Oecologia (2014) 175:747–762
DOI 10.1007/s00442-014-2935-5

PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*

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Received: 25 October 2013 / Accepted: 15 March 2014 / Published online: 3 April 2014

Abstract There is evidence of continued stimulation of foliage photosynthesis in trees exposed to elevated atmospheric CO_2 concentrations; however, this is mostly without a proportional growth response. Consequently, we lack information on the fate of this extra carbon (C) acquired. By a steady application of a $^{13}CO_2$ label in a free air CO_2 enrichment (FACE) experiment, we traced the fate of C in 37-m-tall, ca. 110-year-old *Picea abies* trees in a natural forest in Switzerland. Hence, we are not reporting tree responses to elevated CO_2 (which would require equally ^{13}C labeled controls), but are providing insights into assimilate processing in such trees. Sunlit needles and branchlets grow almost exclusively from current assimilates, whereas shaded parts of the crowns also rely on stored C. Only 2.5 years after FACE initiation, tree rings contained 100 % new C. Stem-respiratory CO_2 averaged 50 % of new C over the entire FACE period. Fine roots and mycorrhizal fungi contained 49–56 and 26–43 % new C, respectively, after 2.5 years. The isotopic signals in soil CO_2 arrived 12 days after the onset of FACE, yet it contained only ca. 15 % new C thereafter. We conclude that new C first feeds into fast turnover C pools in the canopy and becomes increasingly mixed with older C sources as one moves away (downward) from the crown. We speculate that enhanced C turnover (its metabolic cost) along the phloem path, as evidenced by basipetal isotope signal depletion, explains part of the 'missing carbon' in trees that assimilated more C under elevated CO_2.

Keywords Carbon isotopes · Elevated CO_2 · FACE · Forest · Respiration

Communicated by Rowan Sage.

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Introduction

Atmospheric CO_2 concentrations are rising and have now been shown to follow the most extreme of the trajectories predicted earlier (Le Quéré et al. 2009; Friedlingstein et al. 2010). Carbon (C) uptake and transpiration of trees exposed to future atmospheric CO_2 concentrations have been shown to strongly depend on the age of trees (Ceulemans and Mousseau 1994; Medlyn et al. 2001), tree species and tree spacing in a forest (Körner 2000), on soil conditions (Spinnler et al. 2002), and on the duration of the treatment (Idso 1999; Körner 2006; Leuzinger et al. 2011). Therefore, no generally valid response patterns are found for trees exposed to elevated CO_2 (Norby and Zak 2011). The most realistic responses can be expected when trees are experimentally exposed to elevated CO_2 in situ in closed forest stands.

To date, only three free air CO_2 enrichment (FACE) experiments have been completed (with a fourth experiment using whole-tree chambers). A *Pinus taeda*

plantation in North Carolina, USA (Duke FACE; Schlesinger et al. 2006), revealed sustained stimulation of stem growth, which, however, was strongly influenced by high nitrogen (and possibly other nutrients) availability (Oren et al. 2001; Schäfer et al. 2003; McCarthy et al. 2010). The second experiment, a *Liquidambar styraciflua* plantation in Tennessee, USA (Oak Ridge FACE), showed a strong initial but no long-term stimulation of growth and productivity (Norby and Zak 2011). Higher nitrogen demand to keep up with the greater C uptake eventually provoked soil nitrogen limitation, followed by a complete loss of the photosynthetic and NPP response to elevated CO_2 by the end of the experiment (Norby et al. 2010). A third experiment, using adult trees in a mature deciduous forest in Switzerland [Swiss Canopy Crane (SCC) web-FACE], reported no growth response to elevated CO_2 at all (Körner et al. 2005; Bader et al. 2013), although some species-specific water savings induced a soil priming effect (nitrate release; Schleppi et al. 2012). The fourth experiment, using whole-tree chambers on 40-year-old *P. abies* trees in a boreal forest in northern Sweden (Medhurst et al. 2006), showed a growth response to elevated CO_2 only when mineral fertilizer was applied, and none under natural nutrient conditions (Comstedt et al. 2006; Sigurdsson et al. 2013).

In summary, these results suggest that C is not generally a limiting resource for tree growth at current CO_2 concentrations. However, a common observation in most CO_2-enrichment experiments is that leaf photosynthesis remains stimulated by elevated CO_2 (Ainsworth and Rogers 2007; Bader et al. 2010; but see Norby et al. 2010). Even in cases where some downward adjustment had been seen, the diminished photosynthetic stimulation still did not produce a proportional growth response. Thus, the question of the fate of the additionally assimilated C remains (Fatichi and Leuzinger 2013).

Tracing the fate of C in a forest needs a marker that can be applied at canopy scale. The stable ^{13}C isotope is the only suitable marker for such large-scale and long-term in situ labeling investigations. Pulse labeling (a strong, short-term peak with highly enriched $^{13}CO_2$; usually 99 % ^{13}C) is suitable for studying short-term dynamics of the C distribution (Epron et al. 2012). The other option is to expose the foliage continuously with ^{13}C depleted CO_2 originating from fossil fuel (e.g., −30 ‰ relative to the V-PDB standard), permitting the continuous tracing of the fate of novel C over a longer period (Andrews et al. 1999; Hättenschwiler et al. 2002; Körner et al. 2005; Comstedt et al. 2006; Keel et al. 2007; von Felten et al. 2007).

Recent studies on in situ C allocation in field-grown conifers (<25 m tall) used the whole tree/canopy ^{13}C pulse (or extended pulse) method with trees, which are normally growing under ambient CO_2 concentration (Kagawa et al. 2006; Högberg et al. 2008; Endrulat et al. 2010; Dannoura et al. 2011; Kuptz et al. 2011a; Warren et al. 2012). Such short-term pulse experiments in conifers revealed a rapid appearance (a few days) of labeled C in respiratory CO_2 released from soils (Högberg et al. 2008; Ritter et al. 2011), indicating a strong linkage between assimilation and soil CO_2 release. However, transport velocity and basipetal C propagation was found to vary with tree size and season, and is controlled by weather conditions and sink strength, as was demonstrated by repeated pulse labeling of 12-year-old *Pinus pinaster* growing in a plantation (Dannoura et al. 2011; Epron et al. 2011). Moreover, the dilution of new assimilates in large, old non-structural C pools prior to utilization (Keel et al. 2007) led to only a small and seasonally fluctuating contribution of new C to respiratory CO_2 release from stems and coarse roots of a 60-year-old *P. abies* stand in Germany (Kuptz et al. 2011a). Similar C mixing and seasonal patterns for structural C were observed elsewhere (Lippu 1994; Kagawa et al. 2006), and in deciduous trees at our study site (Keel et al. 2007; Bader et al. 2013). Given that tissue formation in evergreen trees profits from a year-round supply of new assimilates (Hansen et al. 1996), we can assume a rapid manifestation of the isotopic signal contained in new photoassimilates, particularly in foliage and adjacent branchlets (branch autonomy; Gordon and Larson 1968; Hoch 2005). Signals of new C should dilute basipetally, e.g., in stem wood, and extend the time until old C is completely replaced by new C (Keel et al. 2007).

Using the SCC web-FACE facility in Switzerland (Pepin and Körner 2002), we started CO_2 enrichment of canopies of mature *P. abies*, and, thus, labeling with ^{13}C depleted CO_2 in a near-natural forest ecosystem on 30 July 2009. The work presented here is not aimed at elucidating tree responses to elevated CO_2, but should illustrate the basic patterns of C allocation in mature forest trees. Establishing the fate of novel C should help in identifying sites and processes that explain 'missing C' in experiments with elevated atmospheric CO_2. We present C distribution patterns using ^{13}C signals, spanning a pre-treatment period and two full growing seasons under elevated CO_2. By consecutive sampling with short intervals in various tree compartments from tree tops to roots, we aim at analyzing (1) seasonal shifts in assimilate allocation, (2) locations of C-investment, and (3) residence times (turnover) of mobile C pools. We expect that the ^{13}C signal would be transferred from needles to respiratory fluxes within a few days, but that it will take several years before new structural biomass such as stem wood consists fully of new C. Although we cannot assess CO_2 effects on new/old C mixing ratios due to missing similarly labeled control trees, we are able to quantify

the main pathways and the duration of C transfer following assimilation for the first time in mature coniferous trees.

Materials and methods

Description of the free air CO_2-enrichment facility

In 2009, a web-FACE system (Pepin and Körner 2002) was installed in five 37-m-tall, then ca. 110-year-old *Picea abies* individuals. With this system, the trees were exposed to increased atmospheric CO_2 concentration at ca. 550 ppm in their natural forest environment. The site is located near the village of Hofstetten (47°33′N, 7°36′E, 500 m a.s.l), 12 km south-west of Basel, Switzerland. All five *P. abies* trees that were subjected to elevated CO_2 are located within max. 5 m distance from another. Five similarly tall trees served as controls. Of these controls, one tree is situated only 6 m from the treated trees (separated from the treated trees by a forest road), whereas the other trees grew at a distance of 17–30 m from the CO_2-enriched trees. The only control tree that was closer to the treated trees was 'unpolluted' by tank CO_2 as evidenced by the ^{13}C data. While the canopies of all CO_2-treated trees and one control tree were accessible with a crane gondola, the crowns of the remaining control trees could only be reached by professional tree climbers.

The CO_2 enrichment started on 30 July 2009, when earlywood and current year foliage formation of that year had been completed. Pure CO_2 (in food industry quality, with a $\delta^{13}C$ of −30 ‰) was released exclusively into the tree canopies (15–37 m above ground) through laser-punched tubes woven around the tree branches. Control of CO_2 release was separated into four sectors per tree to ensure an optimal CO_2 distribution and concentration in response to wind directions: the tree tops, and three 120° sections around the remainder of the crowns (i.e. 4 sectors per tree, 20 sectors in total). Each sector was separately monitored for CO_2 concentrations and was individually supplied with pure CO_2 to ensure a mean CO_2 concentration in the tree canopy of 550 ppm. Each sampling line in the canopy for CO_2 monitoring ended in a triplet, so that each tree crown was sampled at 12 sampling points (60 for all 5 trees). Four extra gas-sampling lines served as a control (at the crane top, 45 m above ground, the sub-canopy, and a calibration gas bottle). CO_2 concentrations were measured with two non-dispersive infrared gas analyzer (IRGA; LI-820; Li-Cor, Lincoln, NE, USA). Using C_4 grasses grown in the canopy, the $\delta^{13}C$ of the CO_2 mixture supplied was monitored (plant isometers, see below). CO_2 release was suspended when temperature was below 6 °C, and when above-canopy photon flux density was below 100 μmol m^{-2} s^{-1}. With this FACE system, we could reach median elevated CO_2 concentrations of 500–560 ppm based on IRGA readings, with a mean 20–50 ppm higher given the skewed frequency distribution.

Table 1 CO_2 concentrations and $\delta^{13}C$ signatures in the canopies of trees subjected to ambient and elevated CO_2 calculated from isometer (C_4 grass *Echinochloa crus-galli*) $\delta^{13}C$ values

	Mean ± SE	*n*	Mean ± SE	*n*
Canopy $\delta^{13}C$ (‰)				
2009	−8.0 ± 0.1	2	−13.7 ± 0.3	110
2010	−8.7 ± 0.1	2	−13.5 ± 0.3	82
2011	−8.5 ± 0.2	2	−13.8 ± 0.3	98
Canopy CO_2 concentration (ppm)				
2009	382 ± 2	2	541 ± 13	110
2010	397 ± 3	2	532 ± 13	82
2011	396 ± 4	2	541 ± 12	98

Ambient CO_2 values were obtained from isometers installed at the crane top. Mean ± SE for the annual FACE period for *n* isometers

C_4 grass isometers

The values from IRGA measurements could be confirmed by the isotopic compositions of canopy-grown C_4 grasses ('isometers'; *Echinochloa crus-galli* in 50-ml flasks filled with sand-clay soil, mounted in elevated and ambient tree crowns, and, thus, freely exposed to the same atmosphere as the tree crowns) in order to obtain the integrated $\delta^{13}C$ values and the corresponding CO_2 concentrations (Table 1; see Pepin and Körner 2002 for calculation). Since C_4 grasses synthesize new biomass entirely from newly assimilated CO_2 without further enzymatic fractionation, the isotopic signal in C_4 grass tissue reflects the long-term $\delta^{13}C$ within the tree crowns (ambient vs. elevated $\delta^{13}C$ differences in 2009, 2010, and 2011 (mean ± SE): −5.7 ± 0.6, −4.8 ± 0.5, and −5.3 ± 0.3 ‰, respectively,). These differences indicate 100 % new C incorporated and served as a yearly reference for the new C fraction in the different tree tissues investigated. Assuming that the isometers capture the same atmospheric CO_2 mixture in the canopy that becomes effective for the treated *P. abies* trees, the difference in the isotopic signals of isometers grown in elevated and ambient CO_2 concentrations should also be observed between the tissues of the treated and control trees (the maximum possible new C integration into tissues). However, the scattered distribution of a limited number (ca. 14) of containers with C_4 grasses within the tree canopies during peak summer only (spring and autumn are too cold for C_4 grasses) may cause a difference between the isometer signal and the maximum tree tissue signal captured over an entire season. Although we are using isometer signals as a 100 % reference, they are still a proxy for the maximum new C integration into

tissues, and needles may spatially experience slightly less or more labeled tank CO_2. A signal >100 % thus indicates that isometers underestimated the signal strength at some locations. In any case, such a signal means that the new tissue was built from novel C only.

Branchlet needle and xylem sampling

During four sampling campaigns (pre-treatment: 1–16 July 2009; treatment periods: 18–27 August 2009; 29 September 2010; 29 July 2011), professional tree climbers collected three sun-exposed and three shaded branchlets from all four cardinal directions of the control trees that were not accessible via the crane gondola. The same kind of samples were collected from the remaining study trees using the crane (24 branchlets per tree, except during the pre-treatment campaign when only sun-exposed branchlets were used). From these branchlets, we investigated sun-exposed and shaded bulk needle material and sun-exposed branchlet xylem tissue. In 2009, we collected needle and xylem samples from branchlet extensions initiated in the years 2008 and 2009. In the years 2010 and 2011, we sampled 2008 and 2009 branchlet parts again, along with the same kind of material from one or two more recent years. We obtained bulk xylem tissue by peeling off bark and phloem immediately after sampling. Additional needle tissue was collected every 2–6 days in August 2009 for 3 weeks following the FACE onset, later we continued with 1- to 3-monthly sampling intervals. Three to five needles of each of three sun-exposed and shaded branchlets per cardinal direction and per tree were collected. Prior to analysis, we pooled the needle and the xylem samples of the three branchlets from all four cardinal directions per treatment since we did not find azimuthal differences within either sunlit or shaded needles. Immediately after sampling, the material was heated in an on-site microwave oven (90 s at 600 W) to stop any metabolic processes (Popp et al. 1996). Canopy CO_2 enrichment could cause a shift in photosynthetic ^{13}C discrimination in both *P. abies* trees and the C_4 grasses that could add to the CO_2-enrichment signal caused by tank CO_2. Since such a biochemical adjustment is likely to be small (<1 ‰) and differ between sun and shade needles, we refrained from assuming a change and applying a uniform data adjustment, but present the data as measured.

Wood core sampling

Wood cores were collected at breast height (1.3 m) in east direction from each tree. Using a 5-mm-diameter increment corer, we took the samples in March 2010 during the trees' winter dormancy after completion of the latewood formation in 2009 (earlywood was formed before the beginning of the CO_2 enrichment on 30 July 2009). Tree rings formed in 2005–2009 were also analyzed for $\delta^{13}C$ values. Except for latewood 2009, we expected $\delta^{13}C$ signals in these tree rings to reflect only pre-treatment conditions before the start of the experiment. There may, however, be a very small influence of storage reserves in older ray parenchyma in the trees receiving CO_2 enrichment since midsummer 2009. Wood cores were again taken in February 2012 to assess the changes of the isotopic signal in the 2008–2011 tree rings throughout the experiment. Earlywood and latewood of each year were separated under a binocular using a razor blade. The transition zones between early and latewood, and between the years were removed in order to obtain a clearly separated isotopic signals. The wood segments were then analyzed for $\delta^{13}C$.

Fine root sampling

In March 2010, 9 soil cores per tree (12 cm in depth × 3.6 cm diameter) were collected in the inner part of the root disk (2 m from the stem) of each tree, adding up to a total of 90 root samples for treated and control trees. The nine cores per tree were grouped into three 'joint' cores collected at 10 cm distance from one another, with the triplets separated at a 120° angle around each tree. The values of these triplicate samples were pooled after the isotope analysis in order to give a more robust signal. The resulting holes were used to install ingrowth cores of the same size (2-mm mesh cylinders), filled with sieved root-free soil from on-site molehills to assess CO_2 effects on fine root growth development (Bader et al. 2009). The bulk density of soil in the ingrowth cores was adjusted to the bulk density of the soil outside (mass to volume ratio). To capture the isotopic signal of newly formed fine roots entirely developed during FACE, the use of ingrowth cores was the preferential method. In December 2011, 20 months after installation, the ingrowth cores were removed using a knife. Both soil and ingrowth cores were stored at −20 °C until processing in January 2012. Prior to fine root separation, cores were placed in cold water and defrosted for 48 h at 4 °C to keep the microbial degradation of fine root biomass as slow as possible. Roots were extracted by wet-sieving (1 mm mesh), and *P. abies* roots were identified using a reference root collection. Since the five trees exposed to elevated CO_2 formed a monospecific group in the stand, the inner part of the treated area belonged almost exclusively to *Picea*, whereas at the periphery of the plot, roots of other species (e.g., *Fagus sylvatica, Carpinus betulus, Quercus petraea*) co-occur. However, *Picea* roots are very distinct and can be clearly identified. Fine roots were separated into three diameter classes (<0.5, 0.5 < 1, 1–2 mm). We could not distinguish between still intact dead and live fine roots.

Fungal sporocarp sampling

During late summer and early fall 2010 and 2011, we collected fungal fruit bodies (sporocarps) within three distance intervals to a tree stem subjected to elevated CO_2 (0–6, 6–12, 12–18 m; the last being considered as an untreated control). The sporocarps were frozen and stored at −20 °C until taxonomic and functional group classification (either saprobiotic, mycorrhizal, or parasitic) and further preparation for C isotope analysis.

Stem and soil air sampling

The CO_2 emitted by stems was captured by four aluminum cups per tree (Ø 8.2 cm, height 2.6 cm; using Terostat-IX; Teroson, Ludwigsburg, Germany) mounted in the four compass directions to each stem at breast height (1.3 m) after removing loose bark. Given the rise in CO_2 partial pressure within the cups due to continuous CO_2 release from tree stems, we assumed that small leaks would not affect the isotope signals. Cups were fitted with an open-ended tube (2–3 cm long, inner Ø 3 mm) through a bottom hole. This tube permitted gas sampling with a syringe, but at the same time reduced gas exchange between the cups' interior and surrounding air. Since the CO_2 concentration in the cups did not reach equilibrium (Ubierna et al. 2009a), we had to account for counter diffusion by atmospheric CO_2. Therefore, the Keeling plot approach was applied to calculate the $\delta^{13}C$ in stem-emitted CO_2 (see below). Note that only the tree canopy at 20–37 m height was receiving labeled tank CO_2, with the understory remaining unaffected.

We used gas wells to collect air in equilibrium with soil air. These wells consisted of PVC tubes (inner Ø 18 mm, 12 cm long) in which small holes were drilled at each ca. 70° angle in five rows between 3 and 11 cm of the gas well height to allow air to diffuse into the tubes from the respective depths (Steinmann et al. 2004). The gas wells were inserted in vertical pre-punched holes in the soil in 2009. The bottom of each well was left open, and the top was covered with an air-tight rubber septum. Ten gas wells per tree were installed in two circles of 2 and 3 m radius around each tree stem (each with five gas wells per circle at ca. 70° angle).

At each sampling time, 15 ml air was extracted through the septum using a 60-ml syringe. The air was injected in a 12-ml pre-evacuated glass vial (Exetainer gas testing vial; Labco, High Wycombe, UK) closed with an airtight rubber septum. The overpressure produced in the vials eliminates contamination with ambient air. These vials containing the gas samples were stored in a N_2 atmosphere prior to analysis, which was performed no later than 7 days after sampling.

One day before FACE launch on 30 July 2009, we collected 15 ml gas from all soil gas wells and all stem cups to receive pre-treatment values. Starting 1 day after FACE onset, two soil gas wells per circle and tree and two cups per stem were randomly sampled. During the first 2 weeks, we sampled daily, thereafter biweekly until August 2009, followed by about a bimonthly sampling interval. Starting in May 2010, the sampling intensity was reduced to one mixed 15-ml sample per soil circle from all five gas wells per circle (each 3 ml per gas well) as well as one mixed sample of all four cups per tree stem (each 3.75 ml per cup).

We employed the Keeling plot approach (Keeling 1958) to obtain as correct as possible $\delta^{13}C$ signals in the mixture of two different CO_2 sources, respiratory and atmospheric CO_2, both diffusing in the opposite direction in the soil. We followed the approach by Steinmann et al. (2004) to obtain atmospheric reference CO_2 concentration data and the corresponding $\delta^{13}C$ values from six different European/Middle Eastern weather stations (Terceira Island, Azores, Portugal; Storhofdi, Iceland; Tenerife, Spain; Mace Head, Ireland; Ocean Station M, Norway; WIS Station, Israel) for the years 1997–2004. Note that FACE was applied to the canopy 15–37 m aboveground only; thus, tank CO_2 did not reach the ground in the test area. For applying the Keeling plot approach, CO_2 and $\delta^{13}C$ values for free air in the years 2009–2011 were needed, which were not available when we analyzed our data. Therefore, we modeled the trend of the data with a seasonal component incorporated, using Holt–Winters exponential smoothing (Holt 1957).

C isotope analysis

Within 3–4 h after sampling, all tissue samples were dried at 80 °C for 48 h, followed by grinding to a fine powder using a steel ball mill (MM 2000; Retch, Haan, Germany). Aliquots of 0.6–0.8 mg were weighed into tin capsules and analyzed for C isotope ratios. The samples were combusted in an elemental analyzer (EA-1110 CHN; Carlo Erba Thermoquest, Milan, Italy). The EA was connected to an Isotope Ratio Mass Spectrometer (IRMS, Delta S; Thermo Finnigan, Bremen, Germany) operating in continuous flow mode via a variable open-slit interface (Conflo II; Thermo Finnigan). The precision for $\delta^{13}C$ was <0.1 ‰. The isotope values are expressed in the δ-notation as deviation from the international standard (Vienna-Pee Dee Belemnite: V-PDB): $\delta^{13}C = R_{sample}/R_{standard} - 1$ (‰) where R is the molar ratio of ^{13}C to ^{12}C for the sample and the standard, respectively. The isotope analyses were performed at Stable Isotope Facility of the Paul Scherrer Institute, Villigen, Switzerland.

Statistical analysis

The unit of replication was 'tree' (five trees under ambient and five trees under elevated CO_2 concentrations). All

statistical analyses (except for fungal $\delta^{13}C$) were performed using linear mixed effects models fitted by restricted maximum likelihood. The significance of the main effects was assessed using a backwards selection procedure based on likelihood ratio tests and the Akaike information criterion. The factor 'tree' was included as random factor (apart from fungal $\delta^{13}C$). In certain cases, a second random factor nested within 'tree' was used (xylem $\delta^{13}C$: 'year of the start of branchlet segment growth'; fine root $\delta^{13}C$: 'cardinal direction of the position of the soil/ingrowth core'). Where appropriate, we corrected for homogeneity violations using adequate variance function structures (power, exponential, and constant variance structures, or a combination thereof). Furthermore, we accounted for independence violations where necessary by applying temporal autocorrelation structures. Diagnostic plots (i.e. residual and quantile–quantile plots) were used for model validation. All statistical analyzes were performed using R, v.2-15-0 (R Development Core Team 2008–2010) and the R package *nlme*.

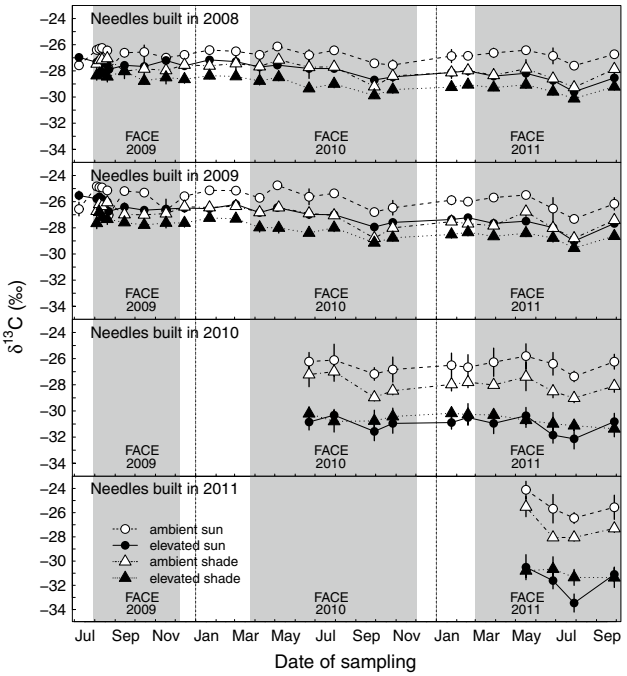

Fig. 1 Seasonal variation of carbon isotope signature ($\delta^{13}C$) in organic matter of sun-exposed and shaded needles of *Picea abies* trees exposed to ambient and elevated atmospheric CO_2 [ambient CO_2: $n = 2$ trees (except for 8 July 2009, 22 August 2009, 29 September 2010, 29 July 2011: $n = 5$ trees), elevated CO_2: $n = 5$ trees, mean ± SE]. *Diagrams* denote needles that were built in the year (from *top* to *bottom*) 2008, 2009, 2010, and 2011, respectively. The *gray-shaded* areas denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only

Results

Canopy $\delta^{13}C$ and CO_2 concentration

Assuming a $\delta^{13}C$ signature of −8.4 ‰ and a CO_2 concentration of 390 ppm in the atmosphere (2009–2011 average; NOAA), data from our regular measurements of tank CO_2 ($\delta^{13}C$ of −30 ‰), and canopy CO_2 data from the IRGA measurements, the expected $\delta^{13}C$ value of the mixed CO_2 in the canopies of treated trees was −13.8 ± 0.3 ‰ (mean ± SE of $n = 3$ years, i.e. 2009–2011). This value corresponds to an expected difference in $\delta^{13}C$ between elevated and ambient CO_2 in the canopies of −5.4 ± 0.3 ‰ (mean ± SE of $n = 3$ years, i.e. 2009–2011). The C_4 grass isometer samples revealed a similar $\delta^{13}C$ value of −13.7 ± 0.1 ‰ (mean ± SE of $n = 3$ years, i.e. 2009–2011) in the canopies of the treated trees ($\delta^{13}C$ difference between elevated and ambient CO_2: −5.3 ± 0.2 ‰), corresponding to a canopy CO_2 concentration of 538 ± 7 ppm (elevated CO_2; mean ± SE of $n = 3$ years, i.e. 2009–2011).

Pre-treatment $\delta^{13}C$ differences

Prior to the onset of FACE (pre-treatment), tissues of trees later exposed to elevated CO_2 concentrations usually showed slightly less negative $\delta^{13}C$ signals compared to control trees. Needles and branchlet xylem of treated trees formed in 2008 and 2009 showed the highest deviations from control trees (0.8 ± 0.2 ‰ in sunlit needles, and 0.9 ± 0.2 ‰ in sunlit branchlets; Figs. 1, 2; no data for shaded needles and branchlets). These canopy signal differences attenuated downstream of the plant body. Tree rings at breast height were first sampled in March 2010. Thus, earlier year rings might store some novel (fixed after 30 July 2009) non-structural carbon in ray tissue causing a slightly more negative bulk xylem signal. Yet, earlywood formed in 2005 to earlywood formed in 2009 of trees later subjected to elevated CO_2 still showed a slightly less (not significantly) depleted $\delta^{13}C$ signal compared to control trees (0.3 ± 0.1 ‰; Fig. 3), suggesting little if any influence of novel C by March 2010. Fine roots were not sampled before FACE. Respiratory CO_2 sampled before FACE showed either no $\delta^{13}C$ differences (0.1 ‰ in soil CO_2 efflux; $P = 0.7$; Fig. 7), or even more negative $\delta^{13}C$ signals (0.9 ‰ in stem CO_2 efflux; $P = 0.03$; Fig. 6) in trees later subjected to elevated CO_2 concentrations versus control trees, thus not matching the pre-treatment signals in branchlet tissues. Given these small and inconsistent trends in pre-treatment $\delta^{13}C$, we did not correct $\delta^{13}C$ signals obtained after the onset of FACE by pre-treatment signals, with the assumption that the deviations fall within the error margins. This approach is conservative, because the pre-treatment trend rather runs counter the direction of tracer signals.

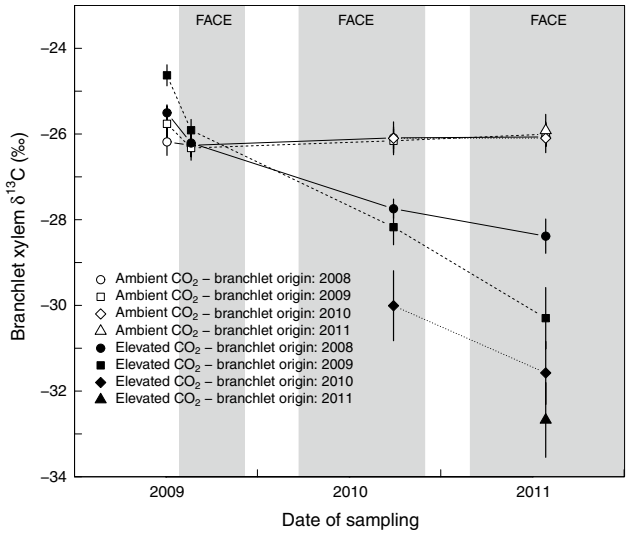

Fig. 2 Time course of the $\delta^{13}C$ of sun-exposed branchlet xylem of *P. abies* exposed to ambient and elevated atmospheric CO_2 (ambient CO_2: $n = 5$ trees, elevated CO_2: $n = 5$ trees, mean ± SE). The *gray-shaded* areas denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only. Note that the branchlet origin indicated in the diagram corresponds to the year when new branch segments were initiated. In the following years, new year rings were produced that add to branch segment thickening leading to dilution of the isotopic signal

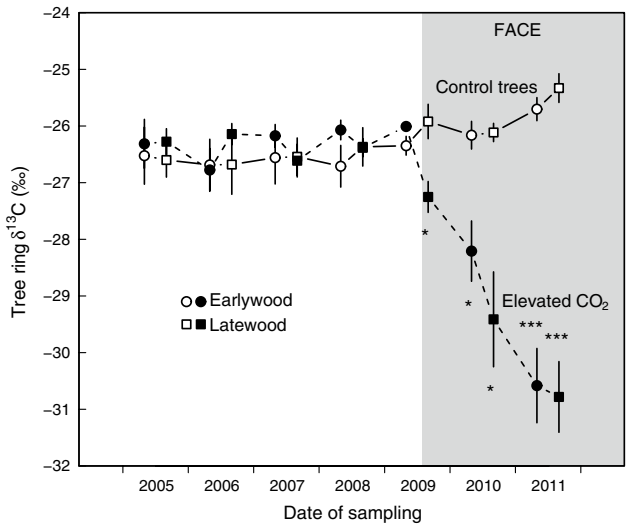

Fig. 3 Seven-year time course of $\delta^{13}C$ values in earlywood and latewood of *P. abies* subjected to ambient and elevated atmospheric CO_2 ($n = 5$ trees, mean ± SE). The *gray-shaded* area denotes the FACE period. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

$\delta^{13}C$ in needles

Generally, needles of trees subjected to elevated CO_2 always showed more negative C-isotope signals compared to trees under ambient CO_2 during the treatment period ('CO_2 treatment signal' effect: $P < 0.004$; Table 2; Fig. 1), with a significant 'exposure × CO_2 × day of year' interaction ($P < 0.001$). Furthermore, signals did not become significantly stronger with time within an age cohort and exposure class ('exposure × day of year' effect: $P = 0.063$). However, isotopic signals became more negative from one needle age class to the next (Fig. 1); the younger the needles, the more pronounced was the $\delta^{13}C$ difference between needles of trees under ambient and elevated CO_2. Sampled during CO_2 enrichment, needles of CO_2-enriched trees which were formed prior to the experiment (e.g., needles formed in 2008 and 2009; Fig. 1), showed more negative $\delta^{13}C$ values by 1.1 ± 0.1 ‰ (mean treatment difference ± SE) compared to controls, irrespective of the position in the canopy, suggesting an exchange of non-structural carbohydrates (NSC) by new ones.

Sampled during the FACE period, shaded needles exposed to elevated CO_2 showed a progressive mean depletion from 1.0 ± 0.1 ‰ (needles formed in 2008 and 2009; Fig. 1), to 2.6 ± 0.2 ‰ (needles formed in 2010; Fig. 1), and to 3.8 ± 0.6 ‰ (needles formed in 2011; Fig. 1) compared to needles in ambient CO_2. Sunlit needles formed during FACE, and sampled after the onset of FACE, were even more depleted under elevated CO_2 by 1.2 ± 0.1 ‰ (needles formed in 2008 and 2009; Fig. 1), 4.5 ± 0.1 ‰ (needles formed in 2010; Fig. 1), and 6.2 ± 0.3 ‰ (needles formed in 2011; Fig. 1) compared to sunlit needles of control trees.

In control trees, shaded needles were always more depleted compared to sunlit needles on the same trees, irrespective of the year of needle formation (1.3 ± 0.1 ‰; $P < 0.001$; Table 2). The same pattern was observed for treated needles formed prior to FACE initiation (i.e. formed in 2008 and 2009), and sampled during FACE (1.1 ± 0 ‰; $P < 0.001$; Table 2). Interestingly, this did not apply to needles grown under elevated CO_2 in 2010 and 2011. Here, shaded needles showed less negative $\delta^{13}C$ values by 0.5 ± 0.1 ‰ compared to sun-exposed needles.

Averaged over samples collected during the entire FACE period, sunlit and shaded needles formed prior to FACE (e.g., 2008 and 2009) incorporated a steady state 20 ± 0.2 % new C during the treatment years 2009–2011. Only 2 years after the FACE onset, we measured 72–117 % new C in needles formed in 2011 compared to the isometer reference signal (see "Materials and methods"). Drawing on new assimilates was intensified in sunlit needles. Overall, the amount of C incorporated did not vary at a given type of position once new needles had fully matured.

$\delta^{13}C$ in branchlet xylem

For statistical analysis, we used only xylem $\delta^{13}C$ of branchlet segments of the most recent shoot expansion, thus

Table 2 Linear mixed effects model results for *Picea abies* ^{13}C signatures of needles, branchlet xylem, year rings, fine roots (soil cores 2010 and ingrowth cores 2010-2011), fungi, and CO_2 efflux from stems and soil under ambient and elevated CO_2

Factor	*df*	*F* value	*P*	
Needle $\delta^{13}C$ (2009–2011)				
Exposition	1, 128	700.6	<0.001	***
CO_2	1, 8	16.3	0.004	**
Day of experiment	1, 128	64.6	<0.001	***
Exposition × day of experiment	1, 128	3.5	0.063	
CO_2 × day of experiment	1, 128	58.8	<0.001	***
Exposition × CO_2 × day of experiment	1, 128	34.0	<0.001	***
Branchlet xylem $\delta^{13}C$ (2009–2011)				
CO_2	1, 8	0.5	0.498	
Day of experiment	1, 9	43.8	<0.001	***
CO_2 × day of experiment	1, 10	96.5	<0.001	***
Year ring $\delta^{13}C$ (2005–2011)				
Latewood/earlywood	1, 124	0.1	0.716	
CO_2	1, 8	16.6	0.004	**
Year	1, 124	0	0.891	
Latewood/earlywood × CO_2	1, 124	8.7	0.004	**
Latewood/earlywood × year	1, 124	0.9	0.336	
CO_2 × year	1, 124	19.1	<0.001	***
Latewood/earlywood × CO_2 × year	1, 124	10.0	0.002	**
Fine root $\delta^{13}C$ (soil cores 2010)				
Diameter	2, 54	12.9	<0.001	***
Fine root $\delta^{13}C$ (ingrowth cores 2010–2011)				
CO_2	1, 8	28.0	0.001	***
Diameter	2, 40	3.8	0.032	*
Soil cores	1, 40	0.1	0.709	
CO_2 × diameter	2, 40	0.8	0.448	
CO_2 × soil cores	1, 40	8.9	0.005	**
Diameter × soil cores	2, 40	1.0	0.370	
CO_2 × diameter × soil cores	2, 40	3.8	0.030	*
Fungal $\delta^{13}C$ (2010 + 2011)				
Distance	1	7.3	0.008	**
Fungal type	1	46.2	<0.001	***
Distance × fungal type	1	8.8	0.004	**
Stem air $\delta^{13}C$ (2009–2011)				
CO_2	1, 8	2.1	0.190	
Day of experiment	1, 334	184.8	<0.001	***
CO_2 × day of experiment	1, 334	22.0	<0.001	***
Soil air $\delta^{13}C$ (2009–2011)				
Stem distance	1, 801	1.7	0.193	
CO_2	1, 8	18.0	0.003	**
Day of experiment	1, 801	0.4	0.510	
Stem distance × CO_2	1, 801	0.8	0.385	
Stem distance × day of experiment	1, 801	35.2	<0.001	***
CO_2 × day of experiment	1, 801	0	0.833	
Stem distance × CO_2 × day of experiment	1, 801	6.9	0.009	**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

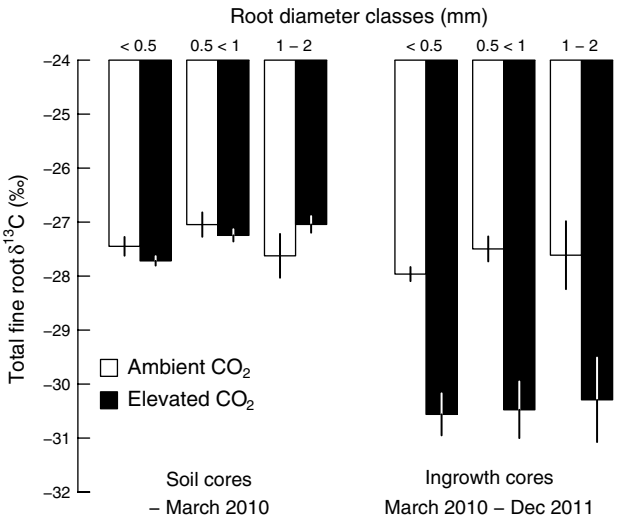

Fig. 4 $\delta^{13}C$ of fine roots under *P. abies* trees exposed to ambient and elevated ^{13}C-depleted CO_2 ($n = 5$ trees, mean ± SE). *Left panel* fine roots were excavated 8 months after the FACE onset on 30 July 2009 (with soil cores). *Right panel* $\delta^{13}C$ of fine roots that were produced exclusively during the FACE experiment from March 2010 to December 2011 (ingrowth core method)

grown exclusively under elevated CO_2 (i.e. without "contamination" of the underlying xylem and reserves from previous years). Over the entire experimental period, branchlet xylem segments collected on control trees showed stable $\delta^{13}C$ signals of −26.1 ± 0.1 ‰, irrespective of the age classes and the sampling date (Fig. 2). In contrast, isotopic signals of branchlet xylem segments formed under elevated CO_2 became consistently more negative over time compared to control tree branchlets ('CO_2 × day of year' interaction: $P < 0.001$; Table 2). This trend was more pronounced, the younger a branchlet segment: when sampled in July 2011, branchlet segments showed $\delta^{13}C$ signals of −28.4 ± 0.4, −30.3 ± 0.7, −31.6 ± 0.7, and −32.7 ± 0.9 ‰ (initiated in 2008, 2009, 2010, and 2011, respectively). These values correspond to $\delta^{13}C$ differences between elevated CO_2 and ambient CO_2 of 2.3, 4.3 5.5, and 6.8 ‰ for branchlet segments initiated in 2008, 2009, 2010, and 2011, respectively. Hence, the isotopic signals of the branchlet segments initiated in 2010 and 2011 were even stronger than would be expected compared to the 100 % new C incorporation estimated by isometer $\delta^{13}C$ signals (44, 81, 103, and 127 % new C relative to the isometer signal).

$\delta^{13}C$ in stem wood

Within just a few months after the start of CO_2 enrichment, newly assimilated C could be detected in latewood

of 2009 (isotopic depletion of 1.3 ‰ compared to control trees; Fig. 3). Signals became more negative over time, leading to a treatment-related difference in $\delta^{13}C$ of 5.5 ‰ in latewood of 2011, which corresponds to full (in fact, 103 % compared to isometers) newly incorporated C only 2.5 years after the onset of FACE. The CO_2 treatment signal was highly significant ($P = 0.004$; Table 2) with a clear 'CO_2 × year' interaction ($P < 0.001$), reflecting the increasing incorporation of new ^{13}C depleted C with time.

$\delta^{13}C$ in tree fine roots

P. abies fine roots from soil cores, collected 8 months after the onset of FACE, did not differ in $\delta^{13}C$ between treatments (not significant), but the $\delta^{13}C$ signals in the three fine root diameter classes were distinct from another ($P = 0.001$; Table 2; Fig. 4).

CO_2 enrichment had a highly significant effect on the C isotope signal in new fine roots that were formed entirely during the FACE period (ingrowth cores over a period of 20 months, installed in March 2010; $P = 0.001$; Table 2; Fig. 4). At 28 months after FACE launch, new fine roots of ingrowth cores were 2.6 ‰ (<0.5 mm), 3.0 ‰ (0.5 < 1 mm), and 2.7 ‰ (1–2 mm) more negative compared to roots sampled from control trees (Fig. 4). These values correspond to 49–56 % new C incorporated into fine roots under elevated CO_2 (relative to isometer signals; Table 2). Further, the $\delta^{13}C$ signals in new fine roots belonging to different diameter classes also differed significantly, irrespective of the treatment ($P = 0.032$).

$\delta^{13}C$ in fungal sporocarps

Altogether, 65 saprobiotic, 51 mycorrhizal, but only 2 parasitic, fungal species were collected during the years 2010 and 2011 in this mixed forest. Only four of the mycorrhizal species are known to be exclusively connected to *P. abies*. Nevertheless, the analysis included all mycorrhizal fungal species. Fungi collected at >18 m distance to the treated trees are assumed not to be influenced by our labeling. Therefore, the isotopic values of these fungi served as a control (Keel et al., in preparation). Mycorrhizal fungi collected closer to the tree base of CO_2 exposed trees showed more negative isotopic signatures compared to fungi collected at greater distance (−27.3 ± 0.5, −26.7 ± 0.3, and −26.0 ± 0.6 ‰ in 2010, and −27.7 ± 0.3, −25.6 ± 0.2, and −25.5 ± 0.3 ‰ in 2011 for the <6, <12, and <18 m distances, respectively; Fig. 5). However, the isotopic differences between the fungi at <6 m distance and the control fungi indicate only 26 % (in 2010) and 43 % (in 2011) new C incorporation. Mean $\delta^{13}C$ in all saprobiotic sporocarps was very similar regardless of the sampling distance from CO_2-enriched trees, suggesting no incorporation of

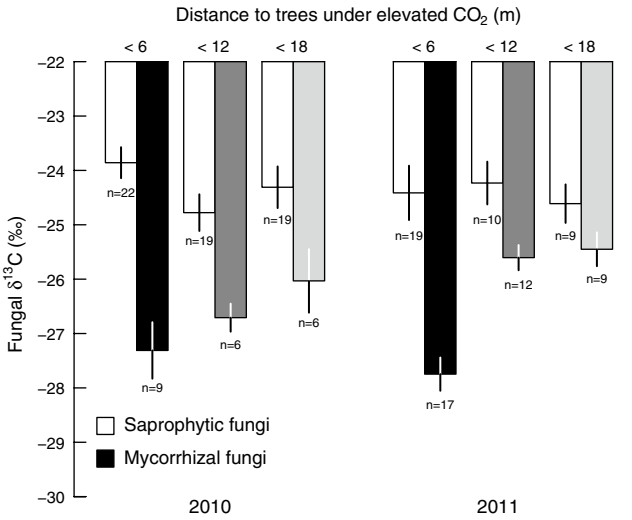

Fig. 5 $\delta^{13}C$ of saprobiotic (*white bars*) and mycorrhizal (*dark bars*) fungal sporocarps (mean ± SE; *n* = number of fungal species) collected in fall of the years 2010 (*left panel*), and 2011 (*right panel*). Sporocarps were sampled at various distances to stems of CO_2-enriched trees, and are grouped into three distance classes

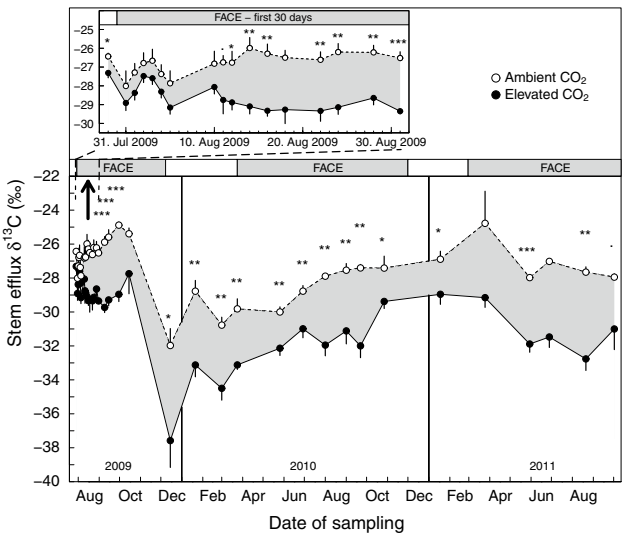

Fig. 6 Seasonal variation of $\delta^{13}C$ in stem CO_2 evolution of *P. abies* trees exposed to ambient and elevated atmospheric CO_2 ($n = 5$ trees, mean ± SE). *Inset* enlarges the first month of the FACE experiment (August 2009). The *gray bars* on *top* of the *panels* denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

new C delivered by the treated trees during that period (−24.4 ± 0.1 ‰ in each year; Table 2; Fig. 5). As a consequence, the isotopic difference between mycorrhizal and saprobiotic fungi increased with proximity to CO_2-enriched trees across all sampled individuals (3.5, 1.9, and 1.7 ‰

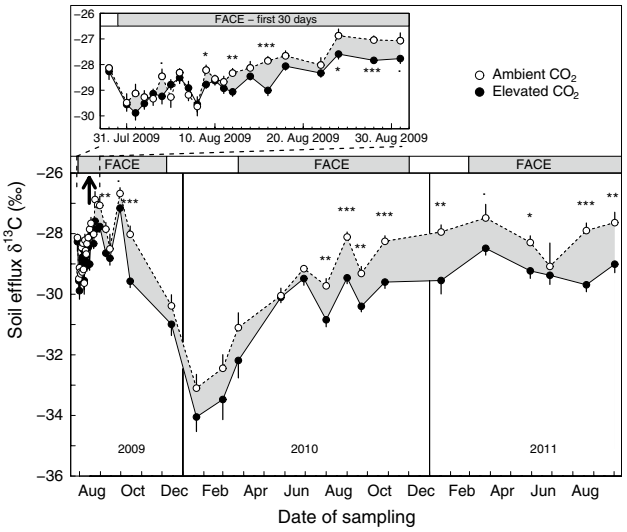

Fig. 7 Seasonal variation of $\delta^{13}C$ in soil CO_2 evolution at 3–11 cm depth under trees exposed to ambient and elevated atmospheric CO_2 ($n = 5$ trees, mean ± SE). *Inset* enlarges the first month of the FACE experiment (August 2009). The *gray bars* on *top* of the *panels* denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

in 2010, and 3.3, 1.4, and 0.8 ‰ in 2011 for the <6, <12, and <18 m distances, respectively). This is supported by a significant 'distance × fungal type' interaction ($P = 0.004$; Table 2).

$\delta^{13}C$ in CO_2 released from stems and soil

Thirteen days after FACE was started, the isotopic signal in stem CO_2 efflux was 2 ‰ lower for trees exposed to elevated CO_2 than in control trees (not corrected for the 0.9 ‰ pre-treatment difference obtained during the 3 days before the onset of FACE). Subsequent measurements (mid-August 2009–October 2011) showed a significantly lower $\delta^{13}C$ value of 3.4 ± 0.2 ‰ (64 % recently fixed C) in CO_2 released from stems of treated trees, with a significant seasonal trend of the isotopic signal ($P < 0.001$; Table 2). A steep drop of the isotopic signal irrespective of the treatment occurred for no obvious reason during winter dormancy 2009/2010 (by 5.9 and 6.6 ‰ for both ambient CO_2 and elevated CO_2, respectively; Fig. 6). Thereafter, stem signals recovered during the course of the 2010 season. In the following winter, no such decline could be observed although weather conditions were similar.

There were clear C-isotope signals in CO_2 released from soils (Fig. 7). First significant deviations between ambient and elevated CO_2 were observed 12 days after the start of the FACE treatment in 2009. The time lap was similar to that in stem air $\delta^{13}C$. However, the mean difference between control and treatment was only 0.6 ‰ by that time (Fig. 7), which accounts for ca. 10 % new C emerging from soil air at this early stage of the experiment. During the entire campaign (starting with the first significant differences 12 days after the onset of FACE), CO_2 enrichment significantly reduced $\delta^{13}C$ in CO_2 released from soil under trees exposed to elevated CO_2 by 1.0 ± 0.1 ‰, corresponding to ca. 15 % new C incorporated ($P = 0.002$; Table 2). During the winter period 2009/2010, a drop in $\delta^{13}C$, very similar to that seen in stem-emitted CO_2, was observed for both ambient and elevated CO_2. This signal even persisted through the early spring season until July 2010. During this period, only minor and non-significant differences in $\delta^{13}C$ between ambient and elevated CO_2 were found. However, from June 2010 onwards, soil air $\delta^{13}C$ under elevated CO_2 was significantly depleted compared to soil air $\delta^{13}C$ under ambient CO_2 (August 2010–October 2011 mean reduction: 1.2 ± 0.1 ‰), which represents 22 % new C. The maximum reduction was 1.8 ‰ in August 2011, which corresponds to ca. 34 % of recently fixed C. Furthermore, we observed stronger ^{13}C signals in soil air collected at 2 m distance to a tree base compared to 3 m distance, further affected by the day of the experiment ($P < 0.001$; Table 2).

Discussion

Canopy-scale CO_2 enrichment has the advantage that the added CO_2 has a low $\delta^{13}C$ value (−30 ‰), which made it possible to quantify the fate of new photoassimilates in 37-m-tall *P. abies* trees in a natural mixed forest ecosystem at a high temporal and spatial resolution. This is the first whole-tree (from the needle to the soil) long-term assessment of the fate of C in tall trees in a natural forest. The intrinsic caveat of using continuous isotope labeling by canopy CO_2 enrichment is that these signals cannot be compared to controls (trees under ambient CO_2 concentrations but exposed to the same isotopic ratio as trees under elevated CO_2). Elevated CO_2 might enhance some transfer processes; hence, the dynamics of C-transfer reported here might be somewhat faster rather than slower, as compared to trees growing under a current atmospheric CO_2 concentration of 395–400 ppm. On the other hand, the current CO_2 concentration is 40 % higher than when these trees were seedlings, and the system (both treated and control trees) may be at or close to CO_2 saturation, suggesting the C-transfer rates observed here may be a reasonable approximation of current 'normal' tree behavior (Leuzinger and Bader 2012). Irrespective of the potential deviations this may incur, our data illustrate the spatial and temporal distribution pattern of freshly assimilated C in such tall trees, and how fast new C is replacing old C.

Our data indicate different rates of integration of new assimilates into the various compartments analyzed. New

foliage and branchlet xylem were built almost exclusively by new photoassimilates when grown in the first spring with FACE. Tree rings took almost 2.5 years until the new tissue consisted entirely of new assimilates. However, latewood of the first season already showed a strong signal a few weeks after the onset of FACE. In contrast, new fine roots seem to utilize more of the old, mobile C pools, and their isotope signal was only half of what we found in the needles after 28 months (ingrowth core data), indicating a slower C turnover than in aboveground tissue. The ^{13}C isotope signal rapidly appears in respiratory CO_2, revealing clear signals from stems and soil within the first 2 weeks of canopy CO_2 enrichment. However, the isotopic signal never reaches the magnitude of that in fresh assimilates (as found in needles, branchlets, and tree rings); hence, it reflects the lasting influence of older stored C sources in these CO_2-releases. In the following, we will discuss in detail the isotope signals observed in the various compartments.

Needles

Sun-exposed needles always showed less negative $\delta^{13}C$ values than shaded needles, which is related to the sun-to-shade difference in the stomatal conductance to photosynthesis ratio (Kaufmann 1982). In shaded plants or plant parts, the ratio of the leaf internal to atmospheric CO_2 concentration (c_i/c_a) is greater than in sun-exposed leaves due to a lower photosynthetic rate and/or higher stomatal conductance (Farquhar et al. 1982). A higher c_i/c_a leads to a higher discrimination of the ^{13}C isotope (i.e. becomes more negative), which is the case with decreasing irradiance and photosynthesis (Livingston et al. 1998).

The initial $\delta^{13}C$ signals in response to FACE in needles produced prior to CO_2 enrichment (i.e. in 2008 and 2009) must reflect the isotopic signature of NSC and other mobile components of needle tissue (Marshall and Linder 2013). In *P. abies*, needles contain ca. 10–27 % of NSC (Hoch et al. 2003; Schädel et al. 2010). This pool was most likely replaced by new C within a few weeks (Keel et al. 2007), corresponding to the 17–23 % of new C found in our bulk sample of old (pre-treatment) grown needles by October 2011, compared to isometer signals. The difference in $\delta^{13}C$ between trees under elevated and ambient CO_2 was clearly bigger in old sun-exposed needles relative to old shaded needles (formed prior to FACE), which is related to higher photosynthetic rates and loading with fresh assimilates (Roberntz and Stockfors 1998), and faster turnover of the non-structural C pools.

In contrast, needles formed during CO_2 enrichment (i.e. in 2010 and 2011) showed much more negative $\delta^{13}C$ values, due to the inclusion of new structural C compounds. Our results indicate that new sun-exposed needles were almost completely self-sustaining (all the new tissue is composed of new C), in line with the branch autonomy theory (Gordon and Larson 1968). In contrast, shaded needles depended more on 'old' C than sun-exposed needles.

All needle age classes exhibited slight seasonal variations with less negative $\delta^{13}C$ values in spring compared to samples analyzed later in the year. We attribute this seasonality in $\delta^{13}C$ to fluctuations in NSC concentration (Flower-Ellis 1993; Hoch et al. 2003; Schädel et al. 2009), resulting in relative shifts of tissue compounds that differ in their isotopic signals. During needle growth and maturation, the concentration in lignin, cutin, waxes, and lipid-related compounds increases. Lignin and lipids show more negative $\delta^{13}C$ signals relative to bulk needles and cellulose (Bowling et al. 2008). Thus, the $\delta^{13}C$ signal of bulk samples should decline even without CO_2 enrichment. Indeed, we found ca. 1 ‰ more negative $\delta^{13}C$ signals in mature, 1-year-old needles, compared to immature needles, which we attribute to the utilization of fresh (early spring) assimilates such as sugars and starch (less negative in $\delta^{13}C$ relative to bulk needles) for primary growth before needles reach C autonomy (Jäggi et al. 2002). A slight age-related decline in photosynthesis (Freeland 1952; Warren 2006) may contribute to that signal.

Branchlet xylem

By the time FACE was initiated on 30 July 2009, earlywood formation in branchlets and stems was already completed; therefore, we assume that only latewood and recently assimilated non-structural C compounds could be responsible for the ^{13}C drop by 0.7–1.3 ‰ in fall 2009. In the following years, $\delta^{13}C$ in branchlets incorporated a stronger signal, since secondary growth adds new xylem layers on top of older-year rings. We assume branchlets to be C autonomous, that is, largely C-supplied by the needles without utilizing stored C from other parts of the tree body (Watson and Casper 1984; Sprugel et al. 1991; Sprugel 2002). Our results suggest that, even in mature trees, primary branchlet formation is mediated almost entirely through new photosynthates produced by new and older needles, with only marginal usage of stored C during the very first stages of branchlet formation (Sprugel 2002; Hoch and Keel 2006).

Tree rings

Tree ring isotope signals indicate a significant but still small contribution (23 %) of novel C to latewood formation in the first treatment season (2009). The gradual $\delta^{13}C$ depletion in tree ring sections of the following years suggests that it takes at least 2 years for novel C to replace all C reserves that feed into xylogenesis. We had not accounted for pre-treatment differences given that we took wood core samples 8 months after the onset of FACE; thus, possible contamination of older-year rings with new assimilates through ray parenchyma cannot

be excluded, but, similar to Marshall and Linder (2013), we see no significant influence of novel C on $\delta^{13}C$ in pre-treatment tree rings. Prior to FACE (i.e. from 2005 until 30 July 2009), there was, however, very little variation in $\delta^{13}C$ signals between early- and latewood given the continuous supply of new photoassimilates due to a nearly permanent photosynthetic season of such evergreen conifers (similar to observations by Jäggi et al. 2002). Accordingly, we found $\delta^{13}C$ of early- and latewood of the same year strongly (1:1) correlated in 2005–2011 ($R^2 = 0.773$; $P < 0.001$).

Fine roots

In control trees, the $\delta^{13}C$ isotope signal of −27.5 ‰ in fine roots marks the midpoint of the range reported by others (Boström et al. 2007; Richter et al. 2009; Andersen et al. 2010), and is 1.1 ‰ less negative than in sunlit needles. This is a general pattern within plants (Hobbie et al. 2002; Badeck et al. 2005), and reflects, for instance, the known downstream post-photosynthetic fractionation processes, resulting in a cumulative enrichment during the assimilate transport from needles to roots and/or plus a change in chemical constituents with less negative $\delta^{13}C$ (e.g., dominance of starch and lignin; Hobbie and Werner 2004; Badeck et al. 2005; Cernusak et al. 2009). The considerable delay in the appearance of new ^{13}C-depleted C in the organic matter of fine roots originating from the added CO_2 suggests that photoassimilates are not directly fed into new root growth. We found no signal in March 2010, although CO_2 enrichment started on 30 July 2009. A rather weak signal compared to other tissues was found at the end of 2011 (2.6–3.0 ‰ difference between elevated and ambient CO_2). This does not contradict the significance of recent photosynthetic activity for root growth, but rather suggests that new assimilates enter a pool of mobile C reserves prior to structural investment in roots (Hansen and Beck 1994; Hobbie et al. 2002). This dilution effect of new C by old C and, therefore, the pool of older photoassimilates can be substantial, as was already concluded for deciduous tree species at the same site (Keel et al. 2007).

The strong incorporation of old C pools (from reserves) in root formation makes it impossible to infer the root age from the fraction of new versus old C in new roots, except with the ^{14}C radiocarbon method. In our case, however, we found that roots could not be older than 20 months (ingrowth cores), but their mixed tissue isotope signal would suggest several years of age (while in reality, some roots may have been only a few weeks old at harvest).

Fungal signals

Ectomycorrhizal basidiocarp formation is believed to be entirely dependent on the provision of new photoassimilates by trees (Högberg et al. 2001, 2010). In the light of this, the C isotope signals of ectomycorrhizal basidiocarps found in the main rooting sphere (<6 m radius) indicate a surprisingly small fraction of only 26–43 % new C provided by our *P. abies* trees in 2010 and 2011, respectively, but it matches the measured ^{13}C content in fine roots. This also holds for fungal species known to be host specific to *P. abies* (in these species, 43–50 % of C is new). Since these fungi may serve several hosts (Brownlee et al. 1983), a signal dilution with C from non-CO_2-enriched trees with overlapping root spheres is likely because our *Picea* trees were neighbored by *Fagus*, *Quercus*, and *Carpinus* trees. This is reflected in the steadily decreasing signal strength with increasing distance to the CO_2-enriched *Picea* trees. Moreover, we assume that the only partial labeling of fungi associated with our treated trees has to do with C-assimilate mixing (new assimilates mixed with old, stored carbohydrates) in the tree before export, possibly isotopically enriched by post-photosynthetic fractionation processes within the plant body, followed by further ^{13}C enrichment during fungal chitin synthesis (sensu Gleixner et al. 1993). As in earlier assessments on this site (Steinmann et al. 2004; Keel et al. 2006), we found a clear distinction between ectomycorrhizal and saprobiotic fungi, with the latter carrying no new C label, hence entirely relying on 'old' C.

Respiratory signals

^{13}C signals in CO_2 released from stems were very similar to those in soil CO_2, both in terms of signal magnitude as well as temporal variation ($R^2 = 0.46$, $P < 0.001$). The close correlation of $\delta^{13}C$ in soil CO_2 and $\delta^{13}C$ in stem efflux may indicate a simultaneous appearance of CO_2 respired from new assimilates in stems and their surrounding soils, despite the different path lengths of assimilate transport. CO_2 diffusion in stem tissues may be slower compared to soil CO_2, caused by stem–internal diffusion barriers (Etzold et al. 2013). Yet it is remarkable that the CO_2 released from metabolic processes appears more or less at the same time, which strongly indicates that the C supply for these processes has a high priority. We assume that the $\delta^{13}C$ contribution of CO_2 transported by sapflow from the rooting zone to the stem surface is small (Ubierna et al. 2009b), and that it would not affect the timing of the signal.

The seasonal trends in isotopic signals differed from year to year, unlike the tri-phase pattern reported for mature *P. abies* by Kuptz et al. (2011b). Since about half of the stem CO_2 efflux results from recent photoassimilates, we assume that the other half is from older storage pools, and that the contribution of these two pools did not significantly change over the study period. We cannot explain the observed $\delta^{13}C$ depression in soil and stem CO_2 efflux in the first but not the second winter, since we can exclude methodological

problems, and moisture or temperature regimes did not differ between the two winters. What makes this phenomenon even more difficult to understand are the results of Maunoury et al. (2007), who found $\delta^{13}C$ values of stem efflux to become more negative with rising temperatures, which is in contrast to the pattern that we observed in our study where values became more negative in winter, despite discontinuation of CO_2 enrichment during the coldest 3 months.

Soil air took 12 days after starting canopy CO_2 enrichment to exhibit a small but clear ^{13}C signal. This confirms a rapid transfer of new C to the rhizosphere. The time lag between C assimilation and soil C release exhibited by our trees is larger than the 5–6 days that were reported for late summer signals in 22-m-high *P. abies* (Ekblad et al. 2005). However, assuming a mean transfer velocity of 0.14 m h^{-1} (Jensen et al. 2012), one would expect 11- to 12-day lags for our 37-m-tall trees, which is consistent with our findings, and what was found by Steinmann et al. (2004) for broad-leaved trees. Our data thus support the significance of path length for C transfer in *P. abies* (Mencuccini and Hölttä 2010). It appears that C release via soil microbiota (including mycorrhiza) and structural growth in roots, both strongly depend on C pools older than 3 years. The rapid signal appearance in soil CO_2 suggests a strong coupling of sinks and sources, but the low fraction of new C in CO_2 release (maximum of 34 % 2 years after FACE onset) highlights the significance of a large, sustained contribution of old C pools to soil CO_2 release (Steinmann et al. 2004; Keel et al. 2006; Kuptz et al. 2011a).

Soil CO_2 in the control plots had more negative $\delta^{13}C$ than roots, a widely observed pattern in C_3 plants (Klumpp et al. 2005; Zhu and Cheng 2011), reflecting additional ^{13}C fractionation (−1.2 ‰) during root metabolism, root decay, and microbial activity (Boström et al. 2007; Werth and Kuzyakov 2010; Brüggemann et al. 2011).

Conclusions

In line with our hypothesis, we conclude that new C is rapidly (within 12 days) allocated to belowground organs and metabolized in respiratory processes. Yet, the further away from the crown (source) the greater the fraction of old C that contributes to new growth and C release (sink). Our work alludes to the branch C-autonomy hypothesis, and warns at expecting a tight isotopic linking between concurrent photosynthesis and tree ring formation (Gessler et al. 2009), and root growth (and, presumably, root metabolism) depending on several years old, stored carbohydrates. A clear distinction needs to be made between the rapidity of the new C signal appearance and the quantitative contribution of new C to sink activity.

Overall, tree growth and metabolic processes in stems and soils reflect a legacy signal of C pools older than 3 years. It does not seem that the delay of novel C investment in stems and belowground sinks such as fine roots reflects a transport problem. We rather suggest that most novel C passes through a large non-structural C pool closely associated with the phloem conduits, rather than being invested directly, irrespective of whether or not trees are C saturated. Such a large turnover of non-structural C pools from phloem to storage and back to phloem must incur a substantial metabolic cost, the tradeoff of which may be a highly buffered C provision system in the case of C limitation under stress. Our data show that the needle C pool is turned over within a few late-season weeks. It has been shown for leaves in the top of a tropical forest in Panama that it takes less than 6 days to completely replace this mobile C pool (Würth et al. 1998). Our analysis underlines the central role of intermediary mobile C pools in tree growth and metabolism. This strategy may be an evolutionary legacy from a predominantly low CO_2 world (only 180 ppm CO_2 at the end of the last glaciation), and may have lost its functional significance under current and future high CO_2 concentrations. We may speculate that the extra C assimilated by trees exposed to elevated CO_2 may cause an enhanced turnover (in terms of mass of C compounds) along the phloem path and associated parenchyma in both xylem and phloem. Distributed across the entire tree, the signal of the associated metabolism (respiration) may be too small to be detected per unit of tissue, but it may be large enough to explain a substantial part of the 'missing carbon' in trees exposed to elevated CO_2 that do not exhibit a growth rate, which is matching their photosynthetic stimulation.

Acknowledgments We greatly appreciate help from M. Saurer and K. Lötscher at the Paul Scherrer Institute for C isotope analyses, E. Amstutz for crane operation and FACE maintenance, Georges Grun for FACE supervision, Olivier Bignucolo and several student helpers for their support in data collection and sample processing (notably Martin Trischler as part of his Master thesis), and Rigobert Keller and members of the Mycological Association Basel for fungal taxonomic classification. This project was funded by the Swiss National Science Foundation (Grants 31003AB-126028 and 31003A_140753, 31-67775.02, 3100-059769.99, 3100-067775.02, and 3100AO-111914/1). The crane was sponsored by the Swiss Federal Office of the Environment (FOEN).

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