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Carbon fluxes to the soil in a mature temperate forest assessed by ^{13}C isotope tracing

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Abstract Photosynthetic carbon uptake and respiratory C release from soil are major components of the global carbon balance. The use of ^{13}C depleted CO_2 ($\delta^{13}C$ = −30‰) in a free air CO_2 enrichment experiment in a mature deciduous forest permitted us to trace the carbon transfer from tree crowns to the rhizosphere of 100–120 years old trees. During the first season of CO_2 enrichment the CO_2 released from soil originated substantially from concurrent assimilation. The small contribution of recent carbon in fine roots suggests a much slower fine root turnover than is often assumed. ^{13}C abundance in soil air correlated best with temperature data taken from 4 to 10 days before air sampling time and is thus rapidly available for root and rhizosphere respiration. The spatial variability of $\delta^{13}C$ in soil air showed relationships to above ground tree types such as conifers versus broad-leaved trees. Considering the complexity and strong overlap of roots from different individuals in a forest, this finding opens an exciting new possibility of associating respiration with different species. What might be seen as signal noise does in fact contain valuable information on the spatial heterogeneity of tree-soil interaction.

Keywords Soil respiration · Carbon turnover · Functional groups · Stable isotopes

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Introduction

Covering nearly one-third of total land surface area, forest ecosystems store more than 80% of the terrestrial biomass carbon and contribute ca. 60% to the global land based net primary production. Flux measurements across a range of (managed) European forests showed that ecosystem respiration amounts up to 80% of gross primary production (Janssens et al. 2001), the rest is in large sequestered into biomass. According to Malhi et al. (1999) soil respiration accounts for 60–70% of total forest ecosystem respiration.

The two major sources of soil respiration are roots living in symbiosis with mycorrhizal fungi (in the following described as autotrophic respiration), and microorganisms with their consumers decomposing dead organic matter (in the following described as heterotrophic respiration). The first is predominantly fed by recent photosynthates, while the latter depends on the rate of decomposition of organic C-compounds in the soil. A wide range of contributions of root respiration to total soil CO_2 evolution (10–90%) is reported in the literature, depending on vegetation type, season, and even on the applied measuring methods (Hanson et al. 2000; Kelting et al. 1997). The large-scale forest girdling study in a boreal pine forest in northern Sweden narrowed the fraction of autotrophic soil respiration to a range of 50–65% (Högberg et al. 2001; Bhupinderpal-Singh et al. 2003), which is in agreement with estimates by Malhi et al. (1999) and Hendrick and Pregitzer (1993).

The age of carbon released by soil respiration is much younger than the age of bulk soil organic matter (Trumbore 2000) and appears to contain a large fraction of recently fixed carbon. Ekblad and Högberg (2001) and Bowling et al. (2002) found a good correlation between the $\delta^{13}C$ signals from respiratory soil CO_2 with relative air humidity due to stomatal response, which occurred 1–4 days before soil air sampling. Based on these results they concluded that recently fixed CO_2 becomes available for root respiration within less than 5 days, which is in good agreement with the girdling experiment, where a 37% decrease in soil respiration was found 5 days after the

phloem was interrupted (Högberg et al. 2001). For a *Pinus taeda* forest (Duke FACE experiment, Andrews et al. 1999) a time lag of 7 days was found until the ^{13}C-depleted CO_2 used for CO_2 enrichment, first appeared in the soil respiration. This experiment also showed that labile carbon pools have a rapid turnover time. After 1 month the CO_2 enrichment was stopped and the tracer signal from ^{13}C depleted CO_2 used for enrichment disappeared from soil-derived CO_2.

Soil respiration varies strongly with season (Hanson et al. 1993) in response to temperature (Widen and Majdi 2001), soil moisture (Pregitzer et al. 2000; Burton et al. 1998) and photosynthetic active radiation (Gärdenäs 2000). All of these factors control the carbon uptake via photosynthesis, tree phenology and hence within tree carbon allocation.

To investigate the dynamics of carbon transfer between tree crowns and the soil we used the isotopic signature produced by ^{13}C-depleted CO_2 applied to the canopy in a free air CO_2 enrichment experiment in Hofstetten near Basel, Switzerland (Pepin and Körner 2002). Since the supplemented CO_2 originated from combustion of fossil fuel it was depleted in ^{13}C ($\delta^{13}C$=−30‰) and thus provided an ideal marker, which could be traced in organic plant material and respiratory CO_2. This offered the unique possibility to address the following questions in a mature, undisturbed forest:

1. How fast is new carbon transferred to the soil compartment, and which fraction of total soil C output is new (carries the new isotope signature)?
2. If there is spatial variability of $\delta^{13}C$ in soil air, can it be explained by the variation in local carbon input via tree roots (identification of tree functional group or tree species effects on CO_2 release)?
3. Is there seasonal variation of new carbon involvement in soil respiration, and if so, what controls this variation?

Materials and methods

Site description

The experimental site is in a mature temperate forest near Basel, Switzerland (47°28′N, 7°30′E, 550 m a.s.l). A canopy crane provides access to the crowns of an 80 to 120-year-old, mixed stand of ca. 30–38 m height and a basal area of ca. 46 m^2 ha^{-1} . The soil is of a mesophilic rendzina type and has a pH of ca. 5.8. The climate is humid temperate with mild winters, moderately warm summers and a precipitation varying between 800 and 1,000 mm $year^{-1}$. The forest is dominated by beech (*Fagus sylvatica* L.) and oak (*Quercus petraea* Mattuschka and *Q. robur* L.), with co-dominant hornbeam (*Carpinus betulus* L.), wild cherry (*Prunus avium* L.), maple (*Acer campestre* L.), and lime (*Tilia platyphyllos* Scop.). Conifers include white fir (*Abies alba* Mill), Norway spruce (*Picea abies* L.), Scots pine (*Pinus sylvestris* L.) and European larch (*Larix decidua* Miller). The understory is rich in *Anemone nemorosa* L., *Mercurialis perennis* L., *Galium odoratum* L., *Arum maculatum* L. and woody species such as *Rubus* sp., *Euonymus europaea* L. and *Lonicera xylosteum* L. together with many tree seedlings, particularly *A. pseudoplatanus* (Pepin and Körner 2002).

Meteorological data

Air temperature was measured at the top of the canopy crane (45 m height) in a time interval of 30 s (shielded temperature sensors). The data were stored as 10 min means using a data logger (DL3000, Delta-T, Cambridge, UK). The soil water content was recorded every hour with TDR-probes at 10 and 20 cm depth (ML2x, Delta-T, Cambridge, UK) outside the CO_2-enriched area and stored with a data logger (DL2e, Delta-T, Cambridge, UK).

CO_2-enrichment system and access to the tree crowns

A new technique suitable for CO_2-enrichment in mature natural forests called “web-Face” was installed in the tree crowns. The web consists of laser punctured irrigation tubes, which are woven in the tree crowns releasing pure CO_2 into the canopy of 14 trees. The CO_2 enrichment was applied not lower than 20 m above ground in the tree canopy (Pepin and Körner 2002). A computer based data acquisition and control system maintains the CO_2 concentration in the canopy at about 530 ppm. CO_2-release started on 1 May 2001. The food-quality CO_2 emitted in the canopy originates from combustion of fossil fuel and is depleted in ^{13}C. Thus the recently fixed CO_2 can be traced in organic plant material and respiratory CO_2. Every new delivery of CO_2 was analyzed for its isotopic composition and the $\delta^{13}C$ remained constant at −30‰ (±1.2‰ SE). A crane was installed in a small gap in March 1999 by helicopter without impacting soils outside the four narrow foundation strips of 0.5×2 m. This crane facility provided access in all three dimensions of the forest canopy within a diameter of 60 m.

Sampling and isotope analysis

Soil air

Soil air was collected with 170 plastic tubes, (12-cm long and 2-cm in diameter, cut from a PVC tube for water supply), which were installed perpendicularly in the topsoil. The top of the tubes was sealed with a rubber septum for sampling the soil air with syringes. From the bottom of the tubes two 8 cm slits were cut to integrate the CO_2 release between 3 and 11 cm soil depth. At the beginning of the experiment the tubes were installed in pre-punched holes prepared with a solid rod of the same diameter (2 cm). During the whole experiment the tubes

were kept installed with the bottom left open for free soil air diffusion. The tubes were spatially distributed in an area of 60 m ×70 m with a grid width of 3 m within the CO_2 enriched zone and 6 m in the surrounding untreated area. Another 18 tubes were placed in a distance of more than 40 m from the experimental area to assure a tracer free reference. Soil air was collected monthly from every soil tube (25 May, 20 June, 3 July, 6 August, 3 September, 1 October and 27 November 2001, the first year of CO_2 enrichment). From every tube 14-ml of air was sampled with a 20 ml syringe equipped with a 6-cm long needle (Discardit 2 syringe and Microlance 3, 23G 1 needle, Becton Dickinson, Fraga, Spain) and injected into a previously evacuated special glass vial (volume of 12 ml, Exetainer gas testing vials, Labco Limited, High Wycombe, UK). The vials were closed with airtight rubber septa. Immediately before sampling, the Exetainers were evacuated to a pressure of 2×10^{-2} hPa in the field with a rotary oil pump (EDM20, Edwards, Knutsford, UK). The overpressure produced by injecting 14 ml of air into 12 ml vials prevented any contamination with ambient air during storage. The gas samples were analyzed within 4 days, using an autosampler (CombiPAL, CTC Analytics, Zwingen, Switzerland). Via the Gasbench II, connected to a mass spectrometer Delta Plus (both Thermo Finnigan Mat, Bremen, Germany) the CO_2 of the gas samples was extracted and the $\delta^{13}C$ was determined.

The CO_2 released by soil respiration leaves the soil by diffusion. During this physical process the lighter $^{12}CO_2$ molecules diffuse faster and therefore more $^{13}CO_2$ molecules remain in the soil gas samples. This type of discrimination, described by Hesterberg and Siegenthaler (1991), and Amundson et al. (1998) systematically biased our measured $\delta^{13}C$ values. Therefore 4.4‰ was subtracted from all measured $\delta^{13}C$ values.

Using a geostatistical interpolation method (point kriging), the soil air $\delta^{13}C$ values, sampled from the irregular spatial lattice, were converted into a grid-file. The contourlines were then calculated from the grid-nodes with equal isotope values. The grid-based data were plotted as a continual surface (Surfer Version 7.00, Golden Software, Golden, Colo.).

The CO_2 concentration of every analyzed gas sample was calculated from the calibration line of standard gas samples with known CO_2 concentrations (0, 2,480 and 4,960 ppm). The area under the voltage signal peak of the mass spectrometer for CO_2 (masses 44, 45, 46) was integrated over time and used as a measure of the CO_2 concentration of the sample.

Leaf material

The $\delta^{13}C$ values of tree foliage were used as a reference value for the composite isotopic signal of source assimilate. We analyzed whole bulk leaf material as well as the water-soluble carbohydrate fraction of leaves. Leaf material was collected three times during summer (1 June, 24 July and 13 August 2001) from six different tree species (three leaves per individual tree pooled, taken from one to nine individual trees per species) from the CO_2-enriched and the non-treated area. To minimize microclimatic effects, only sun-exposed leaves from the south side of each tree crown were harvested.

Besides continuous measurements of the CO_2 concentration with an infrared gas analyzer from 25 locations in the tree canopy, the isotopic composition of canopy air was monitored by growing C_4 grasses in small containers in the canopy (see Pepin and Körner 2002). As long as C_4 plants are neither shaded nor kept in water shortage, their ^{13}C discrimination remains constant (Buchmann et al. 1996). Therefore the monitoring C_4 plants were regularly watered and kept in non-shaded areas. Leaf material from 65 pots with *Cynodon dactylon* (21 of which grown in the CO_2 enriched area) were harvested on 13 June and 23 August 2001. In addition leaf material from 20 understorey plants (*M. perennis*) from both the untreated and the CO_2 enriched area was analyzed for $\delta^{13}C$ (sample date: 23 June 2001). The analysis was carried out to test whether the added, highly ^{13}C-depleted CO_2, affects the isotopic composition near ground and therefore would directly influence the isotopic composition of the soil air. All leaf material was dried at 70°C for 48 h and ground with a steel ball mill (Mixer Mill, Retsch MM 2000, Germany). For the mass spectrometric analysis, between 0.5 and 0.8 mg of dried foliar powder was packed in tin cups. The samples were then combusted in an elemental analyzer (EA-1110, Carlo Erba Thermoquest, Italy). A variable open split interface (Conflo II, Finnigan Mat, Germany) connected the elemental analyzer to the mass spectrometer (Delta S, Thermo Finnigan Mat, Germany) operating in continuous flow mode.

An aliquot of the dried and ground leaf material was used to extract water-soluble compounds of the different tree species. In a first step, 0.2 g powdered leaves were suspended in 10 ml bidistilled water heated to 60°C and then centrifuged (10 min at 9,000 *g*). The eluate was decanted and the same procedure was repeated twice for the residue, but with only 5 ml bidistilled water. All three eluates from one sample were combined and filtered with cellulose acetate filters (Schwarzband-Rundfilter from Schleicher and Schüll, Einbeck, Germany, pore size =0.45 μm). The filtrate was dried in nickel cups at 70°C for 48 h, ground and analyzed for the isotopic composition.

Fine root and ectomycorrhizae

Organic soil samples were taken with a soil corer (7-cm diameter) at 30 locations (15 in the non-CO_2 exposed site, 15 near CO_2-enriched trees) in 10 October 2001. Fine roots (< 2 mm diameter) were picked out by hand from the organic soil samples and washed with deionized water. The dried fine roots (48 h at 60°C) were ground and analyzed for the carbon isotopic composition.

Sporocarps from ectomycorrhizal fungi were sampled in 22 October 2001 (14 and 12 sporocarps in the non-CO_2

exposed area and near CO_2-enriched trees respectively). The fungi were dried for 48 h at 80°C. Material from the cap only (without the stipes) was ground and analyzed for the carbon isotopic composition.

Application of the Keeling plot: conventional and modified

The soil matrix represents a porous system where respired CO_2 is mixed with atmospheric CO_2. Therefore the collected gas samples showed an isotopic signature representing a mixture of both CO_2 sources. To estimate the $\delta^{13}C$ value of pure respiratory CO_2, we applied the Keeling plot (see Keeling 1958), which describes the linear relationship between the reciprocal values of the CO_2 concentrations and the $\delta^{13}C$-values of gas samples. In the usual application of the Keeling plot, a scatter plot ($1/[CO_2]$ vs $\delta^{13}C$) is produced from gas samples, which allows the calculation of the *y*-intercept (where $1/[CO_2]$ is zero) and, thus, $\delta^{13}C$ of the respiratory CO_2 source without quantifying the actual atmospheric mixture (see Fig. 1). This requires the pooling of data of a number of samples. Since we aimed at the determination of the spatial variability of the $\delta^{13}C$ of soil air in the treatment area, we had to know the respiratory isotope signal for each individual gas sampling tube. Knowing the atmospheric CO_2 concentration and its corresponding $\delta^{13}C$ value, we could calculate the $\delta^{13}C$ value of every single soil gas sample. The mathematical description and a sensitivity analysis for the use of the modified Keeling approach are given in the Appendix.

Statistics

For the statistical analysis we defined one tree as a replicate. In order to assign appropriate fractions of ground area to each tree, the experimental area was divided into circular plots centered around tree stems. Since tree size (represented by stem diameter) is highly correlated with horizontal root spreading (correlation coefficient $r>0.9$, see Polomski and Kuhn 1998) we allowed the ground radius (r) of the circles to vary with stem diameter (d) for each tree ($r=f\times0.5$ d, where f was set to 14, which produces an average plot radius of ca. 3 m). We assumed these circular areas to contain the main part of the roots of each considered tree. Therefore the mean of all soil gas samples taken within one of these circles was treated as one replicate assigned to the specific tree (usually one to five gas sampling tubes). Gas sampling tubes, which did not clearly fall into one of the tree circles were disregarded in this analysis. This way, we defined 68 ground circles, 13 of which for the CO_2 enriched area (3 *Fagus*, 4 *Quercus*, 3 *Carpinus* trees plus 1 *Acer*, 1 *Tilia*, 1 *Prunus*). The remaining 55 circles were in the untreated area and were centered on 12 *Fagus*, 12 *Quercus*, 2 *Acer*, 4 *Tilia*, 4 *Carpinus*, 13 *Picea*, 5 *Larix* trees plus 1 *Prunus*, 1 *Abies* and 1 *Pinus*. Since no conifers have grown in the CO_2 enriched area, all tests concerning the difference in $\delta^{13}C$ (and soil air CO_2 concentration) between the enriched and control area, were performed without the soil sample data assigned to conifers. Using an analysis of covariance (Type I sums of squares) we tested the isotopic signal size in the soil air for signals mediated by CO_2 enriched trees. While the CO_2 concentration in the canopy, the tree species and the interaction between the two were used as factors (in this order), $1/[CO_2]$ of the soil gas samples was used as co-variable. In a second analysis, we applied a model for the isotopic composition of the soil gas samples, containing only the CO_2 concentration in the canopy and its interaction with the tree species as factors. The residuals from this model were tested for differences between the tree species using Tukey–Kramer's multiple comparison test. This way we tested a species effect independently of the isotopic tracer treatment. The relationship between $\delta^{13}C$ values of the soil gas (taken only from the untreated area) and climatic factors were examined with a simple regression analysis. Since we expected recently fixed CO_2 from the canopy to show up in root respiration with a certain delay, we correlated the isotopic signals not only with the actual measuring day's climate but also with single day means from 1, 2, 3 up to 15 days before the actual soil air sampling date. The effect of ^{13}C depleted CO_2 in the atmosphere on the isotopic composition of tree foliage and its water-soluble compounds was tested with a two-way ANOVA (Type I sums of squares) where the CO_2 treatment and the tree species as well as their interaction were used as factors. To test whether ^{13}C depleted CO_2 diffuses directly downwards from the canopy towards the ground, leaves of *M. perennis* were tested for a treatment effect by a one-way ANOVA. All statistical analyses were conducted with JMP (Version 4.0.0, SAS, Cary, N.C.).

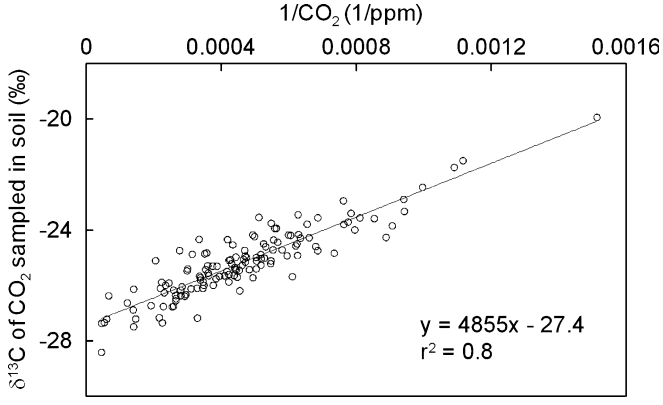

Fig. 1 Keeling plot for data sampled in June 2001 (only data of the non-treated area are shown). The *y-intercept* indicates a $\delta^{13}C$ value of −27.4‰ for the respiratory CO_2 source

Results

Seasonal dynamics

Three weeks after the CO_2-enrichment started, soil air from the CO_2 enriched area showed a slightly more negative $\delta^{13}C$ signal (−28.4±0.3‰ vs −27.9±0.1‰ SE; Fig. 2a). The difference became significant in June (1‰, $P<0.01$) and reached a maximum of 2‰ by the end of the season in October. The C isotopic signature of the soil air showed a seasonal course in both the untreated and the CO_2 enriched area. In the untreated area, the least negative $\delta^{13}C$ value was observed during midsummer, the most negative one by the end of November (a 1.4‰ seasonal amplitude in both, the untreated area and in the CO_2-enriched area). The overall decline of $\delta^{13}C$ in the second half of the season was paralleled in the CO_2-enriched area.

Concentration of CO_2 in the soil showed an early summer peak, with little variation in both "treatments" thereafter (2,000–3,000 ppm; Fig. 2b). The CO_2 concentration was always higher in the CO_2-enriched area compared to the untreated area suggesting higher CO_2 release. However, this difference was statistically significant only in June ($P=0.03$) when concentrations in the CO_2-treated area (and presumably respiratory rates) had reached twice the value that was measured in the control area. Analysis of leaves of the understorey plant *M. perennis* indicates that an artefact caused by direct diffusion of enriched CO_2 from the canopy downwards to the floor can be excluded. Both leaves from the control and the enriched area showed a $\delta^{13}C$ value of −33‰ ($SE_{control}=0.16$, $SE_{elevated}=0.18$). Hence no depleted CO_2 from the enrichment experiment was present in the air 0.5 m above the soil. We recall that CO_2 enrichment was applied not lower than 20 m above ground in the tree canopy.

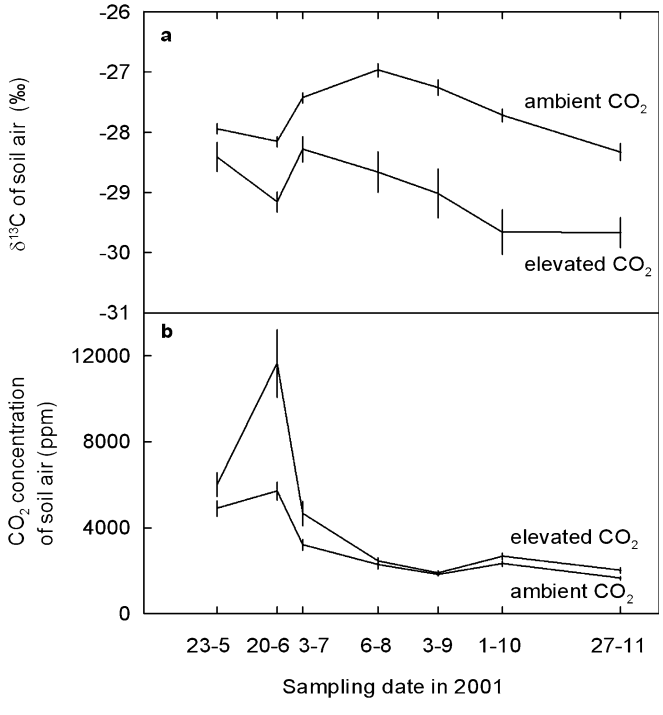

Fig. 2 **a** Seasonal course of soil respiratory $\delta^{13}C$ (calculated with Keeling plot). The difference between $\delta^{13}C_{elevated}$ and $\delta^{13}C_{ambient}$ is significant for all sampling dates except in May. *Error bars* indicate SE. **b** Seasonal course of the CO_2 concentration in soil air. During the whole growing period soil air sampled near CO_2 enriched trees showed a higher CO_2 concentration. Yet the difference between the concentrations of $CO_{2\ elevated}$ and $CO_{2\ ambient}$ was only significant in June (*Error bars* indicate SE).

Correlation with climatic data

The higher the temperature prior to gas sampling the less negative was $\delta^{13}C$. Tested for the control area, the best positive correlation was obtained for daily mean air temperatures of day 4–10 before sampling (see Table 1). As soil moisture increased, $\delta^{13}C$ became more negative. In this case the best negative correlation was found for means from 0–4 days before sampling (highly significant for day 4). The vapor pressure deficit (VPD) is known as a key variable influencing leaf CO_2 exchange. In fact, $\delta^{13}C$ increased with increasing VPD. The positive correlation was significant for the VPD measured 10–11 days before sampling. Because of greater signal noise, trends (which were similar) were not significant in the high CO_2 area.

Table 1 Correlation coefficients for the regression between $\delta^{13}C$ values of soil respiratory CO_2 and meteorological parameters [VPD, mean air temperature, and volumetric soil moisture (TDR1 and TDR2) at 10 and 20 cm depth]. The highest correlations were found for mean air temperature measured 4–10 days before soil air sampling. Bold italic numbers indicate a significant correlation ($\alpha<0.05$). The bold italic and italic numbers between day 4 and 5 indicate a soil process controlled CO_2 release driven by temperature and soil water content whereas the area at days 10 and 11 indicates CO_2 release from the soil as a result from tree physiological processes, controlled by VPD and temperature

Days before sampling	Ambient VPD	Mean ambient temperature	Mean ambient TDR 1	Mean ambient TDR 2
0	0.00	0.80	−0.93	−0.91
1	0.71	0.95	−0.94	−0.91
2	0.07	0.91	−0.94	−0.93
3	−0.03	0.94	−0.93	−0.93
4	0.68	***0.97***	*−0.94*	***−0.96***
5	0.46	***1.00***	*−0.90*	*−0.87*
6	0.32	***1.00***	−0.85	−0.85
7	0.54	***0.96***	−0.84	−0.81
8	0.63	0.83	−0.91	−0.76
9	0.70	0.83	−0.91	−0.76
10	***0.76***	***0.98***	−0.76	−0.76
11	***0.75***	*0.89*	−0.24	−0.32
12	0.56	0.95	−0.17	−0.26
13	0.42	0.81	0.14	−0.06
14	0.69	0.95	0.22	0.04
15	0.49	0.80	0.26	0.25

Spatial patterns

The spatial variability in the carbon isotope ratio of the soil air was very high (variance of 4.7 and 2‰ in untreated and enriched area respectively). The higher variation in the much larger untreated area had to do with the presence of conifers (see below, no conifers in the treated part of the forest). Despite this variability, the more negative tracer signal in soil air from the CO_2 enrichment area was clearly detectable (Fig. 3).

A map of the soil air $\delta^{13}C$ data ($\delta^{13}C$ values not adjusted by the Keeling plot procedure, Fig. 3, left column) illustrates an obvious isotopic fingerprint in the CO_2 enriched area during the first part of the growing period (May–July). During the dry period in August and September the tracer signal was rather small but resumed with October rains. For these data, the more negative $\delta^{13}C$-values in the CO_2-enriched area not only resulted from ^{13}C depleted assimilates but also reflect the higher soil CO_2-concentration (indicating a higher respiratory

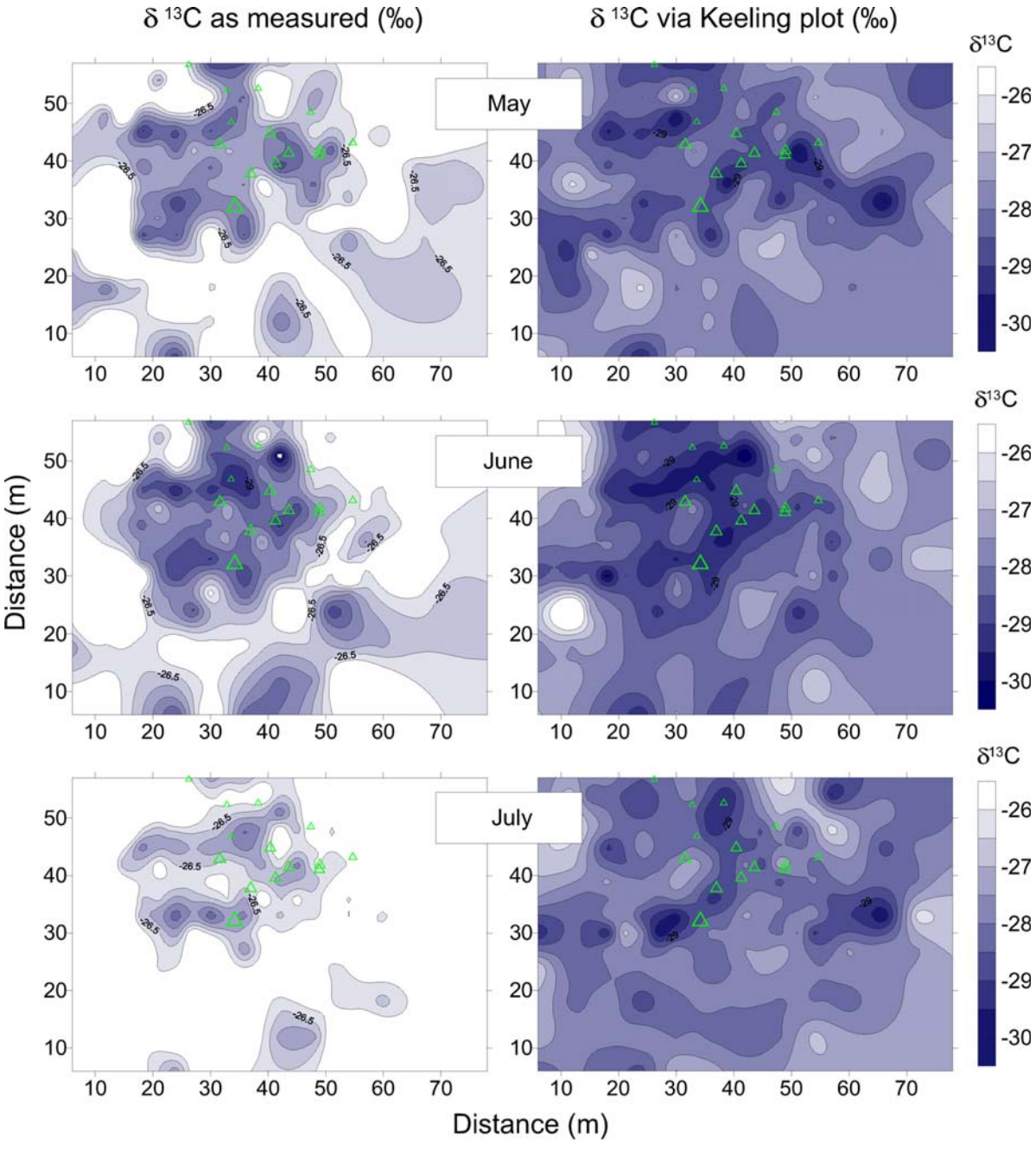

Fig. 3 Spatial and temporal variation of the $\delta^{13}C$ values in soil air. *Triangles* show the position of the CO_2 enriched trees. The size of the triangles varies in proportion with the tree stem diameter. *Left column* directly measured $\delta^{13}C$ of soil air. *Right column* $\delta^{13}C$ values calculated with the modified Keeling plot. In June and towards the end of the growing season (October and November) the soil air near the CO_2 enriched trees was clearly depleted in ^{13}C

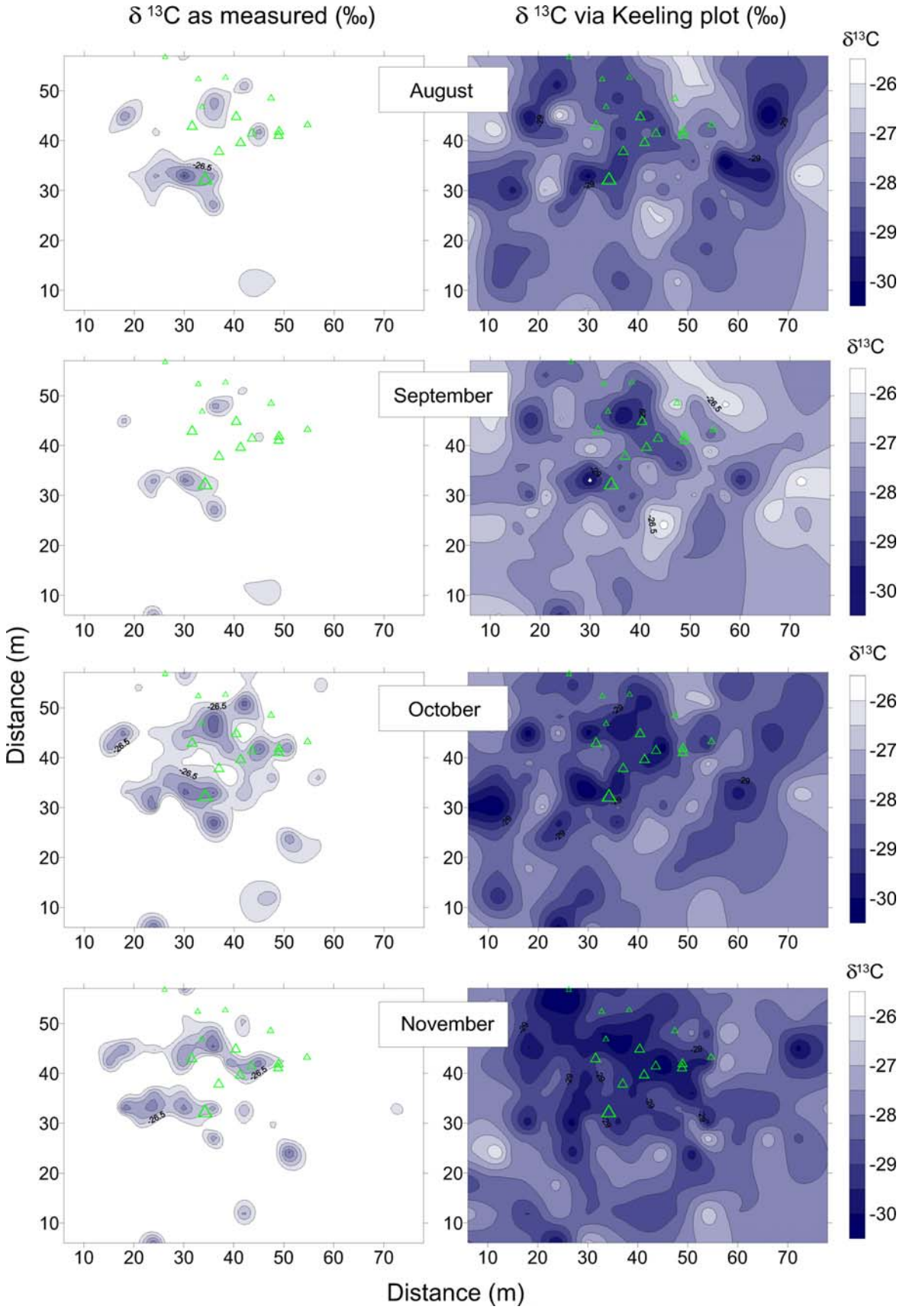

Fig. 3 (continued)

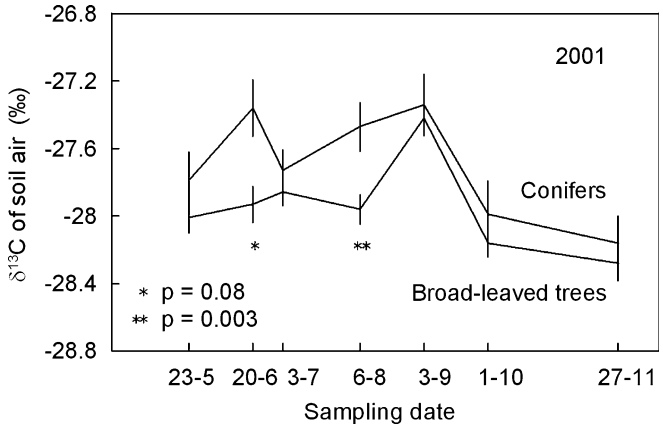

Fig. 4 Seasonal course of the $\delta^{13}C$ values in soil air sampled near conifers and broad-leaved trees. During the whole growing season the CO_2 of soil air near conifers was less depleted in ^{13}C. Only data of the untreated area were used. *Error bars* indicate SE

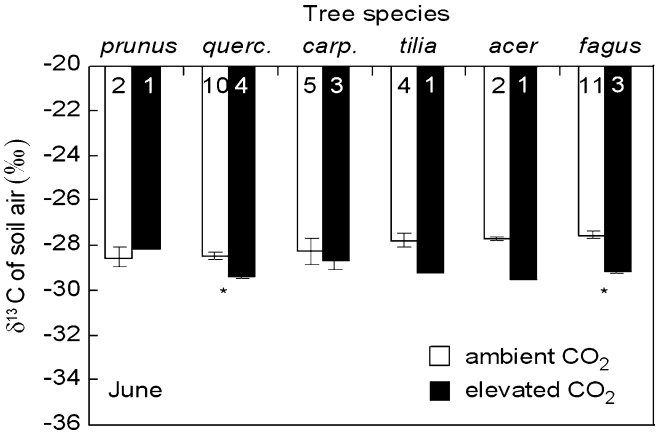

Fig. 5 The $\delta^{13}C$ values of soil air near different tree species. *Carpinus; Quercus*. *Error bars* indicate SE. *Quercus* and *Fagus* (marked with *) differ significantly in their $\delta^{13}C$ values ($\alpha<0.05$), according to Tukey-Kramer's multiple comparison of the residuals of the $\delta^{13}C$ values tested for the CO_2 enrichment effect and its interaction with the tree species. The $\delta^{13}C$ of soil air from the CO_2 enriched area is significantly lower compared to that of the untreated area ($P<0.01$)

activity) compared to the untreated area (Fig. 2b). This twofold influence explains why the δ^{13} C-signal derived from the intercept of the Keeling plot was less pronounced than in the directly measured values (Fig. 3, right column).

In May, the area with a C-isotope signature lower than −29‰ was fragmented over the whole test area (no treatment effect). In June this area was roughly doubled in size, and showed a clear concentration around the CO_2 enriched trees. During the following three relatively dry months (July–September), the treatment signal largely disappeared but recovered in October and November.

To some extent the spatial variability in $\delta^{13}C$ of soil air corresponds with the different tree species, which seemed to influence the $\delta^{13}C$ of soil air in their root area as defined here. Soil air samples collected in the core root zone of broad-leaved trees generally showed more negative $\delta^{13}C$ values compared to soil air samples collected near conifers (Fig. 4). The conifer effect was significant in June and August. However, a significant species effect (including differences among broad-leafed species) on $\delta^{13}C$ of respiratory CO_2 was found in June ($P<0.035$) and July ($P<0.0001$) and was mainly driven by the soil signals around *Quercus* and *Fagus*, the two species which exhibited the most negative and most positive soil air $\delta^{13}C$ respectively (Fig. 5). During the following two (dry) months (August and September) the species effect was smaller but recovered in October, with a lower significance of the species effect ($P<0.078$). A strong tracer signal due to CO_2 enrichment could be found in the soil air near *Fagus* and an even more pronounced one near *Tilia* and *Acer* (but no signal near *Prunus*).

Carbon isotope ratio in foliage

As expected, the bulk leaf material of CO_2 enriched trees showed more negative $\delta^{13}C$ values compared to foliage of untreated trees already in this first season of CO_2 exposure (Fig. 6). Overall the mean $\delta^{13}C$ of all species varied between −27.5 and −26.2‰ (mean SE ±0.2‰) in the untreated group and between −29.7 and −29.3‰ (mean SE ±0.5‰) in the CO_2 enriched group. The average difference between the two groups was surprisingly small (about 2.5‰±0.54‰ SE). *Tilia* however, clearly differed from the common pattern with a difference of about 8‰ between the foliage of the untreated and the CO_2 enriched group. The above-mentioned patterns explain why the treatment × species interaction was significant ($P<0.0001$). Different species seem to have integrated the ^{13}C depleted CO_2 to a differing degree.

Independent of the treatment, there was a species effect on $\delta^{13}C$ in bulk tissue as well as in water-soluble carbon compounds. The monthly mean $\delta^{13}C$ of soluble compounds of all species varied between −27.5 and −25.8‰ (mean SE ±0.3‰) in the untreated group and between −29.4‰ and −29.0‰ (mean SE ±0.6‰) in the CO_2 enriched group (2.42‰ more negative). The positive linear correlation between the $\delta^{13}C$ of bulk leaf material and water soluble carbon compounds was highly significant ($r^2_{ambient}=0.63$, $r^2_{elevated}=0.95$, $P<0.001$ for both treatments), indicating that the relative differences among the two different fractions remained unaffected.

The different ranking of soil and leaf $\delta^{13}C$ (Figs. 5, 6) from different species indicates, that bulk leaf $\delta^{13}C$ (and water-soluble $\delta^{13}C$, data not shown) translates to different degrees into soil air $\delta^{13}C$.

The fate of carbon in the forest

The $\delta^{13}C$ signal in leaves, but also in roots and mycorrhizae, and consequently, in soil CO_2, reflected the spatial investment of recently assimilated carbon. The C_4 plants, which built all their biomass while exposed in the tree crowns, allowed the long term monitoring of the ^{13}C values in the air of the canopy. During the summer the

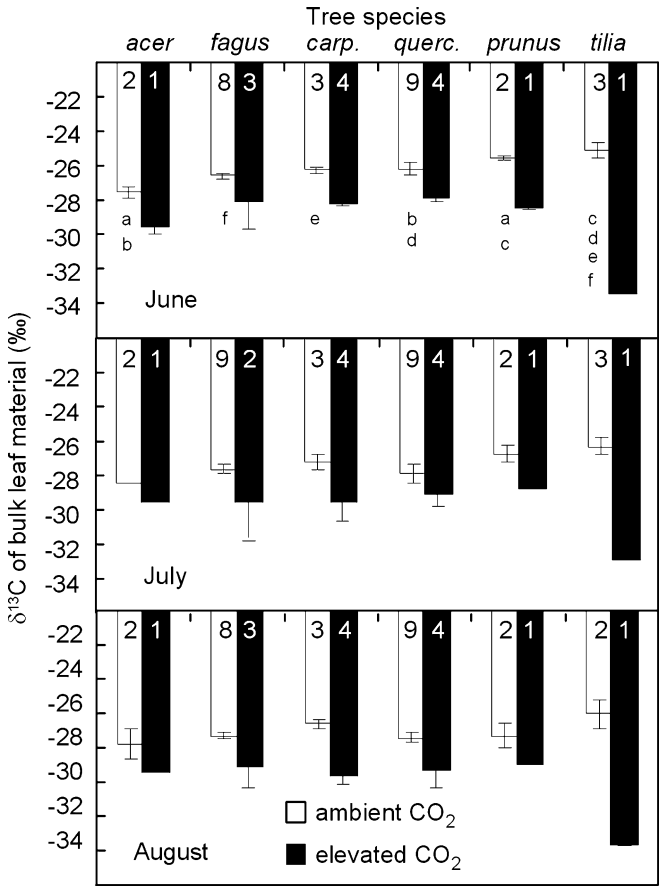

Fig. 6 ^{13}C signatures of bulk leaf material for different tree species. *Error bars* indicate SE. *Numbers* in the bars indicate number of individual trees per species and treatment (whereas three leaves per individual tree were pooled). Species labeled with the same letter significantly differ in their $\delta^{13}C$ values ($\alpha<0.05$, according to Tukey–Kramer's multiple comparison of the residuals of the $\delta^{13}C$ values tested for the CO_2 enrichment effect and its interaction with the tree species)

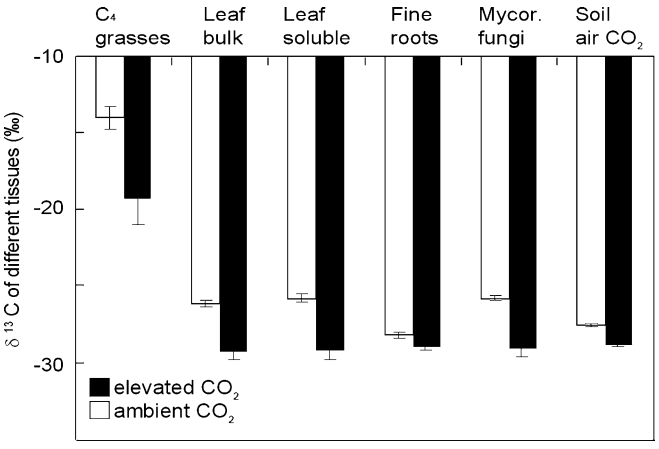

Fig. 7 Comparison of the difference in $\delta^{13}C$ values between different tissues and soil air from the CO_2 enriched area and the untreated area. C_4 grass (*C. dactylon*), *Leaf soluble* water soluble fraction. *Error bars* indicate SE

difference in leaf $\delta^{13}C$ of C_4 plants grown in the CO_2 exposed and the non CO_2 exposed site was constant at 5.3‰ (±1.84‰ SE in June and ±2.32‰ in August, see Fig. 7). This difference suggests a $\delta^{13}C$ of ca. −13.3‰ (−8–5.3‰) in the mixed atmosphere of the CO_2 enriched area. A $\delta^{13}C$ value of ca. −14.8‰ is expected from the mass balance for $\delta^{13}C$ in the enriched area, where atmospheric CO_2 (365 ppm, −8‰) mixes with the added CO_2 (+165 ppm, −30‰). Thus the $\delta^{13}C$ data from the C_4 plants proved to be useful reference values.

The difference in $\delta^{13}C$ between the leaf material of trees from the CO_2 enriched area and the untreated area (sampled in June) was reduced to only 3.1‰ (±0.58‰ SE) in bulk leaf material and 3.36‰ (±0.69‰ SE) in water soluble carbon compounds, which implies a dilution compared to the C_4 grass by a factor of 1.7 (5.3/3.1=1.7). The leaves of trees therefore consisted of about 60% of recently fixed CO_2 and 40% of "old" carbon (carbohydrates stored during the pre-treatment period). With a treated-untreated difference of 0.77‰ (±0.25‰ SE) in the roots, the tracer signal was further diluted by a factor of 4.4 compared to bulk leaf dry matter (3.36/0.77=4.4). Thus ca. 25% of the root carbon came from the recent leaf carbon pool, while the residual 75% of root carbon represent "old" carbon compounds. In contrast sporocarps formed by ectomycorrhizal fungi showed a difference in $\delta^{13}C$ between the CO_2 enriched area and the untreated area similar to that in soluble leaf carbon compounds and averaged at 3.3‰ (±0.49‰ SE; see Fig. 7). This indicates that ectomycorrhizae are fed to nearly 100% by the actual leaf carbon. With an average difference of 1.3‰ (±0.16‰) the tracer signal in the soil air was diluted by a factor of 2.6 (=3.36/1.3) compared to mobile carbon compounds in leaves. Thus, ca. 40% of the CO_2 respired from the soil (a mix of root and microbial respiration) originated from the recent leaf carbon pool.

Discussion

This whole forest isotope labeling study documented a rapid appearance of new carbon substrates for below-ground respiratory metabolism in a mature forest. Tree species identity played an important role. The tracer signal in the soil air translated into a tracer map mirrored the CO_2 enriched canopy area during periods of great tree activity such as in early summer (June/July). The rapidity by which the isotope signal appeared in both, plant tissue (leaf material, roots) and in the soil air confirms earlier estimates by Gärdenäs (2000), Ekblad and Högberg (2001) and Bowling et al. (2002). In the following, we will discuss these results with respect to carbon flows in general, not accounting for potential CO_2 enrichment effects as such, for which a longer treatment period would be required. The higher CO_2 concentration found in the soil air near CO_2 enriched trees does, however, indicate that the carbon input to the soil and hence into soil respiration was increased, particularly in the second month of the treatment. The early spring peak in Fig. 2b almost looks like a burst of excess carbon, soon brought under

control by various possible regulatory processes. But a small positive signal was retained during the rest of the season, in line with common observations of more rapid carbon turnover in model ecosystems exposed to elevated CO_2 (Norby et al. 1992; Körner and Arnone 1992; Hättenschwiler and Körner 1997 and in the FACE study by Andrews et al. 1999).

Carbon investment and turnover

The fast carbon transfer from the tree crowns into the rhizosphere of this mature forest as well as the high proportion of recently fixed carbon in the root- and rhizosphere respiration suggest that the metabolic below ground processes are strongly linked to carbon uptake as has been suggested by Robinson and Scrimgeour (1995) and Raich and Schlesinger (1992). The still surprisingly weak ^{13}C signal in fine roots compared to $\delta^{13}C$ values in respiratory CO_2 and in sporocarps of ectomycorrhizal fungi indicates that roots, once they are formed, mainly serve as transport tissue for carbohydrates from the host trees to their associated ectomycorrhizae. Ectomycorrhizae are known to cause trees to shift the C-allocation into C-pools with a rapid turnover like in hyphae and into root as well as fungal respiration (Rygiewicz and Andersen 1994). Hyphal respiration can contribute 30% of total soil respiration. Högberg et al. (2002) suggested that 75% of the carbon allocated to the roots is used for metabolic activity, leaving only little for renewal of structural tissues. Thus it is no surprise that the tracer signal is more pronounced in CO_2 of soil air than in root tissue. Decomposition of 'old' soil organic carbon may further dilute the signal. Assuming that the tracer signal in fine roots originated from non-structural carbon (NSC) only, a conservative estimation would suggest that ca. 10% of total fine root carbon content is present as NSC (5–20% reported by Pregitzer et al. 2000).

Little "new" carbon seems to have been invested into new root growth during this season, suggesting a considerable mean residence time of fine root biomass as was suggested in earlier studies (Matamala et al. 2003; Gaudinski et al. 2000, 2001). Thus the observed increase in soil respiration under elevated CO_2 cannot be attributed to significantly enhanced fine root growth. The common flush of root growth during leaf expansion in spring (McClaugherty et al. 1982; Fahey and Hughes 1994; Hendrick and Pregitzer 1993) appears to have been nourished predominantly by old, stored carbon compounds.

Climate signals: soil moisture, air temperature, and vapor pressure deficit

The high correlations between $\delta^{13}C$ of soil CO_2 and soil moisture, air temperature, and VPD of few days before soil air sampling suggests that much of the carbon released in the ground is of very recent origin. The seasonal course of both, CO_2 concentration and isotope signal in the untreated area illustrates a combined effect of plant and microbial activity. In the untreated area, topsoil desiccation during dry periods (July–September) strongly reduced the soil CO_2 release and led to less negative $\delta^{13}C$ values. Less negative $\delta^{13}C$ of soil air under dry conditions can result from three effects: (1) a reduced discrimination of ^{13}C in leaves during photosynthesis (reduced stomatal conductance, see Farquhar et al. 1989; Panek 1996); (2) an increase of the ratio of autotrophic (less ^{13}C depleted CO_2, see above) to heterotrophic (micro-organism) respiration, because most microbial activity occurs in the top soil layer, which dries out first; and (3) an increased contribution of respired CO_2 from deeper and wetter soil layers, which are known to carry a less negative $\delta^{13}C$ signal (Hesterberg and Siegenthaler 1991; Amundson et al. 1998). Since high air (and leaf) temperature, high VPD and low soil moisture are mostly concurrent, the degree to which the isotopic ratio in soil CO_2 is impacted, by either plant or microbial activity remains uncertain (see Davidson et al. 1998). The highly significant correlation with VPD data measured 10–11 days before soil air sampling, suggests a minimal carbon residence time of less than 2 weeks. In other studies, where recently fixed CO_2 appeared in the soil respiration within less than 1 week, the trees were much smaller or younger than the trees studied here (see e.g. Ekblad and Högberg 2001).

The correlation coefficients in Table 1 also suggest a time-lag between soil processes (highest correlation between $\delta^{13}C$, ambient temperature and soil water content between day 4 and 5) and tree based processes, which seem to respond later (highest correlation between $\delta^{13}C$, VPD and ambient temperature at day 10 and 11). This indicates that soil respiration and crown-soil carbon transfer processes are not necessarily temporally synchronized. As shown above an increase in $\delta^{13}C$ indicates a reduction in soil CO_2 release, which occurs along with a rising ambient temperature and decreasing soil water content in the topsoil, where the highest microorganism activity takes place. The soil at our study site shows a rather high susceptibility to drought, thus the rapid response of the soil to changes in meteorological parameters. The relatively slow response of $\delta^{13}C$ to changes in VPD and temperature in the tree crown could be a result of a relatively slow phloem transport in these old and tall trees (80–120 years).

The "private sphere" of trees

Our results have shown clearly, that the isotopic signature in the soil air does not only reflect climatic conditions but also reflects the proximity of a certain tree species. In the untreated area functional groups, such as conifers and broad-leaved trees could be identified from $\delta^{13}C$ in soil air, with respiratory CO_2 near conifers always showing less negative $\delta^{13}C$ (mirroring the well-known difference in the biomass $\delta^{13}C$, see Garten and Taylor 1992). The analysis of leaf material of the same trees in 1999 showed

indeed, that leaf $\delta^{13}C$ in broad-leaved trees such as *A. campestre* and *F. sylvatica* was significantly more negative than that in *P. abies* and *Pinus sylvestris* (Chevillat and Siegwolf, unpublished data). Gas exchange data support this finding since conifers show a lower c_i/c_a ratio than broad-leaved species. Yet, it is surprising, that the tree specific isotope signature is strong enough to translate into a soil air signal not confounded by soil organisms, which use a wide range of carbon sources with different $\delta^{13}C$ signatures.

Conclusions

The carbon transfer from tall tree crowns to the root zone occurs within a few days and is very substantial. Almost half of the respired CO_2 emerging from the ground comes from the leaf carbon pool. The leaf ^{13}C signal remains sustained in the soil respiratory CO_2 and even among deciduous species there seems to be a species specific separation. The soil isotope signals obtained could be explained by the combined influence of seasonal changes of climate, leaf isotope discrimination and species composition. As mature and stable this old growth forest may appear, the vigor of the crown to root carbon flux as documented here draws a highly dynamic picture. The crown to root link is very intense and leads us to expect substantial alterations when crowns experience long-term CO_2 enrichment. The study also underpins the significance of a natural tree-rhizosphere coupling should CO_2 enrichment experiments reveal realistic response trends for a CO_2-rich future.

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Appendix

The regression line of a Keeling plot can be derived from a simple mass balance equation, where the isotopic ^{13}C signature of atmospheric CO_2 mixes with the ^{13}C signature of soil respiratory CO_2 (see Eqs. 1, 2):

$$\delta^{13}C_{mixed}[CO_2]_{mixed} = \delta^{13}C_{atmosphere}[CO_2]_{atmosphere} + \delta^{13}C_{respired}\,[CO_2]_{respired} \quad (1)$$

$$[CO_2]_{mixed} = [CO_2]_{atmosphere} + [CO_2]_{respired} \quad (2)$$

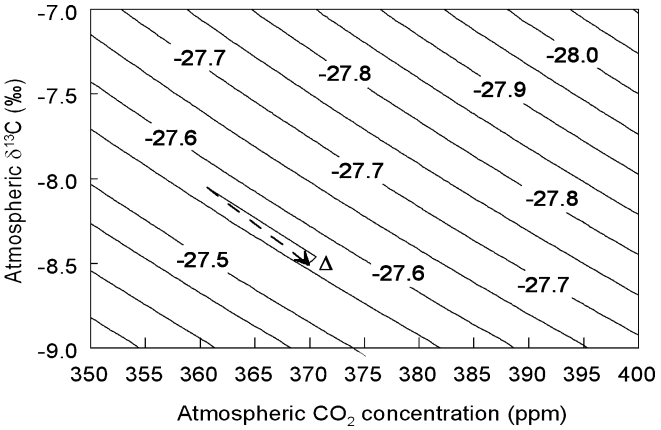

Fig. 8 $\delta^{13}C$ of pure respiratory CO_2 for varying atmospheric CO_2 concentration and atmospheric $\delta^{13}C$. For the simulation typical soil air CO_2 concentration (2,300 ppm) and $\delta^{13}C$ (−20.8‰) were used. A 10 ppm increase in atmospheric CO_2, (roughly related to a decrease in atmospheric $\delta^{13}C$ of 0.5‰, indicated by the *arrow*), results in a potential error of less than 0.1‰ (*open triangle*) in calculated soil respiratory CO_2. See Appendix for details

where $\delta^{13}C_{mixed}$ is the $\delta^{13}C$ of the analyzed soil gas sample, $[CO_2]_{mixed}$ is the CO_2-concentration of the analyzed soil gas sample, $\delta^{13}C_{atmosphere}$ is the atmospheric $\delta^{13}C$, $[CO_2]_{atmosphere}$ is above the canopy the atmospheric CO_2-concentration, $\delta^{13}C_{respired}$ is the $\delta^{13}C$ of respiratory CO_2, and $[CO_2]_{respired}$ is the CO_2-concentration of respiratory CO_2 in the soil.

Except for $\delta^{13}C_{respired}$ and $[CO_2]_{respired}$ all parameters can be measured. Thus the equations can be solved for $\delta^{13}C_{respired}$. The equation solved for $\delta^{13}C_{respired}$ corresponds to an individual Keeling plot based on two points (one point defined by atmospheric CO_2 concentration and its $\delta^{13}C$, the other by the CO_2 concentration of the soil air sample and its measured $\delta^{13}C$). The difference to the classic Keeling approach is that $\delta^{13}C_{atmosphere}$ and $[CO_2]_{atmosphere}$ are assumed to be known.

Air samples collected 5–10 m above the canopy, showed highly variable values in both atmospheric CO_2 concentrations and $\delta^{13}C$. It is likely that this variation was influenced by the vegetation and the CO_2 enrichment experiment. Therefore we extrapolated the CO_2 concentrations and $\delta^{13}C$ values from different monitoring stations (Ireland, Mace Head, Count Galway 53°20′N 9°54′E 25 m a.s.l.; Azores, Terceira Island 38°46′N 27°23′E, 30 m a.s.l; Malta, Gozo 36°03′N 14°11′E 475 m a.s.l; Tenerife, Izana Observatory 28°18′N 2°00′E 7 m a.s.l) to the year 2001. Since both atmospheric CO_2 and its appropriate $\delta^{13}C$ vary seasonally due to a seasonal change in respiratory and photosynthetic activity of the vegetation, we used monthly data of the respective parameters. We assumed that the average of these data taken from different northern latitudes would give an approximate description of the free atmospheric CO_2 around our experimental site and thus could be used for both, calculation of soil respiratory $\delta^{13}C$ in the treated and control area.

Sensitivity analysis of the Keeling approach

Since our new approach of using the Keeling plot is not yet established, we tested its robustness by calculating the C isotopic signature for pure respiratory soil CO_2 for every combination of atmospheric conditions in CO_2 concentration (between 350 and 400 ppm in 1 ppm steps) and $\delta^{13}C$ (between −9 and −7‰ in 0.1‰ steps). In general the sensitivity of the calculated soil respiratory $\delta^{13}C$ towards changing atmospheric conditions decreases with increasing soil air CO_2 concentrations. To be on the safe side, we performed a sensitivity analysis for a soil air sample with a CO_2 concentration of 2,300 ppm (δ^{13} C=−20.8‰), as was found in October and November, the 2 months with lowest soil air CO_2 concentrations. As shown in Fig. 8 the calculated isotopic value for pure respiratory CO_2 changes less than 0.015 ‰ when atmospheric CO_2 concentration shifts from 360 to 370 ppm (a shift which is related to ca. 0.5‰ in atmospheric $\delta^{13}C$). Even when the atmospheric $\delta^{13}C$ is not adjusted to a change in atmospheric CO_2 concentration, a shift of 10 ppm in atmospheric CO_2 is followed by a potential error of less than 0.08‰ in the calculated respiratory $\delta^{13}C$. Therefore in the range of 360 ppm a change in atmospheric CO_2 concentration of 10 ppm does not significantly affect the calculated $\delta^{13}C$ value of respired CO_2. The same modeling applied to a soil air sample with a CO_2 concentration of 1,000 ppm ($\delta^{13}C$=−18.4‰) revealed a shift of less than 0.1 ‰ when the atmospheric CO_2 concentration changed from 360 to 370 ppm. The maximal potential error was 0.2 ‰ when the related shift of 0.5‰ in atmospheric $\delta^{13}C$ was not considered. Less than 3% of all soil air samples had a CO_2 concentration lower than 1,000 ppm. In general the soil samples showed a high enough CO_2 concentration so that the potential error of the calculated respiratory $\delta^{13}C$ was below the measuring precision. Thus we conclude that our method of calculating the $\delta^{13}C$ values of pure respiratory CO_2 of every single soil air sample is sufficiently accurate and can be used to visualize the spatial variability of the biological $\delta^{13}C$ input into the ground.

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