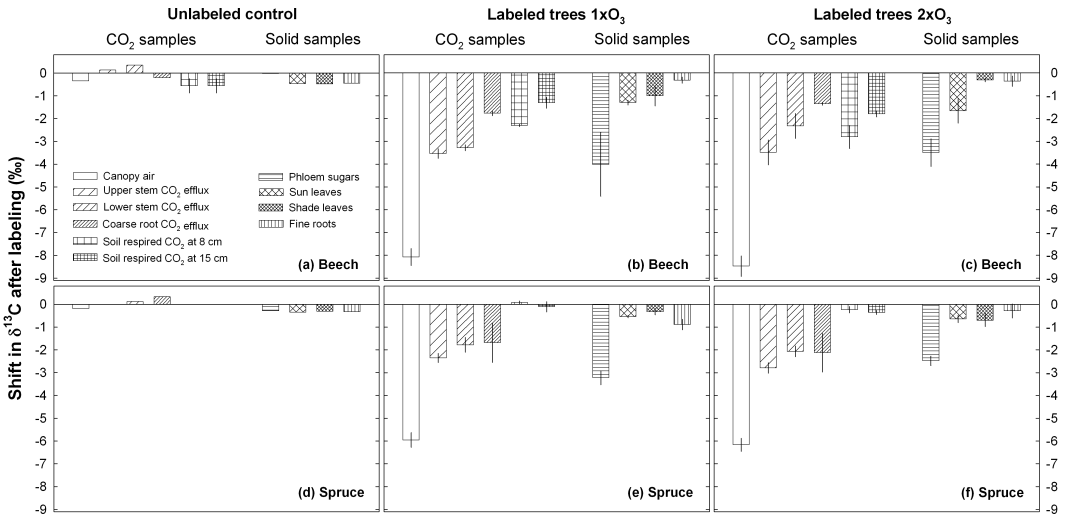

Fig. 4. Shift in $\delta^{13}C$ of canopy air, upper and lower stem CO_2 efflux, soil respired CO_2 at 8 and 15 cm soil depth, phloem sugars, sun and shade leaves as well as fine roots of beech **(a–c)** and spruce **(d–f)** after 2.5 weeks of labeling. Data are shown as means (± SE) for three labeled trees under 1× and 2 × O_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 between O_3 regimes.

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