The fate of recently assimilated carbon in mature deciduous forest trees

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Table of contents

Chapter 1	General introduction	7
Chapter 2	Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest	11
Chapter 3	Strict separation of carbon transfer to mycorrhizal and saprophytic fungi by tall temperate forest trees *Co-authorship in Siegwolf et al., in revision*	23
Chapter 4	Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2 *Co-authorship in Körner et al. (2005)*	33
Chapter 5	Tracing arthropod movement in a deciduous forest canopy using stable isotopes *Co-authorship in Asshoff et al. (2006)*	37
Chapter 6	Rapid mixing between old and new carbon pools in the canopy of mature forest trees	47
Chapter 7	^{13}C-labelling reveals different contributions of photo-assimilates from infructescences for fruiting in two temperate forest tree species *Co-authorship in Hoch & Keel (2006)*	57
Chapter 8	Stomatal conductance of mature forest trees exposed to elevated CO_2	67
Chapter 9	General summary	77
Curriculum vitae		81

General introduction

Forests store half of the terrestrial organic carbon (C) of which two-thirds are contained in soils (Dixon et al., 1994). Nearly all biomass C is assimilated by leaves from where C-compounds are transferred to carbon sinks such as growth and reproduction, maintenance, storage or export (Körner, 2003). How rapidly recently assimilated C is transferred to these various C pools and fluxes is so far largely unknown, in particular it is not well understood at which speed C is allocated to long-lived pools. Depending on where C is allocated to, it stays in the system for minutes (e.g. leaf respiration) to hundreds or thousands of years (e.g. recalcitrant soil C).

Within trees assimilated C is not evenly distributed due to different sink strengths. Carbon is directed to areas of most active growth which strongly vary through the season. For instance the often observed bimodal growth of fine roots, with a first peak in early spring and a second in autumn (Shiroya et al., 1966; Smith & Paul, 1988), is the result of a fine tuned seasonal change in C allocation within the tree.

Indirect measurements based on correlations of the isotopic composition of soil respired CO_2 with climatic conditions of previous days indicate that CO_2 is respired within only 4-10 days after assimilation (Ekblad & Högberg, 2001; Bowling et al., 2002; Steinmann et al., 2004; Knohl et al., 2005). Roughly half of the C assimilated by trees, is rapidly returned to the atmosphere (Högberg et al., 2002) by above ground respiration (Horwath et al., 1994; Nogués et al., 2006) as well as below ground respiration (Andrews et al., 1999; Högberg et al., 2001; Andersen et al., 2005). To assess residence times of pools that are too young for conventional radiocarbon dating the so called 'bomb-$^{14}CO_2$' peak, which is the result of nuclear weapons tests during the early 1960ies can be used as a reference. Using this method fine roots were found to be 3-18 years old (Gaudinski et al., 2001) and more than ten year old C could be detected in saprophytic fungi (Hobbie et al., 2002). Longest lived pools are found in soil organic matter reaching ages up to several thousand years (Trumbore, 2000). However, for most C pools the timing of C allocation still remains largely unknown, because the residence time alone, does not reflect how long carbon remains e.g. in tree stores before it reaches soil organic matter.

On the ecosystem scale the allocation of C is crucial in determining the carbon balance. Only small shifts in the magnitude of C acquisition or loss might have a significant impact on the global carbon cycle (Malhi et al., 1999).

In the early 1970ies many attempts were made to study C allocation, using direct measures of C flows. Mostly ^{14}C was used as a tracer and rarely ^{11}C which due to its half life of 20.4 minutes only allows to study short term C allocation (Thompson et al., 1979; Jahnke et al., 1998). Studies were mainly designed to examine seasonal changes in C allocation and were performed with seedlings and saplings due to easier handling. Since mature trees exhibit large mobile C stores which could interfere with flows of recent C, patterns of C allocation in young trees differ from mature trees.

Labelling tall trees however, is a technical challenge. Whole-tree chambers as they have been used for labelling smaller trees in the field (Horwath et al., 1994) would be too massive for >30 m tall trees with 20 m crown widths. This thesis made use of a specially designed free-air CO_2 enrichment (FACE) system which was set up in a mature deciduous forest near Basel in the year 2000. The so called web-FACE (Pepin & Körner, 2002) complies with the heterogeneous structure of a deciduous forests' canopy and its height of 30-36 m. Due to its fossil fuel origin the stable isotope ratio of the supplemental CO_2 differs from atmospheric CO_2, leaving a distinct signal in all compartments where C enters. Potted C4 grasses grown in the tree crowns served as a reference for the isotope signals (referred to as 'isometers'). Because the grasses consist exclusively of C that originates from the CO_2 they assimilate, with no influence from old C reserves, the $\delta^{13}C$ difference of grasses exposed to labelled air compared with grasses grown in ambient air reflects the actual isotopic signal the canopy is exposed to. Using a rule of proportion where the isometer signal of 5.9‰ refers to 100% new C, the fraction of labelled C which had reached every specific forest compartment could be calculated.

Compared to the conventional FACE systems the web-FACE enriches the tree crowns only. Since the canopy is high enough, no labelled CO_2 reaches the forest floor by direct diffusion or downward draughts as was confirmed by stable isotope analysis of understory herbs showing no ^{13}C signals (Steinmann et al., 2004). This offers to trace the pathway of recently assimilated C through stems into roots, soil and soil air without confounding CO_2 uptake via understory vegetation or direct diffusion.

Although this large scale C allocation study was carried out within a CO_2 enrichment experiment, the main focus here is not on CO_2 effects as such. This thesis rather capitalizes on the unavoidable C-labelling associated with CO_2 enrichment. We assume that elevated CO_2 did not have any major consequences for the speed and direction of C flows or C pools. Under this assumption the data provide an unique opportunity for assessing the fate of carbon *in situ* in a tall forest.

In the first year of the canopy labelling a striking species-specificity in the amount of new C which had been incorporated into leaves was found (Steinmann et al., 2004). While leaves of five of the six study species consisted of 70% new C, leaves of *Tilia* already carried a 100% label. These results suggest that recent photoassimilates are directly invested into new structures only in *Tilia*. In the other five species labelled C had entered an unlabelled mobile carbohydrate pool (likely in wood), was mixed with this pool (dilution) and this mix of old and new C had thereafter been invested into new structures. This mixing process would not only be reflected in growing structures, but also in C allocated to ongoing metabolism. In order to explore whether recently assimilated C is mixed with given C pools, pulse labelling experiments were carried out in the upper canopy on one year old branchlets after termination of shoot growth. We assumed that in species where a high degree of mixing occurred as evidenced by weak leaf signals in the first year of full-canopy labelling, more C would be retained in woody tissue. In species where a low mixing of new with old C was found (*Tilia*) we expected little new C to remain in branchlets after labelling and hence branchlets would represent neutral C pathways.

Related to the overall forest carbon isotope labelling is the question of how much label can actually enter the tree canopy of different forest taxa. This is a matter of

atmospheric coupling and stomatal diffusion. If all trees would exhibit identical stomatal conductance and aerodynamics, the degree of labelling would depend on the rate of photosynthesis only. However, trees and tree species differ in this respect and the CO_2-treatment itself could exert an additional difference by inducing reduced stomatal conductance in a species specific way. It was thus key to know stomatal conductance in this forest canopy. As part of my PhD program I continued pilot work which was started during my diploma thesis in 2001 and monitored stomatal responses of six deciduous species (in later years measurements were confined to the three main species) to elevated CO_2 concentrations.

The following chapters address various aspects of the carbon transfer in a typical central European mixed lowland forest.

Chapter 2 is a four year synthesis of the large scale continuous labelling experiment. Our aim was to examine the most important pools and flows and present a holistic view of C allocation in a mature forest.

Chapter 3 (co-authorship) is focussing on a special part of chapter 2, namely the direct evidence for the contrasting roles of mycorrhizal versus saprophytic fungi in the forest carbon cycle. Saprophytic fungi, which as decomposers present an end-member of the C cycle, were of particular interest. In addition, we wanted to visualize the horizontal distribution of recent C in the fungal network, reflected by labelled sporocarps of mycorrhizal fungi.

Chapter 4 (co-authorship) summarizes the key findings of four years of CO_2 enrichment in this mature deciduous forest. The central question here was whether growth of trees is enhanced in response to elevated CO_2. I contributed to this publication by identifying alternative pools or flows of recently absorbed C to sinks other than structural tree growth.

Chapter 5 (co-authorship) presents the movements of arthropods collected in the canopy based on arthropod-$\delta^{13}C$ which only correlates with leaf-$\delta^{13}C$ if animals are sedentary and feed on leaves or phloem sap. We aimed at studying the dimension of spatial activity.

Chapter 6 presents the fate of recently assimilated C in one year old, fruitless branchlets after complete shoot elongation. Our main question was whether branchlets are only transfer pathways for recently assimilated C or whether an exchange with C present in branch wood occurs.

Chapter 7 (co-authorship) combines the branch labelling with a manipulative treatment where a change in C relations of fruiting branchlets was induced by girdling and/or defoliation. We asked whether leaf-like vegetative infructescence tissue could partly replace assimilation of leaves during fruit development in defoliated, girdled branchlets.

Chapter 8 is a four year synthesis of stomatal responses to elevated CO_2 and includes measurements carried out during an exceptionally dry summer. Beyond assessing *a priori* tree specific differences in stomatal conductance, the main question addressed was whether mature trees in the field show a reduction in stomatal conductance when exposed to elevated CO_2 and whether this reduction is species-specific.

These seven chapters represent the content of research articles either printed, accepted for printing, in revision or submitted to international journals. In cases where I am not the lead author, my contribution was the stable isotope aspect. A general summary is given at the end of this thesis (**chapter 9**).

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2 Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest

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Summary

• How rapidly newly assimilated carbon (C) is invested into recalcitrant structures of forests, and how closely C pools and fluxes are tied to photosynthesis, is largely unknown.

• A crane and a purpose-built free-air CO_2 enrichment (FACE) system permitted us to label the canopy of a mature deciduous forest with ^{13}C-depleted CO_2 for 4 yr and continuously trace the flow of recent C through the forest without disturbance. Potted C_4 grasses in the canopy ('isometers') served as a reference for the C-isotope input signal.

• After four growing seasons, leaves were completely labelled, while newly formed wood (tree rings) still contained 9% old C. Distinct labels were found in fine roots (38%) and sporocarps of mycorrhizal fungi (62%). Soil particles attached to fine roots contained 9% new C, whereas no measurable signal was detected in bulk soil. Soil-air CO_2 consisted of 35% new C, indicating that considerable amounts of assimilates were rapidly returned back to the atmosphere.

• These data illustrate a relatively slow dilution of old mobile C pools in trees, but a pronounced allocation of very recent assimilates to C pools of short residence times.

Key words: carbon allocation, free-air CO_2 enrichment (FACE), fungi, rhizosphere, roots, soil, soil respiration, stable isotopes.

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Introduction

Of all the carbon (C) assimilated by trees, about half is rapidly returned to the atmosphere by respiratory metabolism (Högberg *et al.*, 2002), at least during the growing season. The other part enters various fast- and slow-turnover pools, the residence times of which are largely unknown (Körner, 2003). In particular, it is not known how quickly newly assimilated C compounds are invested into recalcitrant structures (e.g. stem wood), and how intimately (on what timescales) the various C pools (e.g. soil organic matter) and fluxes (e.g. root respiration) are tied to actual photosynthesis. For instance, C allocated to leaf respiration can be released within minutes, whereas C entering the root biomass pool can remain in the ecosystem for months or even several years. Carbon transferred to the recalcitrant soil organic matter pool, for example via root litter, may reside for thousands of years (Trumbore, 2000). We quantified the allocation of newly assimilated C to different forest compartments by taking advantage of the Swiss canopy-crane CO_2-enrichment experiment (Pepin & Körner, 2002; Körner *et al.*, 2005), in which naturally grown deciduous trees receive labelled CO_2. The forest is not a plantation, so trees are of different size and age and live in interspecific competition for above-ground as well as below-ground resources.

Earlier direct quantifications of C allocation have used radiocarbon. However, these studies were either conducted on rather young trees (Hansen & Beck, 1990, 1994; Horwath *et al.*, 1994), or were restricted to single trees (McLaughlin *et al.*, 1979). The first forest-scale attempts used indirect evidence by interrupting phloem transport through girdling (removing or cutting of phloem). These experiments showed that allocation of photoassimilates to autotrophic respiration

represents the largest flux of current assimilates (approx. 50%; Högberg *et al.*, 2002). Autotrophic below-ground respiration is now more often defined by including not only roots, but also mycorrhizal fungi and microbes feeding on root exudates, altogether representing 50–65% of total soil respiration (Andrews *et al.*, 1999; Högberg *et al.*, 2001; Högberg *et al.*, 2002; Bhupinderpal-Singh *et al.*, 2003; Andersen *et al.*, 2005). Stable C-isotope trials using pulse labelling in a grassland revealed that 4–6% of labelled C was respired by mycorrhizal mycelia within 21 h (Johnson *et al.*, 2002). Slightly higher amounts (7–13%) of current assimilates have been found to be lost through exudation in potted tree seedlings (Phillips & Fahey, 2005). Such studies suggest that the largest amount of autotrophic respiration emerges directly from root respiration. Above-ground, assimilates are used mainly for structural growth (leaves, wood and fruits) and for cell metabolism.

The study of C allocation in mature forests is technically difficult without destroying the delicate plant–soil continuum, the widespread hyphal network of mycorrhizal fungi that forms the interface between roots and soil and allows the exchange of carbohydrates and nutrients. Stable isotopes serve as an ideal tracer to study C allocation, as only tiny amounts of tissue suffice for analysis. To apply isotopically labelled C, CO_2-enrichment systems are a convenient tool as the supplemental CO_2 is mostly of fossil fuel origin and therefore contains less ^{13}C than ambient air. Given the many experimental systems in use, it is surprising that labelled C has rarely been used to trace the fate of C in the plant body and the ecosystem (Andrews *et al.*, 1999; Matamala *et al.*, 2003; Pataki *et al.*, 2003; Steinmann *et al.*, 2004). One reason may be that most tests did not last long enough, given that it takes several years until new C signals are detectable in large pools such as soil (Hungate *et al.*, 1996). Furthermore, the assumption has to be made that CO_2 enrichment does not exert major alterations of C allocation. CO_2 effects cannot be determined as such, a long-term labelling of large control trees at ambient CO_2 concentrations is all but impossible.

Here we present data for an array of assimilate pathways in an approx. 100-yr-old, diverse central European forest, studied over four growing seasons. We used 12 mature deciduous trees exposed to approx. 540 ppm CO_2 using a specially designed free-air CO_2-enrichment technology called web-FACE (Pepin & Körner, 2002). This system enriches tree crowns only, and the canopy is at a height that prevents downward draughts and direct CO_2 diffusion from the crowns to the forest floor, as a lack of ^{13}C signals in understorey herbs confirmed (Steinmann *et al.*, 2004). This offers the unique opportunity to trace the fate of C in trees through stems into roots, soil and soil air, without confounding CO_2 fluxes via understorey vegetation or direct diffusion. Therefore there is a clearly defined 'port of entry' for C, with all other parts of the system not directly affected by the label.

To calculate the potential ^{13}C signal strength, we used C_4 grasses grown in small pots, exposed in the tree crowns, as references for the isotope signals ('isometers'). Repeated sampling of different forest compartments over four growing seasons allowed an estimation of the timing and mixing of new C in various C pools. We hypothesize that most of the carbohydrates formed by photosynthesis are invested in labile C pools, and we expect a rapid return of most of this new C to the atmosphere.

Materials and Methods

Site description and CO_2-enrichment system

The experiment was performed in a diverse mixed forest located near Basel, Switzerland (47°28′ N, 7°30′ E; elevation 550 m asl) with tree heights of 30–35 m. The forest is situated on a silty-loamy rendzina and is characterized by a 15-cm-deep rock-free topsoil and a 15–30-cm-deep rocky subsoil (approx. 40% of the subsoil volume is made up of stones), underlain by fragmented limestone bedrock. In the upper 10 cm the soil has a pH of 5.8 (measured in distilled water extracts).

A 45-m free-standing tower crane equipped with a 30-m jib (crane arm) and a working gondola provided access to 62 dominant trees in an area of approx. 3000 m^2. A group of 14 canopy-size broad-leaved trees [three *Fagus sylvatica* L., four *Quercus petraea* (Matt.) Liebl., four *Carpinus betulus* L., one *Tilia platyphyllos* Scop., one *Acer campestre* L. and one *Prunus avium* L.], covering a canopy area of 550 m^2, were selected for CO_2 enrichment. Of these, one slim individual of *Quercus* died, and CO_2 enrichment on the one *Prunus* at the eastern edge of the plot was not sufficient, so these two trees were excluded from the study, leaving 12 individuals for the analysis. Eleven control trees (three *Fagus*, two *Quercus*, two *Carpinus*, two *Tilia*, two *Acer*) were located in the remaining crane area at sufficient distance from the CO_2-release zone. In late September 2000, trees were exposed to a 'warm-up' CO_2 treatment of a few weeks to mitigate the inevitably step-nature of the treatment (Luo & Reynolds, 1999). From spring 2001 onwards, trees were exposed to elevated, labelled CO_2 from around mid-April to roughly the end of October, depending on bud break and leaf shedding. During the night, CO_2 release was interrupted. In total, approx. 300 t pure CO_2 was used per year. A more detailed description of the CO_2-enrichment system is given by Pepin & Körner (2002).

The isotopic composition of the pure CO_2 was monitored every week in year 1 and was found to be identical for all but one week. In year 2, a contract was made with the gas deliverer to guarantee the same source of CO_2, so CO_2 was monitored only at 2–3-wk intervals from year 2 onwards. Because of its fossil fuel origin, it was depleted in ^{13}C relative to ambient atmospheric CO_2 by -29.7 ± 0.3‰ vs approx. -8‰ (Fig. 1a), thus the fate of labelled photoassimilates could be traced. In spring 2004 we analysed honeydew that had been excreted

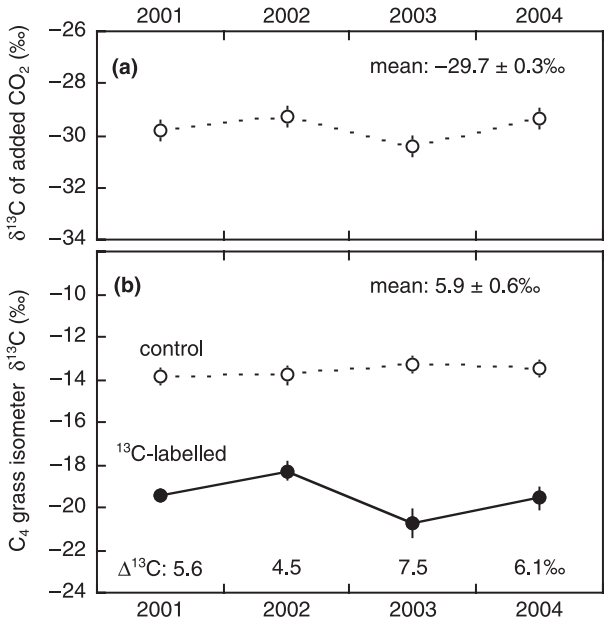

Fig. 1 (a) Mean annual $\delta^{13}C \pm 1$ SE of the pure supplemental CO_2 (n = 6–12 sampling dates). Top right, overall mean ± SE over 4 yr. (b) Mean annual $\delta^{13}C \pm 1$ SE of C_4 grass isometers (2001, *Cynodon dactylon*; 2002–04, *Echinochloa crus-galli*; n = 12–35 pots). Numbers represent differences in $\delta^{13}C$ between grasses grown in control trees and trees exposed to labelled CO_2 for single years; top right, average difference over 4 yr ± SE.

by aphids as a reference for fresh photoassimilates (Pate & Arthur, 1998; Barbour *et al.*, 2005). On average, we found honeydew $\delta^{13}C$ values of −25.7‰ in control and −30.8‰ in labelled trees, which correlated very well with leaf $\delta^{13}C$ ($r^2 = 0.93$). The isotope values are expressed in the δ-notation: $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$ (‰) where R is the molar ratio of ^{13}C to ^{12}C for the sample and the standard, respectively.

C_4 isometers

The abundance of ^{13}C in the CO_2 was monitored with so-called isometers, C_4 grasses [*Cynodon dactylon* (L.) Pers. and *Echinochloa crus-galli* (L.) P. Beauv.] grown in 50-ml containers (in a sand–clay mixture), placed in the tree crowns. In year 1, the grasses were also used to monitor the spatial distribution of the added CO_2 in neighbouring trees. We therefore had more pots in the area surrounding the CO_2-release zone (n = 35) than in the labelled area itself (n = 12). As the CO_2 was concentrated mainly around the labelled trees (Pepin & Körner, 2002), the number of pots in the control area was reduced to 12 and, in turn, the number of pots in the labelled area was increased to 35. We assumed the $\delta^{13}C$ difference between grasses exposed to labelled air compared with grasses grown in ambient air (5.9‰) to reflect the actual isotopic signal the canopy is exposed to, because the grasses consist exclusively of C that originates from the CO_2 they assimilated, with no influence from old C reserves. To calculate the fractions of new (= labelled) C in other compartments, we used a rule of proportion where the isometer signal of 5.9‰ refers to 100% new C. We assumed that ^{13}C fractionation is not influenced by elevated CO_2 (Saurer *et al.*, 2004).

The sensitivity towards ^{13}C discrimination in response to changes in climatic factors is low under well watered and light-saturated conditions in *C. dactylon* (used in 2001) and even lower in *E. crus-galli*, which was used from 2002 onwards (Buchmann *et al.*, 1996). Therefore $\delta^{13}C$ values of these grasses exposed to labelled CO_2 could be used to calculate time-integrated CO_2 concentrations of the labelled CO_2 using the following mixing ratio model with the CO_2 concentration and isotope ratio of its two CO_2 constituents (atmospheric and pure CO_2 gas):

$$c_{elev} \times \delta^{13}C_{elev} = c_{pure} \times \delta^{13}C_{pure} + c_{amb} \times \delta^{13}C_{amb} \qquad \text{Eqn 1}$$

where c_{elev} is the CO_2 concentration of the CO_2-enriched air, and $\delta^{13}C_{elev}$ is the $\delta^{13}C$ isotope ratio of the CO_2-enriched air derived by C_4 grasses ($\delta^{13}C$ of leaves minus a discrimination factor of 5.5‰ for *C. dactylon* and 4.4‰ for *E. crus-galli*; Buchmann *et al.*, 1996). c_{pure} is the CO_2 concentration by which the air was increased and was substituted by $c_{elev} - c_{amb}$, and $\delta^{13}C_{pure}$ is the value of the CO_2 in the tank (Fig. 1a). C_{amb} is the atmospheric CO_2 concentration (assumed to be 375 ppm), and $\delta^{13}C_{amb}$ is the $\delta^{13}C$ of ambient air (assumed to be −8‰). CO_2 concentrations were calculated by rearranging the equation and solving for c_{elev}. The seasonal means of these CO_2 concentrations were compared with the seasonal mean CO_2 concentrations measured with a nondispersive infrared gas analyser (LI-800, Li-Cor, Lincoln, NE, USA).

Tissue sampling

Leaves We collected 20 leaf discs of upper canopy foliage of the five deciduous tree species in August of each year (in 2002 in June/July and September) using a metal puncher. To minimize microclimatic effects, only samples of sun-exposed leaves were harvested. Overall means were calculated by averaging over all trees, thus giving the more abundant species a stronger weight.

Litter Fifty-six litter traps of 0.5 m^2 were placed in a 6-m grid in the crane area. In autumn, the traps were emptied every second week, and litter was sorted by species and weighed. For $\delta^{13}C$ determination, litter of one pretreatment and only one treatment year were chosen for analysis, for reasons of analytical costs (1999 vs 2003). The overall $\delta^{13}C$ for each litter trap was calculated by pooling $\delta^{13}C$ values of all species weighted by their biomass contribution. For comparison with fresh crown litter, five leaves per tree were sampled in autumn 2003, shortly before leaf abscission.

Wood We used wood cores punched in 2004 with a custom-made 4-mm-diameter stainless steel core puncher, creating minimal tree wounding (Asshoff *et al.*, 2006). Yearly growth rings were separated using a scalpel under a microscope.

Fine roots In August 2004, fine roots (<1 mm diameter) were collected at *c.* 10 cm depth for each tree by digging near the stem close to the main roots, to make sure that only roots of a specific tree were included. Fine roots were picked by hand; roots of understorey species (mainly *Hedera helix* L.) and dead roots could be distinguished visually based on their colour, and were excluded. In the laboratory, loose substrate attached to the roots was removed mechanically by gentle shaking and kept for analysis (so-called rhizospheric soil, see below). The roots were enclosed in plastic bags filled with water to remove the remaining substrate in an ultrasonic cleaner (Bransonic 92), then rinsed with deionized water and oven-dried at 80°C.

Fungi All fungal sporocarps on the site, and in the surrounding area within *c.* 100 m from the labelled zone, were harvested. Sporocarps from the unlabelled area were collected with >12 m distance from the edge of the CO_2-enriched zone, which was identified as the demarcation zone based on stable $\delta^{13}C$ values of mycorrhizal fungi. Sporocarps were specified by taxonomic experts and classified as either mycorrhizal or saprophytic, based on the taxonomic literature. Only the caps of sporocarps were used for isotope analysis.

Rhizospheric and bulk soil The sedimented root-attached soil fraction (partly including dissolved organic C) was placed in glass cups and oven-dried at 60°C. Of this, 20 mg was weighed into tin capsules and 80 µl 2 M HCl was added to remove carbonates. Before isotope analysis, the acid-treated samples were air-dried for 24 h. In April 2005, soil cores from 0 to 6 cm depth were collected to analyse the $\delta^{13}C$ in bulk soil ($n = 5$). The samples were washed through a 400-µm sieve, rinsed with deionized water, oven-dried at 60°C and ground. The carbonates were removed from the powder as described above.

Carbon-isotope analysis of organic samples

All organic material was oven-dried at 80°C for 48 h and ground with a steel ball mill (Mixer Mill, Retsch MM 2000, Haan, Germany), and 0.6–0.8 mg dried powder was packaged in tin capsules for $\delta^{13}C$ analysis. Samples were then combusted in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy). Via a variable open-split interface (Conflo II, Finnigan Mat, Bremen, Germany), gas samples were transferred to the mass spectrometer (Delta S, Thermo Finnigan Mat), which was operated in continuous flow mode. The precision for $\delta^{13}C$ analysis was <0.1‰.

Soil air

Soil air was sampled from 170 'gas wells' (permanently installed PVC tubes in the upper soil layer, 12 cm long, 2 cm in diameter). The top was sealed with a silicon septum. The bottom of the tube was open, and two vertical slits on each side were cut from the bottom up to 3 cm below soil surface to integrate the CO_2 released from soil between 3 and 11 cm depth. The gas wells covered a test area of 60 × 70 m, and were placed in a grid of 3 m within the approx. 550-m^2 CO_2-enriched area and in a grid of 6 m in the larger control area. For details on the sampling and measurement procedure, see Steinmann *et al.* (2004).

To determine the $\delta^{13}C$ of soil CO_2, the Keeling plot approach (Keeling, 1958) was applied for each day and CO_2 treatment separately. All data were corrected for isotope fractionation caused by slower gas diffusion of the heavier $^{13}CO_2$ by subtracting 4.4‰ (Hesterberg & Siegenthaler, 1991). To estimate the effect of understorey vegetation on $\delta^{13}C$ of soil air, total above-ground biomass of herbs and small shrubs was cut to the base on four circular plots (1 m radius) centred around the gas wells in July 2004. Measurements of soil-air $\delta^{13}C$ were carried out 2 d before and 1–16 d after understorey removal (daily in the first week, every second day thereafter).

The isotope ratio of the soil air was determined with a gas bench II linked to a mass spectrometer (Delta Plus XL, Thermo Finnigan, Bremen, Germany). The CO_2 concentration of every gas sample analysed was calculated from the calibration line with standard gas samples of known CO_2 concentrations (340 and 5015 ppm). The area of the voltage signal peak of the mass spectrometer for CO_2 (masses 44, 45 and 46) was integrated over time and was proportional to the CO_2 concentration of the air sample. Reference gas samples were included with each series of measurements. Up to 20000 ppm, the CO_2 concentrations agreed well ($y = 1.04x$, $r^2 = 0.99$) with infrared gas analyser readings (Innova 1312, Innova, Ballerup, Denmark).

Statistics

The need for a canopy crane did not permit randomization of the treatment units (it would require several cranes), therefore a detailed investigation of *a priori* differences in physiology and morphology between control trees and those later exposed to CO_2 was performed by (Cech *et al.*, 2003). As no systematic differences between the two groups of trees were found, we could use single trees as treatment units for the statistical analysis.

Our main goal was to identify tree signals irrespective of species ($n = 12$ trees in labelled CO_2; $n = 11$ control trees). In addition, tests were carried out using species as a factor, despite the low replication. Because *Acer* and *Tilia* were represented by only one tree in the labelled zone, they were pooled for the analysis and referred to as 'others'. A repeated-measures ANOVA was applied whenever data were collected in several years, with tree species, CO_2 treatment and their interaction as fixed factors, and year as the repeated factor.

In the case of roots and soil, where data were collected only once, a two-way ANOVA was performed with species and CO_2-treatment as fixed factors. For the analysis of leaf litter data, traps were defined as replicates, and single pots were defined as replicates for canopy isometer analysis. Species were regarded as replicates in the case of fungi, including the fungal type (mycorrhizal or saprophytic) as a fixed factor.

Applying a Student's *t*-test, soil-air $\delta^{13}C$ between treatments was compared using Keeling plot intercepts calculated for each treatment. For the soil-air CO_2 analysis, gas wells were assigned to trees as described by Steinmann *et al.* (2004), resulting in 12 circles in the CO_2-enriched and 35 circles in the control area, the diameter of which varied with tree diameter. These circles were regarded as replicates for the two-way ANOVA, with tree species and CO_2 treatment as fixed factors.

All errors refer to standard errors. Statistical analysis was carried out using R ver. 2.0.1 (R Development Core Team, 2004).

Results

Isotopic composition of supplemental CO_2

A constant isotope ratio of the added CO_2 is a prerequisite for tracing the assimilated C. The 10th and 90th percentiles were −30.4 and −28.9‰, respectively, and reflect the temporal variation. Yearly $\delta^{13}C$ means remained relatively constant (Fig. 1a), resulting in an average of −29.7 ± 0.3‰ over 4 yr.

C_4 isometers

Seasonal mean $\delta^{13}C$ of C_4 grasses grown on control trees showed little variation between the four study years (Fig. 1b). More variation was observed in grasses exposed to labelled CO_2, with significantly lower $\delta^{13}C$ values (−19.6 ± 0.26‰, $P < 0.0001$). The new C signals, represented by the $\delta^{13}C$ difference between grasses in ambient minus $\delta^{13}C$ of grasses exposed to labelled CO_2, did not change significantly between years (CO_2 treatment × year, $P = 0.32$) and reached 5.9 ± 0.6‰ averaged over the 4-yr means.

The isometer-derived CO_2 concentrations for 2001–04 were 514, 519, 596 and 566 ppm. In the first 2 yr, these concentrations corresponded well with independent readings of gas-sampling lines using an infrared gas analyser, and were somewhat higher than infrared gas analyser readings in the last 2 yr (mean CO_2 concentrations for 2001–04: 520, 520, 580 and 550 ppm).

Leaves

In the pretreatment year (1999), trees later assigned to the CO_2 treatment tended to have slightly less negative leaf $\delta^{13}C$ (−26.7‰) than trees later used as controls (−27.5‰; Fig. 2a). A similar difference was found for leaf litter. These pretreatment differences were accounted for when calculating the tissue-specific contribution of new, labelled C. For the overall signal we used a pretreatment correction over all trees, whereas for signals in single species we applied a species-specific pretreatment correction. We have no obvious explanation for this *a priori* difference, because there are no measurable differences in soil parameters, including moisture. Leaves from CO_2-enriched trees were significantly labelled starting from the first full year of treatment, and signals were four times higher than pretreatment differences (Fig. 2a). In August 2001, new C signals were 39% in *Quercus*, 63% in *Fagus*, 66% in *Acer*, 77% in *Carpinus*, and reached 100% in *Tilia*, possibly reflecting differences in branchlet C autonomy. The species-weighted average signal over all trees increased from year to year, reaching 97% new C by year 4.

Leaf litter

Leaf litter collected with litter traps in pretreatment year 1999 was −29.6‰ in the area later used as a control, and −29.0‰ in the area later exposed to labelled CO_2 (Fig. 2b). In 2003, pretreatment-corrected new C signals in litter reached only 28%, averaged over all traps, whereas in freshly fallen litter collected in the canopy, a 90% signal was measured in accordance with fresh leaf signals (Fig. 2a). Litter collected

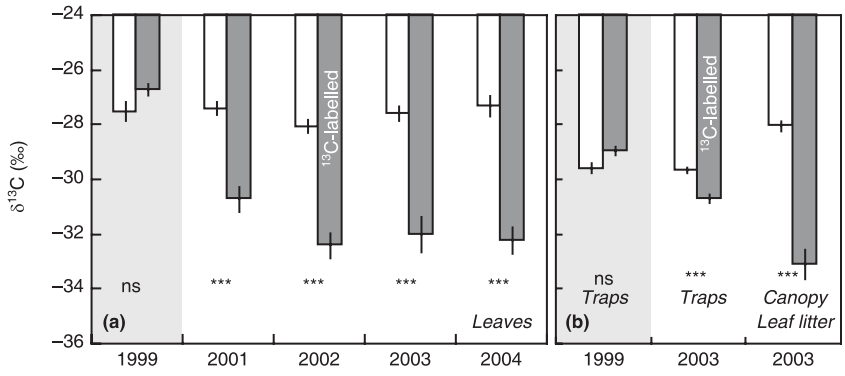

Fig. 2 (a) Leaf; (b) leaf litter $\delta^{13}C$ of five deciduous tree species exposed to ambient (open bars) and ^{13}C-depleted CO_2 (closed bars), including a pretreatment year (1999, shaded area). Means ± 1 SE for each year and treatment are shown (n = 11–12 trees). Litter data are shown for year 3 only (2003), when both fresh litter picked in the canopy and trapped ground litter (0.5 m^2 mesh traps, 30 cm above-ground; 15 traps under control trees and five under labelled trees) were analysed. Leaves were collected in mid-summer; litter was collected in October–November. *P* values for labelling effects (ANOVA): (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

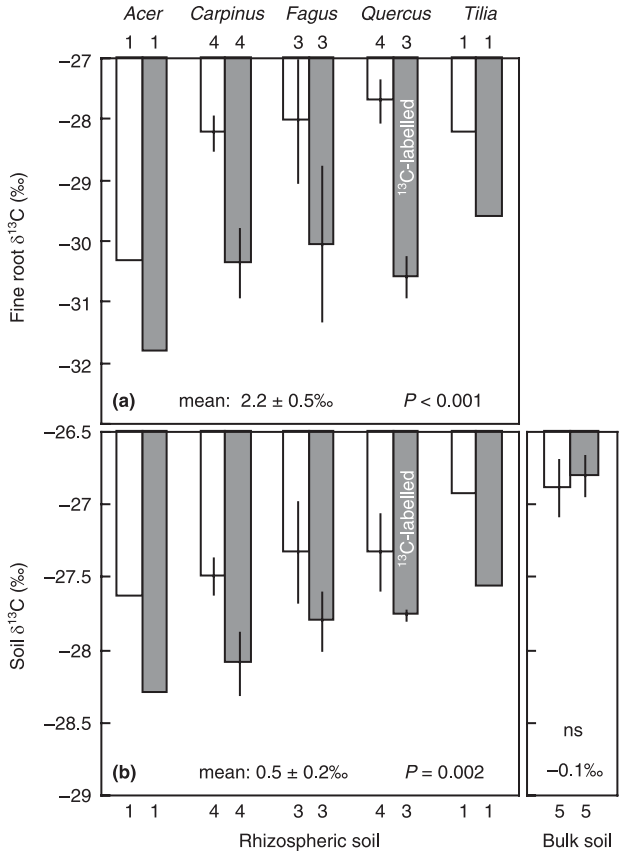

Fig. 3 (a) Mean $\delta^{13}C \pm 1$ SE of fine roots (<1 mm) for five tree species exposed to ambient (open bars) and ^{13}C-labelled CO_2 (closed bars) in year 4 of carbon isotope labelling (2004). Numbers above graph indicate number of trees sampled. (b) Left panel, mean soil $\delta^{13}C \pm 1$ SE, which was attached to fine roots (rhizospheric soil) shown in (a); right panel, bulk soil $\delta^{13}C \pm 1$ SE at 0–6 cm depth in April 2005. Number of samples shown below graph. In the lower part of all panels, mean $\delta^{13}C$ differences ± SE between samples collected in the control and labelled areas are shown with results for the labelling effects of the one-way ANOVAS (ns, not significant).

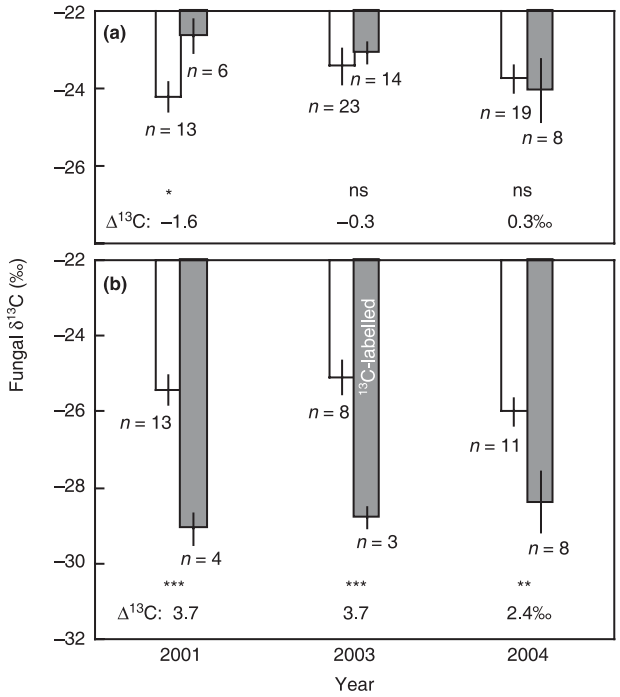

Fig. 4 Mean $\delta^{13}C \pm 1$ SE of fungal sporocarps classified as (a) saprophytic; (b) mycorrhizal species. *n* = Number of species found. Mean $\delta^{13}C$ differences between sporocarps collected under control (open bars) and ^{13}C-labelled trees (closed bars) are shown by numbers in graph. $^{(*)}$, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

with traps near the ground (25–35 m below canopy) had probably been mixed with litter from the surrounding area during autumn storms, which reduced the signal in ground litter compared with litter from the canopy. Ground litter from control trees was therefore collected at sufficient distance from the labelled zone to minimize mixing with labelled material. In 2003, the isotopic signal strength of ground litter in the labelled zone was strongly species-specific and signals were significant in all species except *Fagus*. This, together with large variations in biomass contributions ranging from <1% (*Acer*) up to 90% (*Fagus*), explained most of the variation in $\delta^{13}C$ between different traps.

Wood

Wood $\delta^{13}C$ in trees later exposed to labelled CO_2 was −27.3‰, whereas trees later assigned to the control treatment exhibited slightly less negative values (−27.1‰) in pretreatment year 1999 (data not shown). Over all trees, pretreatment-corrected signals of newly formed wood weighted by species were 71% in year 1, and reached 91% in year 4.

Fine roots

Fine roots consisted of 38% new C over all trees in August 2004, 3.5 seasons from the start (Fig. 3a). *Quercus* exhibited the strongest signals, followed by *Carpinus* and *Fagus*, whereas the weakest signals were measured in the *Acer* tree and, surprisingly, in the *Tilia* tree, which always produced the strongest label in leaves and wood.

Fungi

Over all years, sporocarps of 85 different fungal species were found (33 presumably from mycorrhizal and 52 from saprophytic fungi, of which 11 mycorrhizal and 21 saprophytic fungi were found in the labelled zone). All mycorrhizal species belong to the ectomycorrhizal type. The $\delta^{13}C$ analysis of fungal sporocarps clearly confirmed the taxonomic classification of species into saprophytic and mycorrhizal ($P < 0.0001$), the latter always exhibiting more negative $\delta^{13}C$ values (Fig. 4). In the labelled forest zone, no ^{13}C labels were found in

saprophytic fungi even after 4 yr (Fig. 4a). By contrast, labels in sporocarps of mycorrhizal fungi growing under labelled trees had already reached 62% in year 1. This signal did not increase with time, and was identical in 2003 (Fig. 4b). For no obvious reason, the ^{13}C signals in mycorrhizal fungi were reduced to 41% in year 4, the year following an exceptional drought in 2003. In the reference area, large variations in $\delta^{13}C$ values were found between species of the same type of fungus (−26.6 to −20.7‰ for saprophytic species; −28.2 to −22.4‰ for mycorrhizal species). Also, within the same species and year substantial variation occurred, reaching an extreme range of −27.7 to −21.1‰ in *Mycena crocata*.

Soil

Acidified soil particles that had been attached to the fine-root surface contained 9% new C by year 4 (Fig. 3b, left), whereas no signal was found in acidified bulk soil of the same rooting zone in April 2005, shortly before the CO_2-enrichment system was set in operation for the fifth season (Fig. 3b, right).

Soil air

Already in May 2001, 3 wk after the first full growing season of CO_2 enrichment began, soil air tended to be labelled (Fig. 5a). From June 2001 onwards, new C signals remained statistically significant throughout the study period, including winter data. The contribution of new C increased almost steadily during the first growing season, reaching 29% in October 2001, and was around 35% between June and October during normal years (2002, 2004). In October 2003, at the end of an exceptional drought, new C signals in soil air reached 51%. At the beginning of the growing season (April–May), new C signals were always less pronounced than later in the season. As soil CO_2 labels in 2002–04 remained in the same range as in October 2001, a steady state had already been reached one season after continuous labelling of the canopy commenced. Cutting the understorey vegetation around our gas wells (3.14 m^2) did not alter soil-air signals, suggesting that signals were not affected by the light ground cover and mainly reflected the respiration of tree roots and root-associated microbes/fungi.

During summers with normal weather conditions, CO_2 concentrations of the same gas samples collected for isotope analysis were higher in the area where crowns received CO_2 enrichment. For half the sampling dates, the difference was significant (Fig. 5b). The largest increase in CO_2 concentration (+123%) was measured in October 2002 after a wet summer. During a centennial drought in summer 2003, the canopy CO_2-enrichment effects on soil-air CO_2 concentrations diminished, and were even reversed in December 2003. At the same time the contribution of new C, as assessed by ^{13}C signals, reached a maximum (Fig. 5a).

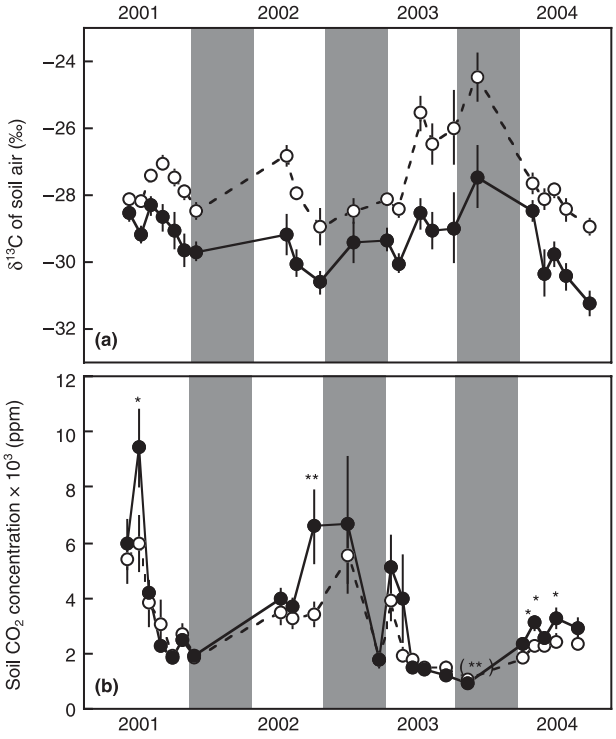

Fig. 5 (a) Seasonal variation of $\delta^{13}C$ in soil air at 3–11 cm depth over 4 growing seasons under trees exposed to ambient (open symbols; n = 59 gas wells) and ^{13}C-labelled CO_2 (closed symbols; n = 25). Values derived from Keeling plot. Except for the first measurement date, all isotope signals were statistically significant as assessed by t-test. Error bars are SE of Keeling plot intercepts. Months between the growing seasons are shaded. (b) Mean soil CO_2 concentrations ± 1 SE of the same samples used for isotope analysis. *, Significantly higher CO_2 concentrations in soil air under CO_2-enriched trees. (*), Lower CO_2 concentrations in the CO_2 enriched area (reverse CO_2 effect). For statistical analysis samples were assigned to circles around trees (n = 35 circles around control trees; 12 around CO_2-enriched trees). P-values for the CO_2-effects of two-way ANOVAs with species and CO_2-treatment as factors are shown. (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Discussion

After labelling photoassimilates in tree canopies with ^{13}C-depleted CO_2 for 4 yr, new C signals were found in all forest compartments investigated except bulk soil and sporocarps of saprophytic fungi (Fig. 6). Our data illustrate a very intense and rapid C flow from canopy to soil biota, a slow penetration of fine roots (suggesting an approx. 10-yr turnover), and an almost complete replacement of old C in new growth rings of trees by year 4. Below we discuss these results separately for each forest compartment.

Canopy CO_2 environment

The vigorous apical growth of top-canopy branches made it necessary to slightly elongate and move the CO_2-release tubing system every year, to maintain the desired CO_2 concentration

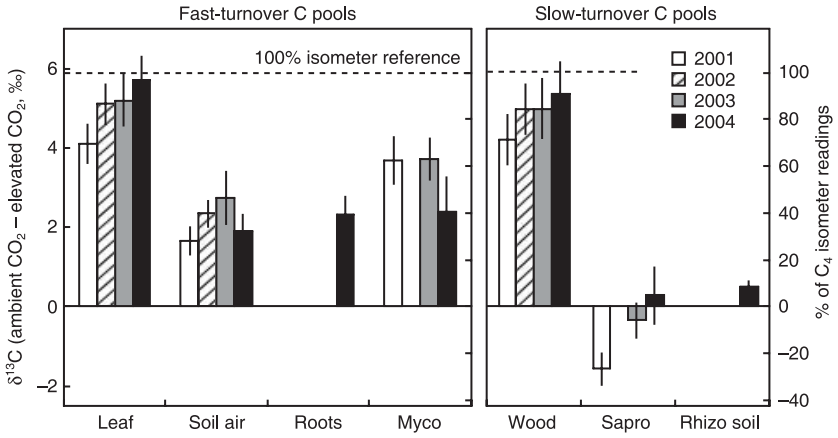

Fig. 6 New carbon (C) signatures as assessed by ^{13}C-tracer signals of forest compartments classified as fast-turnover C pools (left panel) and slow-turnover C pools (right panel). The maximum steady-state C-isotope difference between C_4 grasses (isometers) grown in crowns of control and CO_2-enriched trees is shown as a dashed line and refers to 100% new (= ^{13}C-labelled) carbon. Myco, mycorrhizal fungi; Sapro, saprophytic fungi; Rhizo soil, rhizospheric soil.

around the upper canopy (Asshoff *et al.*, 2006). Isometers had to be newly installed every year. This explains the variation in yearly average CO_2 concentrations measured by infrared gas analysis and isometers across this rough forest canopy. Frequent watering and exposure to the high irradiance in the upper canopy minimized the biochemical ^{13}C discrimination in the C_4 grasses, as evidenced by the rather stable δ^{13}C readings of control grasses. The agreement between CO_2 concentrations derived from isometer signals and infrared gas analysis of air samples confirms the steady exposure of the forest canopy to the ^{13}C-tracer signal.

Delayed leaf signals

Surprisingly, leaf tissue had fully adjusted to the novel stable isotope signal only after 4 yr. During leafing, no decrease of mobile C reserves takes place (Hoch *et al.*, 2003), suggesting that our data should not be seen as evidence for a dependence of leaves on old C to build a new canopy. More probably, the data indicate that new C rapidly enters a given pool of mobile C (presumably in wood tissue) and mixes with this pool before entering leaf construction. In other words, the data suggest substantial turnover of mobile C pools along the assimilate transport path. The resulting dilution process of old by new C can theoretically go on for years, but the degree of dilution (and hence C turnover) is still remarkable, and also points to a large reserve pool compared with annual leaf C needs, which had already been confirmed for this site (Hoch *et al.*, 2003). Based on our first year's results, 30% of C found in new foliage is from previous years. This does not preclude that leaves may still produce three to four times their own C cost per year (Poorter *et al.*, 2006). As the lower part of the canopy is not exposed to elevated CO_2, unlabelled lower-canopy foliage could, alternatively, have dampened the new C label. The largest amount of old C in new foliage was found in *Quercus* leaves (39%), a late-flushing species reaching maximum photosynthetic rates only later in the season (Morecroft & Roberts, 1999).

New carbon signals in leaf litter

Nearly identical new C signals in canopy litter compared with fresh leaves in 2003 suggest that structural biomass contains the same mixture of unlabelled and labelled C as mobile carbohydrates or amino acids, which are recycled during leaf senescence. Therefore the weak signals in leaf litter collected with traps near the ground must have resulted from dilution with litter from surrounding trees during autumn storms.

New carbon signals in tree rings

There may be two reasons why stem wood did not attain a 100% new C signal after 4 yr. First, early wood formation, just like foliage, may draw C from a slowly diluting mobile C pool in stem-storage tissue (see above); second, the lower canopy (<15 m) is not exposed to labelled CO_2, perhaps causing a dilution of the isotope signal. If we attribute the lack of a 100% tree-ring signal at the base of the stem exclusively to the contribution of C from lower-canopy foliage, the data suggest this canopy layer contributes, at most, 9% of total C (because 91% was labelled). This is an interesting result, illustrating the dominant role of upper-canopy foliage for tree growth. Most probably this is true only for upper-canopy trees, as they are exposed to a large light gradient, whereas in the lower canopy light is distributed more evenly. If we also account for unknown mobile C-pool dilution (particularly through ray tissue), the contribution of the subcanopy becomes even smaller.

Fine root signals

Fine roots are often assumed to turn over rapidly, but the bulk fine-root fraction in forests has been shown to last several years (Gaudinski *et al.*, 2001; Matamala *et al.*, 2003). The 38% new C signal in the <1-mm fine-root fraction found here in the fourth season suggests that our samples represent a mixture of new and older (>4-yr) fine roots. However, similarly to

leaves, fine roots could also be built from a slowly diluting C pool (see above). Assuming a linear increase in new C, fine roots would reach a 100% signal in approx. 10 yr (10-yr root C turnover), which is substantially longer than suggested by data for a *Pinus taeda* forest (4.2 yr) and a temperate deciduous *Liquidambar styraciflua* forest (1.25 yr; Matamala *et al.*, 2003).

Contrasting labels in sporocarps of mycorrhizal and saprophytic fungi

Based on the high (a few days) turnover rate of arbuscular mycorrhizal hyphae (Staddon *et al.*, 2003), we assume that ectomycorrhizal hyphae are also rapidly recycled. Therefore the pronounced allocation of new C to ectomycorrhizal fungi might indicate that large amounts of C are rapidly released back to the atmosphere. As hyphae of single genets can cover areas up to 300 m^2 (Bonello *et al.*, 1998), sporocarps collected in the CO_2-enriched area could be linked to trees exposed to elevated as well as ambient CO_2. This might explain why sporocarps consisted of at least 40% old C during the whole study period. A labelling gradient with increasing radial distance from the treated area suggested a signal influence of approx. 20% at 6–12 m outside the CO_2-enriched area. So the fungal signal in the labelled area should reflect the reciprocal influence of nonlabelled trees surrounding the 550-m^2 test area. It is very unlikely that mycorrhizal fungi had acquired C from sources other than their host plant, such as soil or leaf litter (Högberg *et al.*, 2001; Treseder *et al.*, 2006). The variability in $\delta^{13}C$ of mycorrhizal fungi we observed between years might partly reflect C obtained from either overstorey or understorey trees, depending on years. Understorey trees are well known to exhibit more negative $\delta^{13}C$ (for *Fagus*, −34.4‰ in the understorey compared with −28.0‰ in the overstorey), and this signal could translate to their fungal partner (Högberg *et al.*, 1999). Alternatively, fungal species composition might have been altered in response to elevated CO_2 as shown earlier by Fransson *et al.* (2001), resulting in a shift in $\delta^{13}C$ caused by species-specific values.

As no label was detected in saprophytic fungi after four treatment years (Fig. 4a), these fungi decomposed C compounds that were >4 yr old, in accordance with the results of Hobbie *et al.* (2002). This was somewhat surprising, as at least a few of the species found are known to decompose leaf litter (e.g. *Mycena galopus*; Ghosh *et al.*, 2003).

Soil carbon signals

The fact that new C signals in soils were found exclusively in the rhizospheric fraction, but not in bulk soil, suggests that soil C input had taken place mainly via fine roots (exudates, rhizosphere microbes). As these are relatively short-lived compounds, we assume that our signal reflects contributions to the labile C pools, as has been shown in previous studies (Hagedorn *et al.*, 2003; Lichter *et al.*, 2005), and is likely to be accompanied by a stimulation in soil respiration, as shown earlier (Körner & Arnone, 1992; Heath *et al.*, 2005). In contrast to our experiment, an increase in soil C was found in a *L. styraciflua* forest exposed to elevated CO_2 (Jastrow *et al.*, 2005), which is probably the result of strongly enhanced root production and root turnover (Matamala *et al.*, 2003; Norby *et al.*, 2004). In general, bulk soil signals are usually very small (5%; Jastrow *et al.*, 2005) and are therefore difficult to detect (Hungate *et al.*, 1996).

Soil air

Our data suggest that, after reaching a quasi-steady state within a year, new C contributes 35% to respired CO_2 emerging from soil under normal weather conditions during three seasons, which is lower than described earlier (55–65%; Andrews *et al.*, 1999; Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003; Andersen *et al.*, 2005). This may reflect real differences between forests to some extent, but may also have other explanations. For example, during a severe drought in summer 2003, when no plant-available water was present down to 1 m depth (Leuzinger *et al.*, 2005), contributions of current assimilates to total soil CO_2 rose to 51%, similar to the studies mentioned above. We assume that during the drought microbes feeding on older, unlabelled C were less active and contributed less to respired CO_2 (Fig. 5b), whereas root respiration continued, perhaps supported by hydraulically lifted water (Caldwell *et al.*, 1998) or by water from greater depths, and exceeded heterotrophic respiration, thus causing the strong new C signals in this year (Fig. 5a). The more pronounced soil-air signal in this year might also have resulted from the interaction of drought and elevated CO_2 on stomatal conductance. Drought leads to less negative $\delta^{13}C$ in assimilates, but elevated CO_2 relieved drought stress during that extraordinary dry summer. Actually, drought led to higher stomatal conductance in trees exposed to elevated CO_2 (S. G. Keel, unpublished data), causing $\delta^{13}C$ in concurrent assimilates to become even more negative in CO_2-enriched trees, thus adding to the strength of the tracer signal imposed by artificial labelling. The generally smaller signals at the beginning of the season (during leafing) indicate that soil-air signals are driven mainly by current assimilates, which are less abundant under a flushing canopy in April and May than after full canopy development.

Steadily increasing soil-air isotope signals during the first treatment season highlight the velocity by which new C is allocated below-ground, and the importance of recently assimilated C for below-ground metabolism. This is supported by previous studies, which have shown a close temporal linkage between climatic conditions and the isotopic composition of respired CO_2, suggesting that these photoassimilates are respired within <10 d after assimilation (Ekblad & Högberg, 2001; Bowling *et al.*, 2002; Scartazza *et al.*, 2004; Steinmann *et al.*, 2004; Tang *et al.*, 2005).

As demonstrated by increased soil-air CO_2 concentrations under the CO_2-enriched canopy area, roots, microbes feeding on exudates, and/or mycorrhizal fungi respired more CO_2 in response to elevated CO_2. This confirms earlier findings of increased soil CO_2 efflux in response to CO_2 enrichment under more artificial test conditions (Zak *et al.*, 2000; King *et al.*, 2004; Niinistö *et al.*, 2004; Heath *et al.*, 2005). Hence the new C fluxes measured here are likely to have been affected by CO_2 enrichment as they are higher than 'normal'.

Conclusions

This *in situ* ^{13}C-labelling study yielded direct evidence on the path and speed of C flows in mature deciduous forest trees. The data indicate a high degree of mixing between newly assimilated C and old mobile C stores before investment into structural growth. While new tissue such as leaves and fine roots may correspond quantitatively to 100% new C, their actual isotopic composition proves a high degree of dilution with old C; it takes several years to replace old by new C, even in zones of most active growth. On the other hand, new C signals appear strongly and rapidly (within days) in soil CO_2, suggesting a massive flow of new C to the rhizosphere, and fungal symbionts in particular. We conclude that C loaded to the phloem (as indicated, e.g. honeydew $\delta^{13}C$ of aphids) enters the rhizosphere largely undiluted. However, before C is invested in tree tissues, it is rapidly mixed (and diluted) with old C. The size of the C-reserve pool and its mobility thus determine the new C-signal strength in tree tissue. Four years suffice to arrive at 90–100% C replacement in leaves and new tree rings, but fine roots still retain 60% old C, which we attribute to their greater-than-expected longevity.

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3 Strict separation of carbon transfer to mycorrhizal and saprophytic fungi by tall temperate forest trees

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in revision

Mycorrhizal and saprophytic fungi in forest soils depend on recent and past tree photosynthates[1,2]. Continuous ^{13}C isotope labelling of 550 m^2 of canopy in a 100 year old temperate forest enabled us to trace the fate of newly fixed carbon and monitor its transfer time to fungal fruit bodies over five years. By the end of the first labelling season, mycorrhizal fungi were largely built from new, isotope-labelled carbon, retaining the signal strength throughout the testing period. Isotope signals in these mushrooms hardly spread beyond the 6 m periphery of the labelled canopy area. In contrast, no significant fraction of new carbon was found in saprophytic mushroom tissue by the 5th year. Saprophytic fungi obviously metabolize carbon resources largely older than five years, despite a rapid leaf litter turnover. This large-scale experiment evidences a distinct differentiation in the path and speed of carbon transfer from the forest canopy to mycorrhizal and saprophytic fungi.

Carbon assimilated in tree crowns not only provides the resources for metabolism and growth of trees, but also feeds a rich microbial, fungal, and faunal soil food web. Coarse estimates suggest that about half of all carbon metabolized in and released from forest soils in the form of CO_2 comes from tree roots, while the other half is released by microbiota[3,4]. Among these, mycorrhizal fungi living in symbiosis with trees, are predominant direct consumers of recent photoassimilates[5]. Together with dead root biomass and litter input they are a major source of soil humus formation.

Using the Swiss Canopy Crane CO_2 enrichment facility[6,7] we were able to label new carbon assimilates on a 550 m^2 tree canopy area. By releasing two tons of isotope labelled CO_2 per day into the crowns of 12 temperate zone, deciduous trees (30-38 m height and ca. 100 years of age) we enriched the canopy CO_2 concentration to 540 ppm for five consecutive growing seasons (doubling pre-industrial era concentrations). From 4 mm diameter, laser punctured tubes, which were loosely woven into the tree canopy, pure CO_2 was mixed into the atmosphere of the tree crowns. The concentration was computer controlled by monitoring the CO_2 level at 24 different canopy positions. The study makes use of the ^{13}C signal in the added CO_2 which originated from fossil fuel (4 year mean $\delta^{13}C$ =-29.7±0.3‰ instead of -8‰). The mixture of ambient air and fossil derived CO_2, which was assimilated by photosynthesis was depleted of ^{13}C by ca. 5.8 ± 0.6‰ relative to the ambient air[6,7].

By the end of each growing season (2001, 2003, 2004, 2005) all mushrooms in and around the labelled forest (a total of 97 species of Basidiomycota; supplementary online material) were harvested, identified, and analyzed for their isotopic composition. We distinguished mushroom locations as (1) from directly underneath the 550 m^2 labelled tree crowns, (2) from a 12 m wide ring around the labelling zone divided into two concentric sections of 6 m width each, called the "transition zone", and (3) from locations at > 12 m distance from the labelled zone (reference). The mushrooms were classified as either ecto-mycorrhizal or saprophytic, based on the taxonomic literature[8].

The mycorrhizal fungi data demonstrate a direct and immediate tree to mycorrhizal fungus carbon link in this undisturbed temperate forest (Fig. 1), which is also strongly reflected in the isotopic composition of respiratory CO_2 released from soils[9]. The time lag of 2 - 10 days resulting from the tree crown to soil-CO_2 carbon

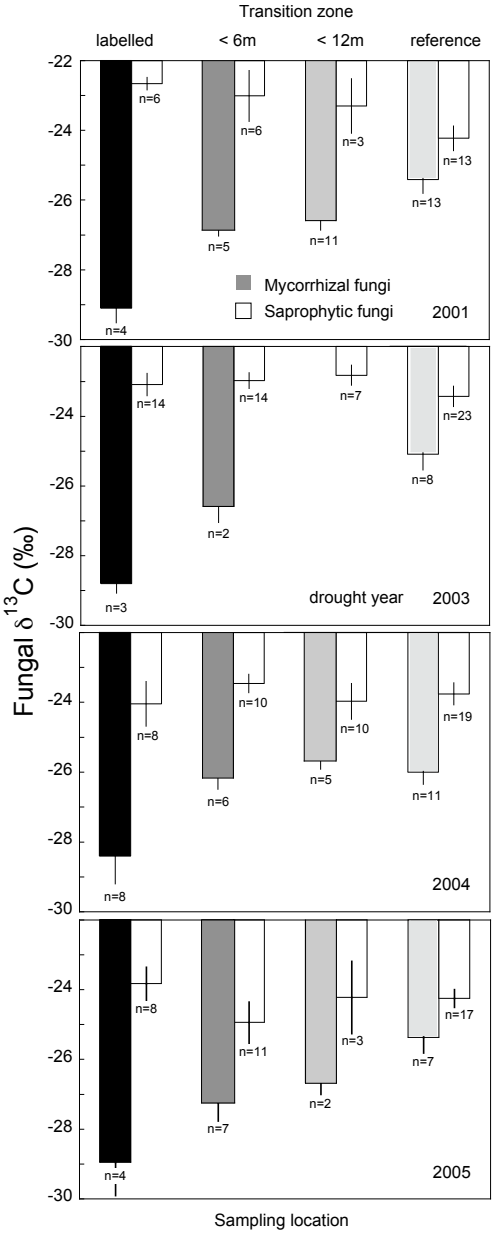

Fig. 1: Mean $\delta^{13}C$ in the fruit bodies of mycorrhizal (shaded bars) and saprophytic fungi (open bars), as found in fall of the years 2001 (upper graph) until 2005 (subsequent graphs; no fungi were collected in 2002). The x-axis represents the increasing distance from the labelled site. Error bars indicate standard errors. In the reference area $\delta^{13}C$ of mycorrhizal fungi was consistently lower compared to saprophytic fungi as shown by one way ANOVA's (2001, 2003, 2004, 2005: P=0.035, P=0.0065, P<0.0001, P=0.029). $\delta^{13}C$ values of the mycorrhizal fungi in the labelled site, but not in the transition zone are significantly lower than in the reference site in all years (α=0.05, according to Tukey's HSD post hoc test). Datasets that were not normally distributed (according to Shapiro Wilk's test for normality) were transformed prior to analysis (x^{-1}).

transfer agrees well with other soil CO_2 studies[10], with ecosystem respiration[11] and phloem sap[12, 13]. Mycorrhizal hyphae are known for a turnover of 5-6 days[14] whereas fine roots may live as long as 11 years[7, 15]. Such a rapid turnover of hyphae should also lead to rapid transfer of new C to the sporocarps, which is strongly supported by our data.

We assume that 100% new carbon in the fruit bodies would result in a 5.8‰ more negative isotope signal than in those from the ambient area[6, 7]. The incorporated amount of new carbon is calculated from the difference in the isotope signal of the fruit bodies from the elevated CO_2 area minus those grown under control trees. If no mixing of new with old carbon had occurred along the phloem path the mycorrhizal mushrooms would consist of 62% newly assimilated carbon. If there was sugar exchange between phloem and adjacent tissue along the transfer trajectory (for which we have evidence (S. Keel, unpublished data)), the calculatory net carbon input would even be higher. After one season, the mycorrhizal ^{13}C isotopic signal was 3.7‰ more negative relative to the controls, and was clearly more pronounced than in any tree tissue, including water soluble compounds from leaves, or CO_2 in soil air[9], all still containing greater fractions of pre-treatment assimilates. The pronounced decline in the isotope signal of mycorrhizal fungi between the labelled and the unlabelled zone indicate a clear spatial association with source trees. The signals dropped by 2‰ in the 6 m innermost transition zone, and by another 0.4‰ in the outer one. In both transition zones signals were indifferent from controls beyond the 12 m ring (i.e. a distance of ca. three times the crown radius from the last labelled stem).

The categorization into mycorrhizal versus saprophytic fungi was sharply reflected in the ^{13}C isotope data. The saprophytic fungi outside the treatment area were always less ^{13}C depleted than mycorrhizal fungi reflecting their higher trophic status[16,17] (Fig. 1). This functional difference was strongly enhanced by the labelled CO_2, which we applied to the tree crowns. While mycorrhizal fungi produced fruit bodies predominantly made of newly assimilated (and labelled) carbon, no such signal could be detected in the saprophytic fungi at any time. Our data also provide no evidence for a mixed saprophytic/mycorrhizal strategy among the studied fungal taxa. At no time during the five-year test period did saprophytes capture any significant ^{13}C signal, indicating a substantial (>5 years) lag between production and saprophytic consumption of plant litter. Obviously these fungi metabolize carbon resources older than five years, despite a rapid leaf litter turnover.

This slow C-transfer to the saprophytes is consistent with the low turnover of dead tree organic matter where the turn over time can range from years to centuries[18]. Our data strongly suggest that the newly assimilated carbon, which is transferred to the below ground compartment is mostly invested into labile forms of carbon, serving as "fuel" for the microbionts in the soil, a large part of which is recycled to the atmosphere through respiratory processes. The long time gap between the production of new carbohydrates and the appearance of this carbon in the saprophytic fungi also suggests a rather slow transfer of assimilated carbon to the recalcitrant of the soil carbon fraction.

Methods

For carbon isotope analysis only caps of the fruit bodies were used. They were oven-dried at 80 °C for 48 h, ground with a steel ball mill (Mixer Mill, Retsch MM 2000, Germany) and 0.6 to 0.8 mg dried powder was wrapped in tin capsules. Samples were combusted in an elemental analyzer (EA-1110, Carlo Erba Thermoquest, Italy). Via a variable open split interface (Conflo II, Thermo Finnigan Mat, Germany) gas samples were transferred to the mass spectrometer (Delta S, Thermo Finnigan Mat, Germany), which was operated in continuous flow mode. The precision for $\delta^{13}C$ analysis was < ±0.1‰.

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Supplementary online material

Table 1 ^{13}C abundance in fungal fruit bodies in a mixed temperate forest. The transition zones between the labelled and the reference site are represented by two rings (<6 m and <12 m) around the labelled zone. Each species is represented by its mean $\delta^{13}C$, standard error (s.e.), and number of fruit bodies (n) found in each specific area.

Species	Exposition	Year	$\delta^{13}C$ (‰)	s.e.	n
Mycorrhizal fungi					
Cortinarius (telamonia) pulchripes	reference	2005	-24.21		1
Cortinarius (telamonia) spec	reference	2001	-22.42		1
Cortinarius (telamonia) spec	reference	2005	-23.71		1
Cortinarius anomalus	reference	2004	-26.64		1
Cortinarius ophiopus	reference	2004	-25.94		1
Cortinarius spec	reference	2005	-24.91		1
Cortinarius torvus	reference	2004	-27.58		1
Hebeloma crustiliniforme	reference	2004	-26.00		1
Hebeloma sinapizans	reference	2001	-25.96		1
Hebeloma spec	reference	2001	-25.85	0.36	3
Hygrophorus chrysodon	reference	2004	-23.63		1
Hygrophorus cossus	reference	2001	-24.79	0.23	4
Hygrophorus discoxanthus	reference	2001	-25.19	0.10	4
Hygrophorus eburneus	reference	2001	-26.17	0.68	6
Hygrophorus eburneus	reference	2003	-24.47	0.25	3
Hygrophorus eburneus	reference	2004	-26.01	0.37	7
Hygrophorus eburneus	reference	2005	-25.93		1
Hygrophorus spec	reference	2004	-25.06	0.72	3
Hygrophorus unicolor	reference	2003	-27.41		1
Inocybe cincinnata	reference	2003	-24.78	0.38	3
Inocybe geophylla	reference	2003	-24.54	0.01	2
Inocybe geophylla	reference	2004	-26.66	1.23	4
Inocybe geophylla	reference	2005	-26.11	0.65	3
Inocybe petiniosa	reference	2005	-27.00		1
Inocybe spec	reference	2001	-26.72	0.58	3
Inocybe spec	reference	2004	-26.57		1
Inocybe spec	reference	2005	-25.76	0.53	4
Lactarius fluens	reference	2003	-26.48		1
Lactarius pallidus	reference	2001	-26.32	0.85	2
Lactarius pallidus	reference	2004	-27.14		1
Lactarius quietus	reference	2001	-24.11	0.11	9
Lactarius rubrotinctus	reference	2004	-24.79		1
Lactarius semisanguiflus	reference	2001	-24.69		1
Lactarius spec	reference	2003	-25.27		1
Lactarius subdulcis	reference	2003	-24.20		1
Russula chlorides	reference	2001	-24.70	0.16	2
Russula mairei	reference	2001	-28.22		1
Suillus granulatus	reference	2001	-25.23	0.08	2
Tricholoma terreum	reference	2003	-23.56		1
Cortinarius spec	<12 m	2001	-25.63	0.06	2
Cortinarius spec	<12 m	2004	-25.32		1
Hebeloma crustiliniforme	<12 m	2004	-26.27		1
Hygrophorus discoxanthus	<12 m	2001	-26.08	0.08	3
Hygrophorus eburneus	<12 m	2001	-26.87	0.32	7
Hygrophorus eburneus	<12 m	2004	-25.43	0.06	4
Inocybe geophylla	<12 m	2001	-27.26		1

Inocybe geophylla	<12 m	2004	-24.98	0.36	4
Inocybe geophylla	<12 m	2005	-26.34		1
Inocybe spec	<12 m	2001	-27.25		1
Inocybe spec	<12 m	2004	-26.27		1
Inocybe spec	<12 m	2005	-27.00		1
Lactarius deciptans	<12 m	2001	-25.59	0.17	2
Lactarius fluens	<12 m	2001	-26.84		1
Lactarius fulvissimus	<12 m	2001	-28.31		1
Lactarius quietus	<12 m	2001	-25.60	0.22	2
Russula mairei	<12 m	2001	-27.45		1
Tricholoma lascivum	<12 m	2001	-25.43		1
Amanita ceciliae	<6 m	2001	-27.25	0.14	3
Cortinarius (telamonia) spec	<6 m	2005	-26.90	0.77	3
Cortinarius spec	<6 m	2005	-25.26		1
Cortinarius spec	<6 m	2004	-25.28		1
Hygrophorus eburneus	<6 m	2001	-27.05	0.05	2
Hygrophorus eburneus	<6 m	2004	-26.42	0.17	2
Hygrophorus spec	<6 m	2004	-24.97		1
Hygrophorus unicolor	<6 m	2004	-26.59	0.29	3
Inocybe asterospora	<6 m	2003	-26.12		1
Inocybe corydalina	<6 m	2005	-25.85		1
Inocybe geophylla	<6 m	2001	-26.53		1
Inocybe geophylla	<6 m	2003	-27.05	0.75	2
Inocybe geophylla	<6 m	2004	-27.17	0.25	4
Inocybe geophylla	<6 m	2005	-28.94	0.68	7
Inocybe spec	<6 m	2001	-26.33	0.21	3
Inocybe spec	<6 m	2004	-26.44		1
Inocybe spec	<6 m	2005	-27.04	0.26	2
Laccaria amathystea	<6 m	2005	-28.44		1
Lactarius vellerus	<6 m	2001	-27.12		1
Tricholoma inamoenum	<6 m	2005	-28.29		1
Cortinarius spec	labelled	2003	-29.10		1
Cortinarius spec	labelled	2004	-27.32		1
Hygrophorus spec	labelled	2004	-26.14		1
Hygrophorus unicolor	labelled	2004	-28.18	0.85	2
Inocybe asterospora	labelled	2003	-28.25	0.27	5
Inocybe fuscidula	labelled	2004	-24.85		1
Inocybe geophylla	labelled	2001	-28.04	1.04	4
Inocybe geophylla	labelled	2003	-29.04	0.16	13
Inocybe geophylla	labelled	2004	-29.52	0.48	18
Inocybe geophylla	labelled	2005	-29.64	0.60	6
Inocybe phaeodisca	labelled	2005	-30.46	0.45	2
Inocybe spec	labelled	2005	-26.16	0.81	2
Lactarius quietus	labelled	2001	-28.77	0.58	4
Lactarius spec	labelled	2001	-29.70		1
Lactarius spec	labelled	2004	-30.71		1
Tricholoma inamoenum	labelled	2004	-31.64		1
Tricholoma inamoenum	labelled	2005	-29.56		1
Tricholoma lascivum	labelled	2001	-29.86	0.54	2
Tricholoma lascivum	labelled	2004	-28.79	0.50	2

Saprophytic fungi					
Baeospora myosura	reference	2003	-21.89	1.26	2
Baeospora myosura	reference	2004	-21.70		1
Calocera viscosa	reference	2003	-20.96		1
Clavariadelphus pistillaris	reference	2004	-26.61	0.39	7
Clavariadelphus pistillaris	reference	2005	-25.39		1
Clavulina cinerea	reference	2005	-25.58		1
Clavulina rugosa	reference	2005	-26.34		1
Clitocybe gibba	reference	2004	-24.22	0.12	2
Clitocybe nebularis	reference	2005	-23.40		1
Clitocybe phaeophtalma	reference	2005	-23.53	0.25	3
Clitocybe spec	reference	2005	-24.62		1
Collybia butyracea var. asena	reference	2004	-23.33		1
Collybia confluens	reference	2001	-24.43	0.09	2
Collybia confluens	reference	2005	-23.80	0.36	2
Collybia putilla	reference	2003	-26.09		1
Coprinus comatus	reference	2001	-21.83		1
Coprinus comatus	reference	2005	-23.53		1
Coprinus micaceus	reference	2003	-24.35	1.21	2
Coprinus spec	reference	2001	-23.41		1
Coprinus spec	reference	2004	-22.70		1
Coprinus xanthothorix	reference	2005	-26.11		1
Cystolepiota hetieri	reference	2004	-24.67	0.18	5
Cystolepiota hetieri	reference	2005	-24.46	0.29	2
Entoloma rhodopolium	reference	2001	-24.49	0.47	2
Entoloma spec	reference	2001	-25.46		1
Galerina spec	reference	2003	-24.96		1
Gymnopus dryophila	reference	2003	-23.62		1
Gymnopus peronata	reference	2004	-24.84		1
Hypholoma fasciculare	reference	2001	-23.07	0.19	4
Hypholoma fasciculare	reference	2003	-22.80		1
Hypholoma sublateritium	reference	2003	-24.82		1
Lepiota cristata	reference	2001	-23.02		1
Lepista nebularis	reference	2004	-22.64		1
Lycoperdon perlatum	reference	2001	-24.01		1
Lycoperdon perlatum	reference	2005	-23.28		1
Lyophyllum rancidum	reference	2004	-24.68		1
Macrolepiota mastoidea	reference	2003	-23.98	0.03	2
Macrolepiota mastoidea	reference	2004	-24.30	0.10	5
Macrolepiota mastoidea	reference	2005	-23.71		1
Marasmius alliaceus	reference	2003	-23.19	0.55	5
Marasmius alliaceus	reference	2004	-24.55	1.50	2
Marasmius androsaceus	reference	2003	-23.96		1
Marasmius cohaerens	reference	2003	-25.00		1
Marasmius torquescens	reference	2003	-24.07	0.25	7
Marasmius torquescens	reference	2004	-24.81		1
Marasmius torquescens	reference	2005	-24.24	0.27	3
Melanoleuca graminicola	reference	2003	-23.28		1
Mycena crocata	reference	2003	-23.23	0.38	18
Mycena crocata	reference	2004	-23.46	0.59	8
Mycena crocata	reference	2005	-23.80	0.38	10
Mycena galopus	reference	2003	-23.71	0.16	23
Mycena galopus	reference	2004	-23.70	0.26	10

Mycena galopus	reference	2005	-24.07	0.20	3
Mycena leptocephala	reference	2003	-20.74		1
Mycena polygramma	reference	2003	-25.18	1.23	3
Mycena pura	reference	2001	-24.55	1.41	3
Mycena pura	reference	2003	-21.58		1
Mycena sanguinolenta	reference	2004	-22.32		1
Mycena sanguinolenta	reference	2005	-22.69	1.10	2
Mycena spec	reference	2001	-23.27		1
Mycena spec	reference	2004	-22.29		1
Mycena vitilis	reference	2003	-23.15	1.14	4
Mycena vitilis	reference	2004	-25.67		1
Mycena vitilis	reference	2005	-23.66	0.04	2
Mycena zephirus	reference	2003	-22.35		1
Pluteus cervinus	reference	2004	-21.47		1
Psathyrella conopilus	reference	2003	-24.32		1
Pseudoclitocybe ciatiformis	reference	2001	-25.44		1
Stropharia cyanea	reference	2001	-25.53	0.11	2
Tubaria nivalis	reference	2004	-23.38		1
Xerula radicata	reference	2001	-26.45		1
Xerula radicata	reference	2003	-21.60		1
Clavariadelphus pistillaris	<12 m	2004	-26.68		1
Clavulina cristata	<12 m	2004	-26.52		1
Conocybe semiglobata	<12 m	2005	-23.78		1
Coprinus micaceus	<12 m	2004	-21.77		1
Entoloma rhodopolium	<12 m	2005	-26.20		1
Entoloma spec	<12 m	2001	-24.73	0.17	3
Galerina spec	<12 m	2004	-22.42		1
Hydropus subalpinus	<12 m	2003	-22.30		1
Marasmius alliaceus	<12 m	2003	-21.89		1
Marasmius torquescens	<12 m	2004	-24.04	0.80	2
Mycena crocata	<12 m	2003	-22.78	0.80	2
Mycena crocata	<12 m	2004	-22.99	0.35	13
Mycena crocata	<12 m	2005	-22.69	1.49	2
Mycena galopus	<12 m	2003	-22.62		1
Mycena galopus	<12 m	2004	-23.54	0.23	5
Mycena pura	<12 m	2001	-23.14	0.21	2
Mycena pura	<12 m	2004	-23.31		1
Mycena sanguinolenta	<12 m	2003	-22.48		1
Mycena sanguinolenta	<12 m	2004	-23.62	0.42	3
Mycena vitilis	<12 m	2003	-23.78	0.80	2
Mycena zephirus	<12 m	2004	-24.81		1
Psathyrella spec	<12 m	2001	-22.03	0.22	2
Tubaria furfuracea	<12 m	2003	-23.91		1
Clavulina cinerea	<6 m	2005	-28.06	0.76	2
Clavulina cristata	<6 m	2005	-27.52		1
Collybia peronata	<6 m	2001	-23.49		1
Conocybe silenophylla	<6 m	2005	-25.23		1
Conocybe spec	<6 m	2004	-23.47		1
Coprinus lagopus	<6 m	2005	-26.15		1
Coprinus spec	<6 m	2005	-25.84		1
Entoloma rhodopolium	<6 m	2005	-25.95	0.73	3
Entoloma spec	<6 m	2001	-26.27		1
Hypholoma fasciculare	<6 m	2001	-22.36	0.05	5

Hypholoma fasciculare	<6 m	2003	-21.93	0.30	2
Hypholoma fasciculare	<6 m	2005	-22.98	1.36	2
Lepiota spec	<6 m	2004	-23.96		1
Marasmius alliaceus	<6 m	2003	-22.87	0.52	4
Marasmius torquescens	<6 m	2003	-22.78	0.18	2
Marasmius torquescens	<6 m	2004	-24.33		1
Megacollybia platyphylla	<6 m	2003	-22.86		1
Megacollybia platyphylla	<6 m	2005	-22.40		1
Mycena crocata	<6 m	2001	-22.39	0.18	2
Mycena crocata	<6 m	2003	-22.74	0.61	7
Mycena crocata	<6 m	2004	-22.49	0.38	9
Mycena crocata	<6 m	2005	-22.80	0.30	15
Mycena galopus	<6 m	2003	-24.27	0.45	3
Mycena galopus	<6 m	2004	-24.09	0.63	5
Mycena galopus	<6 m	2005	-23.30	0.40	2
Mycena polygramma	<6 m	2001	-21.00		1
Mycena polygramma	<6 m	2003	-22.78		1
Mycena polygramma	<6 m	2004	-22.00	0.90	2
Mycena sanguinolenta	<6 m	2003	-23.53		1
Mycena sanguinolenta	<6 m	2004	-23.48	0.25	5
Mycena spec	<6 m	2003	-23.94	0.43	5
Mycena spec	<6 m	2004	-24.11	0.06	3
Mycena vitilis	<6 m	2003	-23.87	0.55	5
Mycena vitilis	<6 m	2004	-24.07	0.47	9
Mycena vitilis	<6 m	2005	-24.06	0.27	7
Pholiota mutabilis	<6 m	2003	-22.33		1
Pholiota squarrosa	<6 m	2003	-22.29		1
Polyporus varius	<6 m	2005	-24.81		1
Psathyrella spec	<6 m	2003	-21.51		1
Stropharia caerula	<6 m	2003	-23.98		1
Stropharia caerula	<6 m	2004	-22.57		1
Stropharia cyanea	<6 m	2001	-22.57		1
Conocybe spec	labelled	2001	-23.26		1
Coprinus hemerobius	labelled	2003	-22.06		1
Coprinus spec	labelled	2003	-22.89		1
Entoloma rhodopolium	labelled	2005	-26.15		1
Galerina sideroides	labelled	2001	-22.02	0.13	3
Hydropus subalpinus	labelled	2005	-22.44		1
Hypholoma fasciculare	labelled	2001	-22.58	0.14	6
Hypholoma fasciculare	labelled	2004	-22.57		1
Macrocystidia cucumis	labelled	2003	-25.31		1
Macrolepiota mastoidea	labelled	2001	-22.49		1
Marasmius torquescens	labelled	2003	-24.30		1
Marasmius torquescens	labelled	2004	-24.06		1
Megacollybia platyphylla	labelled	2003	-23.41		1
Megacollybia platyphylla	labelled	2005	-23.80		1
Mycena crocata	labelled	2003	-22.68	0.41	7
Mycena crocata	labelled	2004	-23.25	0.51	8
Mycena crocata	labelled	2005	-23.13	0.39	12
Mycena galericulata	labelled	2003	-21.80	0.27	3
Mycena galericulata	labelled	2005	-22.87		1
Mycena galopus	labelled	2003	-24.00	0.94	4
Mycena galopus	labelled	2004	-25.18	0.35	11

Mycena polygramma	labelled	2001	-22.53	1.27	2
Mycena sanguinolenta	labelled	2003	-22.20		1
Mycena sanguinolenta	labelled	2004	-25.27	0.76	2
Mycena spec	labelled	2001	-23.06	1.31	2
Mycena spec	labelled	2003	-23.34	0.48	10
Mycena spec	labelled	2004	-23.56	0.62	5
Mycena vitilis	labelled	2003	-23.44	0.32	18
Mycena vitilis	labelled	2004	-24.73	0.74	11
Mycena vitilis	labelled	2005	-24.27	0.59	4
Pholiota squarrosa	labelled	2003	-22.48		1
Psathyrella gracilis	labelled	2005	-25.30		1
Psathyrella spec	labelled	2003	-24.51	1.73	3
Psathyrella spec	labelled	2004	-26.36	0.59	2
Psathyrella spec	labelled	2005	-22.65		1
Strobilurus tenacellus	labelled	2003	-20.81		1

4 Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2

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Materials and Methods
Figs. S1 to S11
Tables S1 to S5
References

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Carbon Flux and Growth in Mature Deciduous Forest Trees Exposed to Elevated CO_2

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Whether rising atmospheric carbon dioxide (CO_2) concentrations will cause forests to grow faster and store more carbon is an open question. Using free air CO_2 release in combination with a canopy crane, we found an immediate and sustained enhancement of carbon flux through 35-meter-tall temperate forest trees when exposed to elevated CO_2. However, there was no overall stimulation in stem growth and leaf litter production after 4 years. Photosynthetic capacity was not reduced, leaf chemistry changes were minor, and tree species differed in their responses. Although growing vigorously, these trees did not accrete more biomass carbon in stems in response to elevated CO_2, thus challenging projections of growth responses derived from tests with smaller trees.

How forest trees, the largest biomass carbon (C) pool on Earth, will respond to the continued rise in atmospheric CO_2 is unknown (*1*). Is there a potential for more growth, and perhaps more C storage, as a result of CO_2 fertilization (*2*)? Are trees in natural forests already carbon saturated, given that CO_2 concentrations have already reached twice the glacial minimum concentration (*3*)?

Experimental ecology has made important advances in recent years answering such questions, but unlike grass and shrub vegetation, adult forest trees do not fit any conventional test system with elevated CO_2. Free air CO_2 enrichment (FACE) is currently applied to fast-growing plantations (*4*–*7*), but to date, tall trees in a natural forest have not been studied because of overwhelming technical difficulties. We solved this problem with a technique called web-FACE (*8*) that releases pure CO_2 through a fine web of tubes woven into tree canopies with the help of a construction crane (Fig. 1) (*9*). Here, we present responses of 32- to 35-m-tall trees in a near-natural deciduous forest in Switzerland (*9*) to a 530 parts per million (ppm) CO_2 atmosphere over 4 years. Given the size and species diversity of the study trees, this project inevitably is tree- and not ecosystem-oriented, in contrast to other FACE projects, which use smaller trees. Because plant responses to CO_2 are species-specific (*10*, *11*), insight from single-species approaches remains limited, no matter how large the test scale. The statistical power of our approach is limited at the species level because of reduced intraspecific replication, but tree-ring analysis helps to account for much of the variation in tree vigor. The ultimate effect of rising CO_2 will remain concealed within our limited time scales, yet knowing the dynamics of tree responses over a number of years helps to estimate the nonlinear, longer-term trends. The project is thus a compromise between realism and precision, given that there is no way to maximize both (*12*).

Web-FACE uses CO_2 gas with a constant carbon isotope composition ($\delta^{13}C$) of $-29.7 \pm 0.3‰$ (mean ± SE of four annual means). Mixture with ambient-air CO_2 ($\delta^{13}C = -8‰$) results in a ^{13}C tracer signal in photoassimilates that was monitored at ~50 canopy positions with "iso-meters" (small containers planted with a grass using the C_4 photosynthetic pathway), which yielded a 4-year mean of 5.8 ± 0.5‰ ^{13}C tissue depletion. Once assimilated by leaves, the signal penetrates the various biomass compartments and allows one to track the fate of carbon. During the first season, leaves accreted 40% (*Quer-*

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Fig. 1. Canopy CO_2 release by a web of porous tubes (left), with "iso-meters" (front) and gas sampling lines (back) for control, all operated from a 45-m-high crane (right).

cus), 65% (*Fagus*), 67% (*Acer*), 79% (*Carpinus*), and 100% (*Tilia*) of new carbon as compared with the iso-meter mean (*9*). By the end of the first season, new assimilates contributed 71% and 73% to newly formed leaf and wood tissue across all trees (Fig. 2), suggesting very close coupling between leaf and tree-ring construction (*13*). Tree rings of *Quercus* and *Tilia* consisted almost completely of new carbon after the first year, whereas in *Fagus*, the tree-ring signals were weaker and lagged. By the end of the fourth year, 99% of all leaf mass and 91% of all wood mass consisted of new C. After 4 years the new <1-mm fine root fraction (*9*) reached 40% (2.3‰ ^{13}C depletion in 2004), suggesting a ~10-year fine root turnover, similar to the data for other deciduous forests (*14*, *15*).

Soil CO_2 sampled in 170 gas-wells at 3- to 11-cm soil depth across the crane area was 1‰ more depleted after 7 weeks in the treatment zone and 1.8‰ after 4 months, and remained at 2.2 ± 0.2‰ during the remaining test period, indicating a near steady-state efflux of new C (38% of total CO_2 emitted). Soil CO_2 concentrations were 25% higher in the elevated CO_2 area as compared with the control area (P = 0.03) in June 2001 and reached +44% in 2002. Mycorrhizal fungi carried a 64% new carbon signal already by the end of the first year (*16*). CO_2 enrichment thus rapidly produced isotopic fingerprints in the whole leaf-soil continuum. The strong soil-air signal indicates a rapid flux of new carbon through this system, and increased soil CO_2 concentrations provide clear evidence for enhanced metabolic activity in soils under CO_2-enriched trees (*16*), as was found in smaller test systems (*17–19*) and in four forest FACE studies (*4*, *20*).

We found no downward adjustment of photosynthetic capacity in response to growth in elevated CO_2 (*21*), similar to findings for other forest FACE experiments (*22–24*) but contrasting with those for some smaller test systems (*25*). Hence, it is not surprising that leaf nitrogen concentrations in the canopy were little affected (Table 1). Only *Carpinus* showed a significant mean reduction in leaf N by 15%, and across all trees the mean depletion was not significant when the dilution by nonstructural carbohydrates (NSC) was accounted for. *Quercus* and the subdominant taxa showed a significant +22% to +25% (P = 0.005) increase of NSC in leaves in elevated CO_2, similar to that for sweetgum in the Oak Ridge FACE (*23*). Across all trees, NSC increased by 12% (Table 1). NSC concentrations in branch wood were not significantly affected. The slight reductions in N and increases in NSC across trees were largely driven by the presence of two taxa, whereas the 5 to 8% reduction in specific leaf area (SLA) was not species-specific. *Fagus*, the only species with a periodic growth response (see below), did not show a CO_2 response in any of these traits.

Foliage per unit land area (leaf area index, LAI) and leaf duration did not exhibit consistent changes. Elevated CO_2 increased mean leaf duration for 2002 to 2004 in *Carpinus* and *Fagus* by 5 to 6 days and reduced it by 5 days in *Quercus* (marginally significant for the species × CO_2 interaction for *Quercus* and *Fagus*; P = 0.068). Annual litter production did not respond to CO_2 (Fig. 3). It indicates a constant LAI of ~5, in line with data for younger monocultures (*7*, *17–19*, *26*).

Total C and N in fresh fallen litter did not change significantly (*9*), but NSC concentrations (varying from 1.8 to 5.7%) rose, suggesting an overflow of photoassimilates channeled to the decomposer pathway (Table 2). Lignin concentrations ranging from 6.5 to 15% across species in ambient CO_2 were significantly reduced in elevated CO_2, largely driven by *Fagus* and the subdominant taxa. *Quercus* litter was hardly affected. These results indicate a shift in carbon fractions from recalcitrant to more labile compounds. Consequently, litter mass loss was enhanced after 220 days of in situ decay (largely driven by *Carpinus* and the subdominant taxa). In summary, litter became richer in starch and sugar, poorer in lignin, and decomposed faster, but the effect was species-specific. Ecosystem consequences will thus reflect species presence and abundance.

Using pretreatment radial growth rates of trees (tree-ring analysis) (*9*), we standardized basal stem area increments during the treatment period derived from girth tapes. *Fagus* showed a sharp growth stimulation in the first year (+ 92%, P = 0.026), whereas no effects were observed in the other taxa at any time (Fig. 4). The CO_2 response in *Fagus* became insignificant in 2002, but recovered in 2003 (P = 0.028) during a centennial heat wave during which elevated CO_2 improved plant water relations (*27*). The cumulative 4-year response of *Fagus* was only marginally significant at P = 0.07. We performed a second analysis for trees irrespective of species that yielded no significant basal area effect, because the *Fagus* signal was diluted by other species signals and, hence, the cumulative CO_2 signal for all trees in 2004 was zero (P = 0.79), suggesting C saturation (*3*, *28*). Tree-ring chronologies illustrate that the four treatment seasons included a pair of best and worst years over 9 years of growth data, partly explaining the greater variation. Tagged and revisited leading shoots in the top canopy showed no consistent length growth response across species and years (mean annual increment ~15 cm). Fruiting was un-

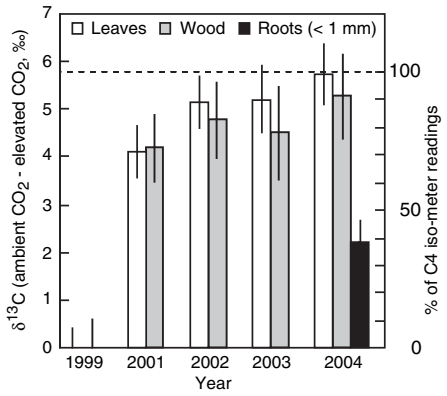

Fig. 2. New carbon in leaves, tree rings, and fine roots as assessed by ^{13}C tracer signals (means ± SE). The maximum steady-state carbon isotope difference of 5.8‰ between ambient and elevated CO_2 is represented by the iso-meter reference value (C_4 grass growing in the canopy, straight dashed line).

Table 1. Leaf nitrogen (N), nonstructural carbohydrates (NSC), and specific leaf area (SLA) across taxa for 13 trees growing in elevated CO_2 versus 16 control trees [repeated measures analysis of variance (ANOVA), with pretreatment values for each individual tree as covariable]; data are for 2001 to 2004, and means for early and late summer are pooled. Dry matter was used as a common reference, either including or excluding NSC.

Leaf trait	Difference (%)	P-value	Main driver
Leaf NSC	+12	0.007	Quercus, TAP*
N, total	−10	0.027	Carpinus, TAP
N, NSC-free	−7	0.136	Carpinus
SLA, total	−8	0.002	Nonspecific
SLA, NSC-free	−5	0.025	Nonspecific

*TAP is a mix of rare taxa of the genera *Tilia*, *Acer*, and *Prunus*.

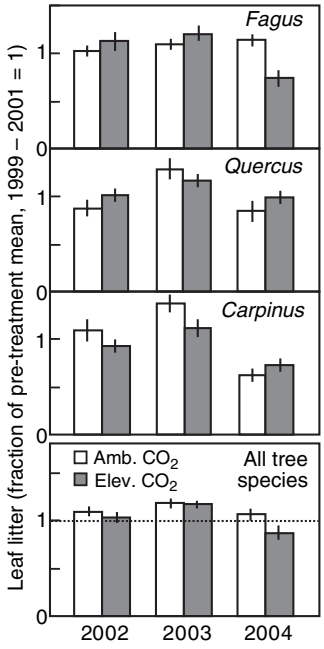

Fig. 3. Leaf litter production standardized (divided) by the mean for two pretreatment years and the year 2001 (*9*) for each litter trap (218 ± 9 g m^{-2} for 56 traps) to account for a priori spatial variability (means ± SE).

affected except for a gain in a single masting year in *Carpinus* (Fig. 5).

The data suggest no lasting growth stimulations by CO_2 enrichment in these mature trees after 4 years. It should be emphasized that the dominant study trees have reached only half of their average life span, have reached about two-thirds of their maximum size, and are growing vigorously by forestry standards (*9*). Other forest FACE experiments with younger trees have either shown a continuous growth stimulation (*Pinus taeda*) (*4*) or a transitory response, very similar to the one shown here for *Fagus* (*Liquidambar styraciflua*) (*5*). A lack of a response in stem growth or leaf litter production does not preclude a faster rate of below-ground (root) production, as was shown for the Oak Ridge FACE (*15*), but these are transitory C pools, which could translate into more recalcitrant forms of carbon in soil humus, mineral nutrients permitting.

Initially we asked whether forest trees will reduce their C uptake or trap more carbon in a CO_2-rich world. Our data suggest that they instead "pump" more carbon through their body. The few secondary effects found varied with species. Had we studied only single species, each would have led us to draw different conclusions: one species reducing its protein concentration, another consistently increasing its leaf carbohydrates, litter being affected in one group of species and not in others, and one species showing a transitory growth stimulation and others not. The Swiss forest FACE study thus points at the crucial role of tree species identity (*29*) and so far does not support expectations of greater carbon binding in tree biomass in such deciduous trees. The lack of a growth response or the transient response in one species is unlikely to be associated with N shortage (*30*), given the overabundance of N in this region (*9*). Broader stoichiometric constraints, soil microbial feedback (*18*, *31*), or counteracting ambient ozone (*6*, *32*) may hold answers. In summary, we find no evidence that current CO_2 concentrations are limiting tree growth in this tallest forest studied so far.

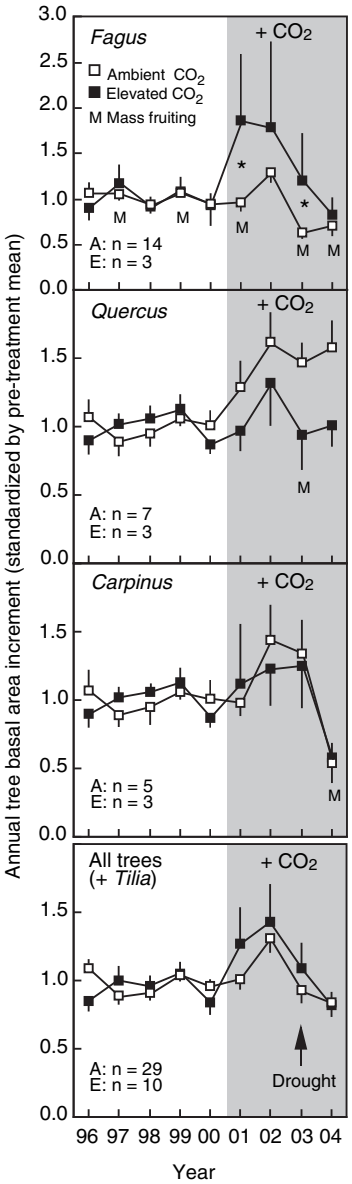

Fig. 4. Stem basal area (BA) growth of trees, standardized by the mean pretreatment BA increment for each tree. The two asterisks in *Fagus* indicate the only significant stimulation; 2003 was a drought summer; no significant $CO_2 \times$ year interaction in any species). The bottom diagram is for all trees that showed measurable growth, i.e., 10 in elevated CO_2 versus 29 controls (means ± SE).

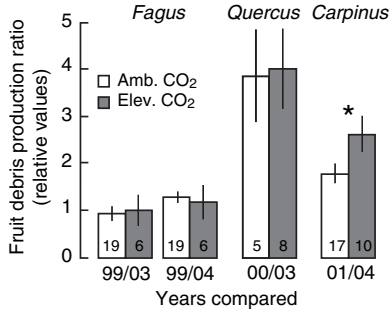

Fig. 5. Fruit debris collected with litter traps for three different tree species in years of mass fruiting. Pretreatment-to-treatment ratios for years with mass fruiting were used to account for spatial variability (numbers within bars for *n* traps). For *Fagus* we show the two latest mass fruiting episodes. The height of the bars denotes the degree of mass fruiting in the respective years ($P = 0.047$ for *Carpinus* in 2004; mean ± SE).

Table 2. Change in litter composition (% dry matter) as well as 220-day decomposition of fresh leaf litter collected in the canopy (two-factorial ANOVA with CO_2 and species as fixed factors).

Litter trait	Difference (%)	*P*-value	Main driver
Total C	+2	0.03	Nonspecific
Total N	−1	0.73	–
NSC*	+21	0.002	*Fagus*, TAP†
Lignin‡	−11	0.007	*Fagus*, TAP
Litter mass loss	+14	0.001	Carpinus, TAP

*Nonstructural carbohydrates (sugar, starch). †TAP is a mix of rare taxa of the genera *Tilia*, *Acer*, and *Prunus*. ‡Correcting for NSC-free litter mass changed the relative difference in lignin between CO_2 treatments from –10.7 to –10.0% ($P = 0.01$) but had no influence on the relative difference in nitrogen concentration.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5739/1360/DC1
Materials and Methods
Fig. S1

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5 Tracing arthropod movement in a deciduous forest canopy using stable isotopes

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Abstract

Using the Swiss canopy crane we investigated arthropod movement patterns in the canopy of a species-rich temperate forest. Since late September 2000, 14 trees of six tree species were exposed to elevated CO_2 using the web-FACE method (a special method of Free Air CO_2-Enrichment) and simulating future atmospheric CO_2 concentrations during the growing season. The CO_2-enrichment gas had a specific $\delta^{13}C$ signature, which could be traced in leaf tissue and in higher trophic levels (e.g. herbivores). Using this $\delta^{13}C$ signal we investigated the mobility of arthropods between the CO_2-enriched and the control areas. In the phytophagous groups studied we found significant correlations between $\delta^{13}C$ levels of leaf tissue with those in the body tissue of aphids, lepidopteran caterpillars, bush-cricket nymphs, true bugs (nymphs) and leafhoppers (nymphs and adults), suggesting that these groups display limited spatial shifts between the CO_2-enriched and the control areas. In contrast, these correlations were not found with fully winged adults of bush-crickets and true bugs, which suggested a higher degree of mobility in the canopy. Omnivorous earwigs revealed a marginally significant correlation between $\delta^{13}C$ of body tissue and that of leaves, whereas no correlations were found for predators (zoophages), which is likely to be due to a wide prey spectrum, which diluted the $\delta^{13}C$ signature.

Zusammenfassung

In der vorliegenden Arbeit untersuchten wir die Mobilität verschiedener Arthropoden im Kronendach eines typischen europäischen Mischwaldes. Wir nutzten hierzu den Swiss Canopy Crane (SCC) bei Hofstetten (Solothurn, Schweiz), der einen leichten Zugang in die Kronenregion ermöglicht. Seit Ende September werden die Kronen von 14 ausgewachsenen Bäumen (6 Baumarten) einer erhöhten atmosphärischen CO_2-Konzentration ausgesetzt. Das benutzte CO_2 Gas besitzt eine spezifische $\delta^{13}C$ Signatur, die in Blätter und höhere trophische Ebenen (z.B. Herbivore) eingebaut als Tracer verwendet werden kann. Wir bestimmten den $\delta^{13}C$ Wert verschiedener Arthropodengruppen als Maß für deren Mobilität im Kronendach. Von den untersuchten Arten hatten Laubheuschrecken (Larven), Wanzen (Larven), Schmetterlingsraupen, Blattläuse und Zikaden (Larven und Adulte) einen geringeren Aktionsradius, als die adulten, geflügelten Laubheuschrecken und Wanzen. Bei omniphagen Gruppen (Spinnen, Ohrwürmer und Ameisen) fand sich kein oder nur ein geringer Zusammenhang zwischen dem $\delta^{13}C$ Wert der Blätter und der darauf gefundenen Individuen. Dies kann in erster Linie durch das breit gefächerte Nahrungsspektrum dieser Gruppen erklärt werden, durch welches das $\delta^{13}C$ Signal stark beeinflusst wurde.

Introduction

Stable carbon isotopes have become an indispensable tool for tracing the fate of carbon in ecosystems. Among the earliest attempts to assess the feeding behaviour and mobility of animals was that by Tieszen et al. (1979) in Africa, who documented the intake of forage by grazers from habitats dominated by C3 or C4 grasses. Links between breeding and wintering grounds of birds have been established by analysing $\delta^{13}C$ of different populations (cf. Rubenstein and Hobson 2004). Food-web studies, particularly the assignment of different animal groups to trophic levels, have adopted isotope signals successfully (Ponsard and Arditi 2000, Eggers and Jones 2000).

Until now, this technique has been applied rarely to arthropods in forest canopies (*e.g.* Blüthgen *et al.* 2003), although they play a key role in ecosystem functioning (Ozanne *et al.* 2003). Previous studies concerning arboreal arthropods, considered changes in seasonal abundances of arthropod communities in canopies (*e.g.* Wagner 2001) or with the spatial distributions of certain insect taxa (*e.g.* Simon and Linsenmair 2001).

Arthropod diversity was assessed in temperate European forest canopies, by Schubert (1998), Floren and Schmidl (1999), Kraus and Floren (2002) and Arndt (2004) and the spatial distribution of canopy herbivores was studied (*e.g.* leaf galls) by Kamplicher and Teschner (2002). The influence of elevated atmospheric CO_2 on insect guilds was explored by Altermatt (2003). However, the mobility of different insect taxa in the canopy has yet to be investigated. Here, we capitalise on a large forest isotope tracer experiment which permitted us to study insect movement patterns from and to a canopy area which received CO_2-enrichment and thus produces $\delta^{13}C$ depleted food.

The experiment was conducted at the Swiss Canopy Crane (SCC) site, where long-term responses of temperate deciduous forest trees to atmospheric CO_2-enrichment are explored (*e.g.* Cech *et al.* 2003, Leuzinger *et al.* 2005, Zotz *et al.* 2005). For arthropods of little mobility there should be a close correlation between the isotope signatures of arthropods and the leaves on which they feed. For highly mobile insects, we expect that $\delta^{13}C$ of arthropods would not correlate with host plant tissue values. High mobility enables to switch between different trees and different nutrient quality, which can affect life history traits. Insects, which possess limited mobility, either during their nymphal stages or as adults, are forced to feed on one specific tree (or a limited area in the canopy), whereas more mobile insects can change trees and search for alternative food resources.

In this study we quantified the net outcome of mobility for different insect groups in a temperate forest canopy. We differentiated insects by developmental stages and explored whether wingless insects (or nymphs) are able to move between trees. We hypothesize, that (1) due to different feeding types (phytophagous, zoophagous or omnivorous) there are differences in correlations between $\delta^{13}C$ of insect and host plant leaf tissue and that (2) insects which are capable of flight feed on a more diverse set of trees and thus show greater movement in the canopy, reflected in no or poor correlations between $\delta^{13}C$ of leaves and insects.

Material and Methods

Study site (Swiss Canopy Crane SCC)

The experimental facilities are located in a forest close to Hofstetten, 15km south of Basel, Switzerland (47° 28′ N 7° 30′ E, 550m a.s.l.). Tree species composition is dominated by approximately 80-100 years old beeches

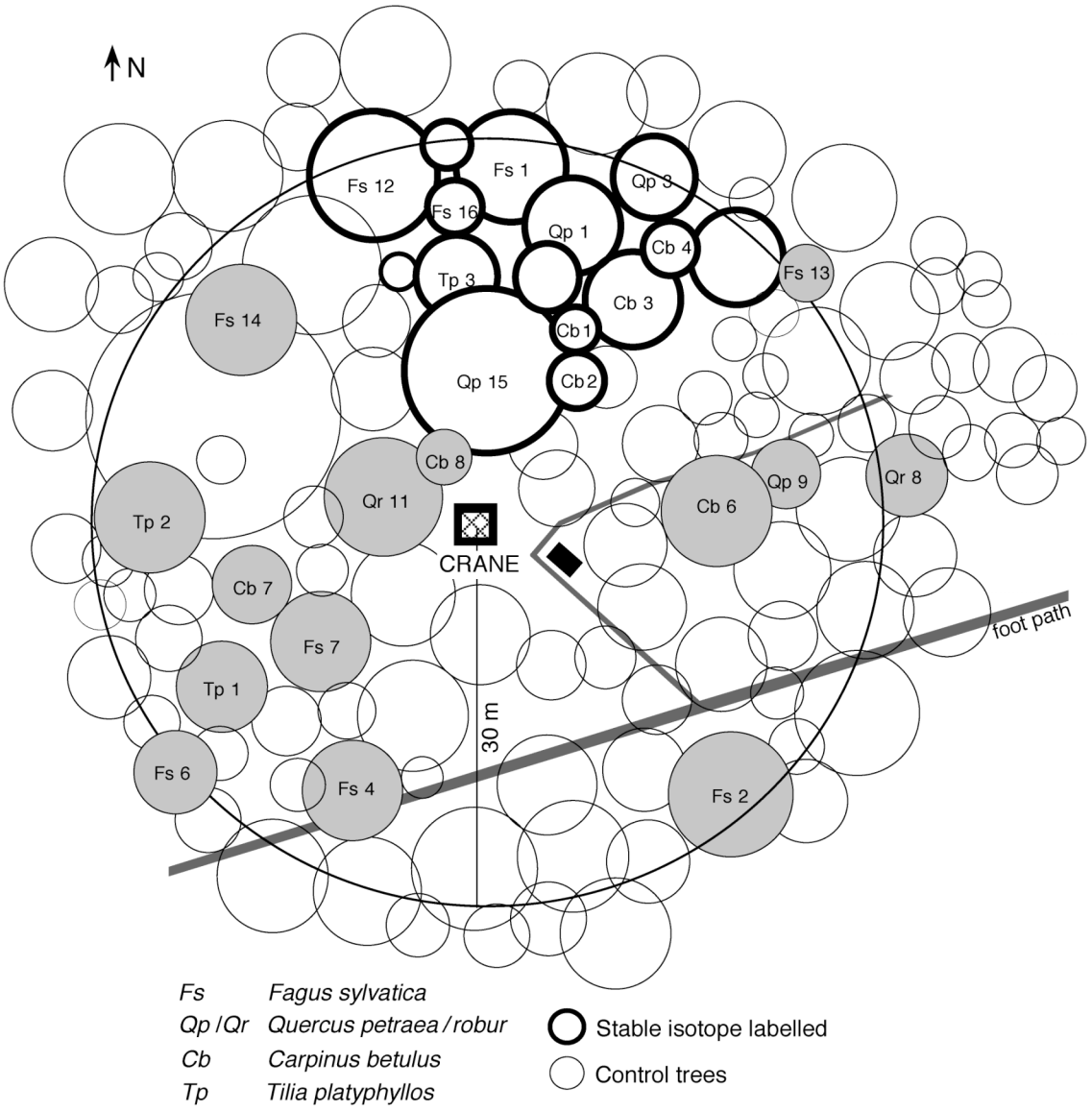

Fig. 1 Map of the SCC site near Basel, Switzerland. Numbered trees were sampled.

(*Fagus sylvatica* [L.]) and oaks (*Quercus petraea* [L.]) with heights between 30 to 36 m. Companion species are *Carpinus betulus* (L.), *Tilia platyphyllos* (L.), *Acer campestre* (L.), *Prunus avium* (L.) and some conifers (*Larix decidua* [Mill.], *Picea abies* [L.], *Pinus sylvestris* [L.], and *Abies alba* [Mill.]). A total of ca. 110 trees could be reached from the crane which covered a ground area of 2800m^2 (Fig. 1, Pepin and Körner 2002, Körner and Zotz 2003). Since September 2000, 14 trees of six tree species were exposed to elevated CO_2 levels using the web-FACE method (a special method of Free Air CO_2-Enrichment) for simulating future atmospheric CO_2 concentrations (Pepin and Körner 2002). This method uses 4mm wide plastic tubes woven into the tree crowns (300-1000m tubing per tree), which release pure CO_2 through laser-punched holes. The CO_2 release is controlled by a computer, connected with a 25-channel infrared gas analyser (IRGA), sampling air from triplet end tubes (a total of 75 sampling points). Within the upper crown of trees exposed to high CO_2 the daily mean concentration was between 526 and 566 µmol mol^{-1}, compared to controls at current ambient CO_2 concentrations of circa 375 µmol mol^{-1}. Three individuals of *Fagus* and four of *Quercus*, and *Carpinus*, and one each of *Tilia*, *Acer*, and *Prunus* are growing in elevated CO_2. Using a gondola, a 45m high construction crane provided access to every position in the forest canopy. Due to its fossil fuel origin, the CO_2 used to enrich the canopy carried a strong stable carbon isotope label ($\delta^{13}C$ of *ca.* -31‰ in comparison with a level of CO_2 in ambient air of -8‰) and thus serves as a tracer. Comparing $\delta^{13}C$ values between samples collected in the CO_2-enriched and the control area permitted identification of the origin of carbon. In addition, the differences in $\delta^{13}C$ between the two treatments were related to the amount of new C in a specific C-pool.

Insect sampling

On two sampling dates in 2003 (30 May and 19 August), the third year of CO_2-enrichment, a total of 25 trees of 4

Table 1 List of collected taxa and species in the Hofstetten forest canopy. * used for isotope studies, L: nymphs, I: Imagines. Zoo: zoophagous, phyto: phytophagous, om: omnivorous. Lepidoptera: caterpillars only. *C*: *Carpinus betulus*, *F*: *Fagus sylvatica*, *Q*: *Quercus petraea, T*: *Tilia platyphyllos*

Order	Family	Genus/Species	Feeding guild	Host trees			
				C	*F*	*Q*	*T*
Araneida*	Araneidae		Chewer, zoo	x	x	x	x
Araneida *	Araneidae	*Araniella c.f cucurbitina*	Chewer, zoo				
Araneida *	Philodromidae		Chewer, zoo		x		
Araneida *	Salticidae		Chewer, zoo		x		
Araneida *	Tetragnathidae	*Tetragnatha*	Chewer, zoo		x		
Auchenorrhyncha*	Cercopidae	*Aphrophora alni*	Sucker, phyto	x			
Auchenorrhyncha*	Cicadellidae	*Oncopsis*	Sucker, phyto			x	
Auchenorrhyncha*	Cicadellidae	*Iassu lanio*	Sucker, phyto		x	x	
Blattodea	Blattidae	*Ectobius sylvestris*	Chewer, phyto	x		x	
Coeleoptera	(Cantharoidea)		Chewer, phyto	x	x		
Coeleoptera	Coccinellidae	*Coccinella*	Chewer, phyto	x	x	x	
Coeleoptera	Curculionidae	*Curculio nucum*	Chewer, phyto	x	x	x	
Coeleoptera*	Curculionidae	*Rhynchaenus fagi*	Chewer, phyto		x		
Coeleoptera	Elateridae		Chewer, phyto	x	x		
Coeleoptera	Scolytidae		Chewer, phyto			x	
Dermaptera*	Forficulidae	*Chelidurella guentheri*	Chewer, om	x		x	x
Diptera				x			
Ensifera	Phaneropteridae	*Barbitstes serricauda*	Chewer, phyto	x		x	x
Ensifera	Phaneropteridae	*Leptophyes punctatissima*	Chewer, phyto			x	
Ensifera*	Meconematidae	*Meconema meridionale*	Chewer, zoo	x	x	x	
Ensifera*	Meconematidae	*Meconema thalassinum*	Chewer, zoo	x		x	x
Heteroptera*	Miridae	*Dryophilocori lavoquadrimaculatus*	Phyto/zoo			x	
Heteroptera*	Miridae	*Psallus*	Phyto		x		
Heteroptera*	Miridae	*Rhabomiris striatellus*	Phyto (L)/zoo (I)		x		
Heteroptera*	Pentatomidae	*Dolycoris baccarum*	Phyto	x			
Heteroptera*	Pentatomidae	*Palomena prasina*	Phyto	x	x	x	
Heteroptera*	Pentatomidae	*Palomena viridissima*	Phyto	x			
Heteroptera*	Pentatomidae	*Pentatoma rufipes*	Sucker, phyto		x		x
Hymenoptera*	Formicidae	*Lasius fuliginosus*	Chewer, om	x	x	x	x
Lepidoptera*			Chewer, phyto		x	x	x
Planipennia	Chrysopidae		Chewer, zoo			x	
Sternorrhyncha*	Drepanosiphidae	*Phyllaphis fagi*	Sucker, phyto		x		

species were sampled [7 *Carpinus* (4 in elevated CO_2, 3 control trees), 9 *Fagus* (3 in elevated levels of CO_2, 6 control trees), 6 *Querucs* (3 in elevated levels of CO_2, 3 control trees) and 3 *Tilia* (1 in elevated levels of CO_2, 2 control trees)] (Fig. 1). Samples were collected on warm, sunny days without precipitation. Three randomly chosen branches per tree in the upper canopy were shaken, while holding a round fabric-frame (∅60 cm) underneath the branch. Arthropods were transferred into plastic tubes and their guts were emptied within 12 hours prior to freezing at -30°C. Aphids were sampled only in May. They were collected with tweezers and transferred directly to tin cups prior to oven drying. All insects were classified to their family or, where possible, to their genus or species and separated based on their developmental stage. After oven drying at 70°C, insects were ground with a steel ball mill (Mixer Mill, Retsch MM 2000, Germany). Very small arthropods were measured as a whole to minimize the loss of biomass during grinding. Insect extremities and wings were not included. For $\delta^{13}C$ analysis 0.6-0.8 mg of dried powder was wrapped in tin cups. The samples were then combusted in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Italy) and transferred via an open split interface (Conflo II, Finnigan Mat, Germany) to a mass spectrometer (Delta S, Thermo Finnigan Mat, Germany).

In total, 262 arthropods were collected and the $\delta^{13}C$ was determined for each specimen (Table 1). For the mobility study, we selected the main groups only and investigated 32 spiders (adults), 9 earwigs (nymphs), 29 ants (workers only), 13 lepidopteran caterpillars, 28 weevils (17 *Rhynchaenus fagi* (L.), Coleoptera, Curculionidae, adults), 29 bush-crickets (Meconema sp., Orthoptera, Tettiginiidae, nymphs and adults), 64 true bugs (40 Miridae (data not shown), 24 Pentatomidae, nymphs and adults) and 34 cicadae (mainly Iassus lanio (L.), Hemiptera, Cicadellidae, nymphs and adults, Table 1).

Leaf sampling

In August 2003 five leaf discs per tree were collected at random in the upper canopy and pooled prior to grinding. Samples were analysed for $\delta^{13}C$ as described above.

Table 2 Results of linear regressions for $\delta^{13}C$ of insects and $\delta^{13}C$ of host plant leaves. L: nymphs, A: adults. *P*-value indicates whether the slope of the regression differs from zero. *<0.05, **<0.01, ***<0.001

Insects	L/A	N	R^2 ($\delta^{13}C$)	*F* ($\delta^{13}C$)	*P* ($\delta^{13}C$)
Aphidae (*P. fagi*)	L	7	0.83	$F_{1,18}$ = 90.2	< 0.001***
Araneida	A	19	0.08	$F_{1,30}$ = 2.7	0.11
Auchenorrhyncha	L	10	0.62	$F_{1,19}$ = 30.9	< 0.001***
Auchenorrhyncha	A	9	0.40	$F_{1,11}$ = 7.2	0.02*
Coleoptera (*R. fagi*)	A	11	0.21	$F_{1,15}$ = 4.0	0.06
Dermaptera (*C. guentheri*)	L	2	0.42	$F_{1,7}$ = 5.0	0.06
Ensifera (*Meconema*)	L	8	0.47	$F_{1,17}$ = 14.9	< 0.01**
Ensifera (*Meconema*)	A	5	0.00	$F_{1,8}$ = 0.0	0.99
Heteroptera (*Pentatoma*)	L	2	0.81	$F_{1,3}$ = 18.3	0.02*
Heteroptera (*Pentatoma*)	A	13	0.00	$F_{1,14}$ = 0.1	0.78
Hymenoptera (*L. fuliginosus*)	A	16	0.02	$F_{1,15}$ = 0.3	0.58
Lepidoptera	L	7	0.61	$F_{1,9}$ = 14.2	< 0.01**

In addition, the leaves upon which aphids were found, were also collected.

Statistical analyses

Linear regressions were used to correlate $\delta^{13}C$ values of insects with the $\delta^{13}C$ signal of leaves of the respective host tree.

Leaf samples were available from August only. Consequently, we plotted our $\delta^{13}C$ values of insects from either May or August against those of leaves collected in August. Since this was the third year of CO_2-enrichment, we expected a similar mix of old and new carbon in all species growing in elevated CO_2. However, there was an unexpectedly high amount of new carbon in the specimen of *Tilia* (Keel et al., unpublished data). $\delta^{13}C$ of leaves is known to be higher in spring (ca. 1‰, Keel et al., unpublished data, Damesin and Lelarge 2003), most probably due to the accumulation of $\delta^{13}C$-enriched starch. During the growing season, $\delta^{13}C$ decreases due to the incorporation of photoassimilates with more negative $\delta^{13}C$ values in comparison with starch.

Assuming the same trend for all tree species, the correlations between $\delta^{13}C$ values of leaves and insects should be affected by this trend in a systematic way. Only the slopes of the regressions would have changed. A significant correlation between $\delta^{13}C$ values of arthropod and those of host plant suggests that arthropods mobility is poor. Differences in $\delta^{13}C$ values were analysed using ANOVA with the tracer treatment as fixed factor.

Results

$\delta^{13}C$ values *in leaf tissue and arthropods*

Leaves of trees growing in elevated CO_2 concentrations revealed a significantly lower $\delta^{13}C$ value in comparison with the controls, based on the results of a two-way ANOVA with the CO_2 treatment and species as fixed factors. The difference between leaves of ambient and elevated concentrations of CO_2 was highly significant [-27.3±0.2‰ (n=14) in comparison with -31.9±0.8‰, (n=11) $F_{1,17}$=53.9, *P*<0.001], whereas no significant difference of $\delta^{13}C$ values between tree species was found [*Carpinus*: -29.7±0.9‰ (n=7), *Fagus*: -28.4±0.8‰ (n=9), *Quercus*; -30.3±1.4‰ (n=6), *Tilia*: -29.5±2.7‰ (n=3), $F_{3,17}$=2.1, *P*=0.14], and no significant interaction between the both factors occurred ($F_{3,17}$=2.4, *P*=0.10). Differences reflect tree species-specific uptake of the $\delta^{13}C$ tracer and, in part, different degrees of exposure to elevated CO_2, and hence, to the isotope signal [$\delta^{13}C$ (ambient CO_2) - $\delta^{13}C$ (elevated CO_2): *Carpinus*: 4.1‰, *Fagus*: 2.9‰, *Quercus*: 5.9‰, *Tilia*: 8.1‰.

Comparing arthropod individuals found in the CO_2-enriched and the control areas, we found significant differences in $\delta^{13}C$ values for aphids (diff.: 3.8‰, $F_{1,18}$=19.73, *P*<0.001), lepidopteran caterpillars (diff.: 3.9‰, $F_{1,9}$=25.31, *P*<0.001), true bugs (nymphs, diff.: 3.0‰, $F_{1,3}$=10.23, *P*<0.05), leafhoppers (nymphs, diff.: 3.2‰, $F_{1,17}$=14.22, *P*<0.01 and adults, diff.: 2.4‰, $F_{1,3}$=11.22, *P*<0.01), bush-crickets (nymphs, diff.: 2.0‰, $F_{1,18}$=34.43, *P*<0.001), coleopterans (diff.: 1.8‰, $F_{1,15}$=17.57, *P*<0.001) and dermapterans (diff.: 1.6‰, $F_{1,7}$=5.60, *P*<0.05). No significant differences were observed for spiders, true bugs (adults) and hymenopterans (ants). When examined for each CO_2 treatment separately, $\delta^{13}C$ differed between insect taxa within both treatments. However, differences in $\delta^{13}C$ values were more pronounced in the CO_2-enriched area (ambient: CO_2: $F_{11,97}$=1.64, *P*<0.10, elevated: CO_2: $F_{11,84}$=9.76, *P*<0.001). In groups where nymphs as well as adults were collected (bush-crickets, heteropterans and leafhoppers) $\delta^{13}C$ signatures were higher in adults in comparison with nymphs. Again, the difference was more pronounced in insects collected in the CO_2-enriched area in comparison with the control.

Arthropod movement

In aphids (*Phyllaphis fagi* (L.) Hemiptera, Aphididae, lepidopteran caterpillars, and bush-cricket nymphs (*Meconema* sp.) $\delta^{13}C$ values of leaves and insects correlated closely, whereas $\delta^{13}C$ of leaves correlated only marginally with coleopteran (*R. fagi*) and dermapteran species (*Chelidurella guentheri* Galvagni, Dermaptera, Forficulidae; Fig. 2, 3, Table 2).

The correlation between $\delta^{13}C$ values of host plant leaf tissue and that of aphids was most pronounced, most probably due to the fact that they are (1) the least mobile group and (2) that leaves on which aphids were found were also those used for analysis. In contrast, we found no correlation for spiders (Araneida) and ants (Hymenoptera, Fig. 3, Table 2).

With regard to the insect taxa where nymphs and imagos were found, a $\delta^{13}C$ relationship with leaf tissue occurred in bush-crickets (nymphs), true bugs (nymphs)

and leafhoppers (nymphs). The correlation between leafhoppers and leaf tissue remained in imagos, although it was somewhat weaker, whereas none was found in adult heteropteran and bush-crickets (Fig. 3, Table 2).

Interestingly, when testing the relationship of arthropods' $\delta^{13}C$ and $\delta^{13}C$ of leaf tissue separately, either for the control or the CO_2-enriched areas, we found that, in the latter, there were still significant correlations, probably due to the higher variability of $\delta^{13}C$. Significant or marginally significant correlations in elevated CO_2 were found in bush cricket ($F_{1,10}$=3.37, P<0.1) and leafhopper nymphs ($F_{1,7}$=5.23, P<0.06), ants ($F_{1,6}$=26.9, P<0.01) and aphids ($F_{1,11}$=42.7, P<0.001).

Discussion

Using $\delta^{13}C$ as a tracer, we found different mobility of arthropod taxa in the canopy of temperate trees. The $\delta^{13}C$ values of host plant leaf tissue and insects correlated significantly in declining order for aphids, leafhoppers (nymphs and adults), bush-crickets (nymphs), lepidopteran caterpillars, and true bugs (nymphs). The significance of these correlations was driven by the differences in $\delta^{13}C$ values between leaves and arthropods collected in the CO_2-enriched and control areas. Thus, we conclude that these arthropods remain in a specific area of the canopy and do not shift between CO_2-enriched and control trees. However, since different tree species within the same treatment differed little in their leaf $\delta^{13}C$ values, it is possible that insects moved from tree to tree within the same treatment area, as shown for leafhopper nymphs and bush-cricket nymphs. Apart from aphids, it is likely

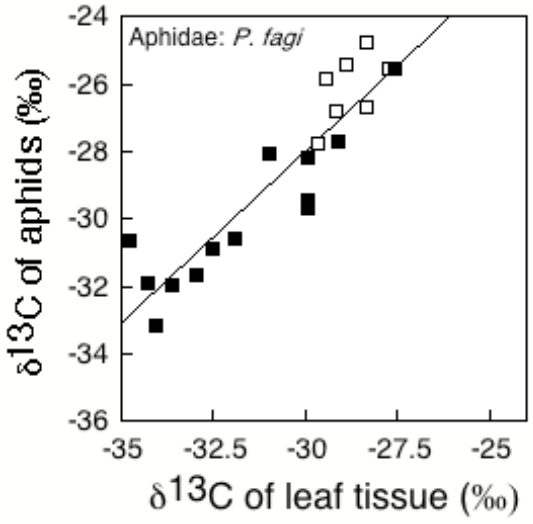

Fig. 2 Linear regressions of $\delta^{13}C$ content of aphids (*P. fagi*) and $\delta^{13}C$ of host plant (leaf tissue). Black symbols: arthropods found in the CO_2-enriched area, white symbols: arthropods found in the control area.

that all arthropods studied here moved to a certain extent, from one branch to another or even from one tree to another. However, in some groups there are shifts between CO_2-enriched and control areas indicating that these arthropods moved between these areas. *A posteriori* it is not surprising that the correlations were strongest in phytophagous insect groups due to the $\delta^{13}C$ signature being reflected in $\delta^{13}C$ tissue of insects, in contrast with zoophagous spiders or omnivorous insects, consuming a wider range of food material with different $\delta^{13}C$ values. In the following section we discuss the examined groups in more detail. Of course, we cannot assign the results found for one species to the complete taxa. Different species have different life histories, which must be considered when studying insect mobility in the canopy. For example, within the bush-crickets the phytophagous (e.g. *Barbitistes serricauda* Fabricius, Tettigoniidae, Phanopteridae) and zoophagous species *Meconema thallasinum*, Tettigoniidae, Meconematidae, will differ in their mobility since the former is incapable of flight, in contrast with the latter.

(1) Groups showing a low degree of mobility

Aphidae (aphids): the strongest relationship between $\delta^{13}C$ values of leaves and insects was found in wingless nymphs of *P. fagi*. Since this species forms colonies on beech leaves, it is most likely that the nymphs remain on the same branch or even on the same leaf and therefore their entire biomass was synthesised at a specific site in the crown.

Auchenorryncha (leafhoppers): we found positive correlations for both leafhopper nymphs and adults. Typically, the three studied species here (*Oncopsis* sp., Auchenorrhyncha, Cicadellidae, *Aphrophora alni* Fallén, Auchenorrhyncha, Cercopidae, *Iassus lanio*) are found on woody plants or in forests (Waloff 1973, Günthard 1987, Nickel 2003). Although these species are able to fly, our results suggest that nymphs and adults do not move a lot. Similarly, Blüthgen *et al*. (2003) found that $\delta^{13}C$ values of homopterans (aphids and leafhoppers) correlated significantly with those of their host plant foliage.

Lepidoptera (butterfly caterpillars): since many caterpillars are known to be strongly host specific (Ebert 1993) and they cannot fly, $\delta^{13}C$ of leaf tissue should directly be reflected in the $\delta^{13}C$ signature of the caterpillars as our results have shown.

(2) Groups showing a higher degree of mobility

Coleoptera (beetles): the weak correlation between values of $\delta^{13}C$ in *R. fagi* and in host plant leaf tissue suggests that this beetle is mobile. In spring, nymphs of this species mine in beech leaves. By the end of July, adults migrate to adjacent overwintering sites (*e.g*. spruce trees) or into forest litter. They do not feed until spring of the next year. Feeding and reproduction starts after bud break on beech leaves (Grimm 1973, Hiltbrunner and Flückiger 1992). However, it is somewhat surprising that this beech specialist does not show a more pronounced relationship with the $\delta^{13}C$ signature of leaves, despite its ability to move during certain parts of its live cycle. For these analyses we collected individuals from their over wintering habitats where they started feeding on unfolding leaves of *Fagus*.

Araneida (spiders): we found no significant correlation with $\delta^{13}C$ of spiders and host plant leaf tissue. Although web-building spiders are sedentary (except when their offspring move passively by wind (Southwood 1962), their prey belongs to the highly mobile category of canopy arthropods. Yet, most of the catch by spiders of the treatment zone seems to come from the treatment area, indicated by a clearly more negative $\delta^{13}C$ signature, than found in the control area.

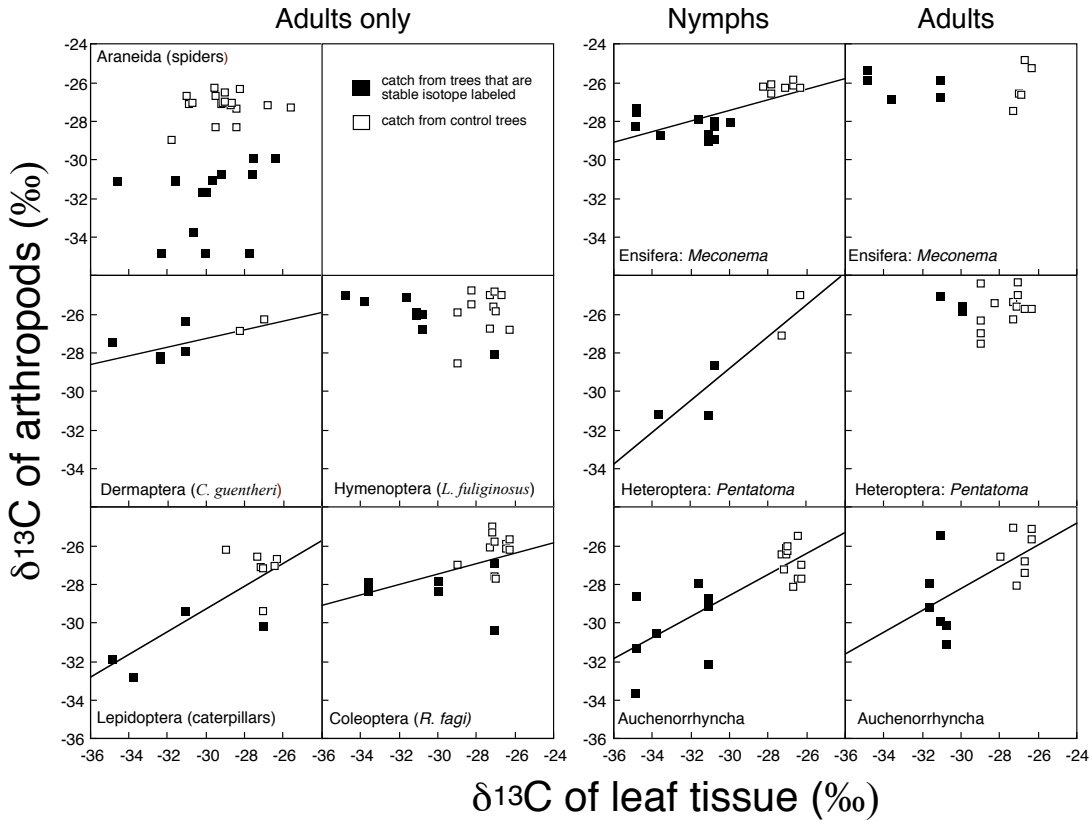

Fig. 3 Linear regressions of $\delta^{13}C$ levels (insect nymphs and imagos) and those of hostplant (leaf tissue) for the respective groups. (Statistical results are given in table 2, black symbols: arthropods found in the CO_2-enriched area, white symbols: arthropods found in the control area.) Those for *Meconema* were published in Asshoff and Amstutz (2004).

But the $\delta^{13}C$ signal was only marginally significant (P = 0.11, Fig. 3).

Dermaptera (earwigs): fifth instar nymphs of the omnivorous *C. guentheri* showed a marginally significant correlation with leaf tissue of the host plant. This species lives primarily, but not exclusively, from living and dead plant matter. In addition, the nymphs consume dead and living insects and other organisms. Given the wide spectrum of food sources, we expected a weak $\delta^{13}C$ signal in these insects and, therefore, a low correlation with $\delta^{13}C$ levels of leaves. Thus, the good correlation, comes as a surprise. As hypothesised previously, the predominant movement of earwigs is vertical (up and down one tree), rather than horizontal (i.e. between trees: Franke 1985, Asshoff and Amstutz 2004), which is in line with our data.

Hymenoptera (ants): the food spectrum of *Lasius fuliginosus* Latreille, Hymenoptera, Formicidae, is wide (a trophobiotic association with aphids and coccids, but also zoophagous, Seifert 1996). Even if feeding on honeydew, the nest may receive sugar from various trees. Therefore, it is not surprising that we did not find a clear correlation. Ants build their body from larval diets that incorporate a mixture of the entire colonies foraging activity. Blüthgen *et al*. (2003) working with ants in the canopy and understorey found a correlation between $\delta^{13}C$ values of ants and those of plant species from which they were collected. Similar to earwigs, ants may consume food from the canopy and from the forest floor thereby diluting the $\delta^{13}C$ signal. We assume, but cannot prove, that the movement pattern of ants is more vertical than horizontal, comparable to that of earwigs. However, it is known that individual ant workers can show a high fidelity to trees and even the same leaves (Mody and Linsenmair 2003) and, in turn, this corresponds with our significant correlation of $\delta^{13}C$ of ants found in the CO_2-enriched area with leaf tissue. However, the studies of Mody and Linsenmair (2003) and Blüthgen *et al*. (2003) were conducted in systems differing greatly from our temperate forest system and, therefore, it is not clear whether results are comparable.

(3) Groups showing differences in mobility in different developmental stages

Ensifera (bush-crickets): young *Meconema* (instar 2) are not very mobile in the canopy and stay on the tree where they hatched, as was evidenced by the high isotope correlations. This bush-cricket mainly feeds on aphids (Ingrisch and Köhler 1997, U. Simon, pers. comm.). In adults of this species, no correlation between $\delta^{13}C$ of body tissue and $\delta^{13}C$ of leaf tissue was found, suggesting that imagos change hosts regularly, most probably by flying, once wings become fully developed (*M. thallasinum*) or by climbing and running (*M. meridionale*, Asshoff and Amstutz (2004).

Heteroptera (true bugs): our correlations proved that the phytophagous heteropterans display limited movement in their nymphal stage, whereas the opposite was observed in adults. Besides walking there are two

other forms of locomotion such as (1) carriage in the upper air by wind and (2) low level flight, typically described as migration (Southerwood 1962). It is probable that winged adults migrated in and out of the CO_2-enriched and the control areas.

A wide range of studies showed that rising atmospheric CO_2 concentrations impaired food quality for insect herbivores (Arnone *et al.* 1995, Lindroth 1996, Hättenschwiler and Schaffelner 2004, Asshoff and Hättenschwiler 2004). In some of these studies insects switched food plants under elevated CO_2 in search for food of higher quality, Immobile insects might suffer from CO_2-induced, species-specific changes in leaf quality, because they cannot escape from a given tree. However, this conclusion is perhaps relevant for experimental conditions with a step change in CO_2. The gradual increase of CO_2 concentration in nature might permit adaptive changes also in relatively immobile insects. For example, in the long term species could change their host plant preferences (and also places for egg deposition) or their physiology may be modified to deal with poorer nutrition quality. Resultant tree species-specific abundance of certain feeding guilds may differ from those observed at current CO_2 concentrations.

To test whether insects switch host trees due to poorer quality under elevated CO_2, it would be interesting to investigate whether species showing significant correlations with leaves in our study will be sustained in the CO_2-enriched area in the future. If not, this could be evidence for a higher degree of arthropod mobility in order to ensure adequate food supply (by utilising different nutrition with different $\delta^{13}C$ values).

In summary, our results suggest that stable carbon isotopes are a valuable tool with which to study arthropod movement patterns in a forest canopy, particularly for phytophagous insects. The most interesting result of our study is that juveniles of bush-crickets, true bugs and leafhoppers are rather sedentary, although they certainly have the possibility to distribute in the canopy.

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6 Rapid mixing between old and new carbon pools in the canopy of mature forest trees

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Abstract

Branch autonomy theory suggests carbon self sufficiency. Does this imply direct investment of new photoassimilates into new structures and ongoing metabolism? Or is new carbon (C) first mixed into older mobile C-pools before this mix is re-allocated to sinks? If the latter is true, new C should be mixed with old mobile C at any time during the growing season. To test this hypothesis, we pulse-labelled one year old branchlets of nine European forest tree species in the canopy of 30 m tall trees after branch elongation had been terminated. In all species labelled C was found in woody tissue within two to nine hours after labelling. Four months later very small signals were left in *Tilia* in contrast to unchanged or even increased signals in *Fagus, Prunus* and *Quercus*, reflecting different degrees of mixing of new with old C. Stronger branch wood labels reflect more intense mixing (e.g. *Fagus*) whereas weak signals occurred in species in which low mixing takes place (*Tilia*). This mixing of new with old C-pools is likely to mask year specific linkages between tree-ring formation and climate and has considerable implications for tree-ring climate reconstruction using stable isotopes as proxies for actual climatic conditions.

Key words

carbon allocation, stable isotopes, labelling, branch autonomy, tree ring, temperate forest, autocorrelation

Introduction

Carbon (C) makes up roughly half of all plant dry matter. Most of this C is tied up in structural pools such as cellulose, hemicellulose and lignin, or in mobile compounds such as starch, sugars, lipids or aminoacids. Daytime carbon fixation commonly exceeds the dissipation capacity of photosynthetic tissues, leading to transitory deposition of starch in chloroplasts as observed already by Sachs (1887), who used iodine to visualize starch in leaves. Carbon transfer away from the photosynthesizing leaf to active C-sinks is the central prerequisite for continued photoassimilation. In addition to instantaneous daytime phloem transport, these transitory starch pools in the leaf are reduced during the night and C is exported to other parts of the tree (Zimmermann & Brown, 1971; Dickson, 1991). Our current understanding of C-transfer in trees is based on seedling and sapling studies (using ^{14}C markers; see below). For mature trees, in a natural setting the timing of these C translocation processes is unknown. It is also unclear, how nearby woody tissue is interlinked with canopy leaves, after shoot elongation has been completed in summer. Are mature branches representing neutral transfer pathways toward the large C-sinks in the trunk or below ground? Is there lateral assimilate exchange between phloem and branch C-pools?

Numerous C transfer experiments with seedlings or saplings have been carried out in the 1960ies and 1970ies using the then newly available radioactive markers (e.g. Balatinecz, Forward & Bidwell, 1966; Rangnekar, Forward & Nolan, 1969). Most of these seedling experiments focused on seasonal rates of signal dissipation, requiring high initial label, achieved by several hours or even days of ^{14}C-labelling (e.g. Shiroya *et al*., 1966; Gordon & Larson, 1968; Smith & Paul, 1988). We are not aware of works in which the immediate C-transfer had been studied in tall trees and which had quantified the amount of labelled C which entered woody tissue. Radiography, which was often used to detect the tracer, possibly constrained quantitative analysis of such studies.

As an example of such works with orchard species, rapid C uptake into woody tissue was found in one year old apple trees, where labelled C was detectable in shoots immediately (i.e. after six hours of labelling), regardless of the season (May, Jul, Aug, Sept, or Oct; Hansen, 1967). One hour after a 3-5 hour labelling, up to 3% of the applied C was retrieved in the axis of eight year old *Pinus sylvestris* trees (Hansen & Beck, 1994). This suggests rapid and intense exchange of new carbon between leaves, phloem and adjacent stem tissue.

One of the advantages of studies with seedlings or young trees is that the whole plant, including roots can be sampled and analyzed and thus high recovery of C across the tissues can be achieved (Rangnekar & Forward, 1972; Hansen & Beck, 1994). In contrast, branchlets on a big tree represent 'open systems' that export C to a large plant body, where the ultimate fate of C remains undetectable. Inversely, the branch can also receive C from proximal stores, though it is unlikely on fully sun-exposed, mature branches in the upper canopy. Loss of recent C through respiration reduces C recovery in both seedling and branch labelling studies, and may contribute >50% to dark respired CO_2 in mature *Fagus* trees (Nogués *et al*., 2006). The emission of volatile organic C (in trees mainly isoprene, terpenes) represents an additional though small pathway through which C is lost. For temperate forest trees isoprene emissions range from 4×10^{-8} to 3×10^{-4} μmol g^{-1} s^{-1} relative to leaf dry weight (Kesselmeier & Staudt, 1999), which is 2-5 orders of magnitude smaller than photosynthetic uptake.

A forest scale full season C-labelling experiment (Keel, Siegwolf & Körner, 2006) revealed surprising delays in the rate at which new carbon appeared in new structural tissue. For instance, it took up to four full seasons for a complete replacement of 'old' carbon signals in leaves. It was concluded that new assimilates are rapidly mixed with old mobile carbon pools in branch wood and that it is this mix which is re-translocated to the active structural sinks.

Although new assimilates would suffice (quantitatively) to support all new growth (Hoch, Richter & Körner, 2003), they are obviously first mixed into the old mobile C pool leading to a continuous 'dilution process'. For leaves of tropical forest trees, 6 days sufficed to turn over the mobile C pool often enough to complete the dilution process and all non structural carbon was new carbon (Körner & Würth, 1996).

In this study we aimed at exploring such 'local' transfer processes of recently assimilated C in nine tall European forest tree species using the Swiss canopy crane (Pepin & Körner, 2002; Körner *et al*., 2005). We used three conifers and six broad-leaved species. One of the conifers is deciduous (*Larix decidua*) as all the broad-leaved species are and two conifers are evergreen. Evergreen conifers and broad-leaved deciduous trees differ in many leaf and wood traits. Conifers have sclerophyllous leaves which assimilate C for several years, whereas broad-leaved trees have softer and mostly thinner leaf tissue with a longevity of only a few months. Conifer phloem is cellular, with supposedly slower transport rates than in angiosperms. Within the broad-

leaved trees there are also significant differences between species. *Quercus petraea*, has thicker leaves with higher assimilation rates than the other taxa. All these differences could affect the speed of short term C translocation and the size of the pool into which new C is mixed and thus 'diluted'.

We pulse-labelled one year old branchlets of ca. 30 m tall trees in the upper canopy using a crane and a bagging technique. Tissue samples were collected at noon and in the evening of the same day (i.e. two and nine hours after labelling) which should reveal the degree of instantaneous coupling between leaves, phloem and xylem in the forest canopy across the most important forest tree taxa in Europe. In addition wood and bark samples were collected four months after labelling (in broad-leaved species only). Based on the short replacement time of old C by new C found in *Tilia* (Keel *et al.*, 2006), we hypothesize that very low mixing of new C with old C takes place before C is reinvested into new tissue in this species. As a result we hypothesize that less new C is found in the branch wood of *Tilia* since after shoot maturation C is flowing through the branch with a lower lateral C exchange. In contrast, *Quercus* and *Fagus* are expected to show a higher exchange of carbohydrates between phloem and woody tissue due to the long replacement time of old by new C that we found in these species (Keel *et al.*, 2006). Therefore more pronounced signals in woody tissue of labelled branchlets are expected in *Quercus* and *Fagus*. The broad comparison across a suite of typical European forest taxa should provide insight into the immediate and longer-term (4 months) fate of carbon at the branch level after assimilation in contrasting tree types.

Material and methods

Site description

The experiment was carried out in a diverse mixed forest located near Basel, Switzerland (47°28' N, 7°30' E; 550 m asl) with tree heights of 30-35 m. The forest is situated on a silty-loamy rendzina and is characterized by a 15 cm deep rock-free topsoil and a 15-30 cm deep rocky subsoil underlain by fragmented limestone bedrock. In the upper 10 cm, the soil has a pH of 5.8 (measured in distilled water extracts).

A 45 m freestanding tower crane equipped with a 30 m jib (crane arm) and a working gondola provided access to 62 dominant trees in an area of ~3000 m^2. The forest is species-rich and is dominated by *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl., *Larix decidua* Mill., and *Picea abies* L. with *Carpinus betulus* L., *Tilia platyphyllos* Scop., *Acer campestre* L., *Prunus avium* L., *Pinus sylvestris* L., and *Abies alba* Mill. as companion species. *Abies* was not included in the study because none of the individuals reaches the upper canopy. Since spring 2001 fourteen broad-leaved trees of this forest were exposed to elevated CO_2 (Körner *et al.*, 2005), an experiment which is not associated with the current analysis, but reduced the number of trees available for the present study.

^{13}C pulse labelling

We labelled fully sun-exposed, one year old, fruitless branchlets of nine species in the upper canopy. However, branchlets were too big in *Prunus* (only the upper part of one year old branchlets were labelled), too small in conifers (additionally part of the previous year's branch segment was included), and carried fruits in *Tilia* (fruits were removed with scissors before labelling). Since the amount of assimilating leaf tissue should be comparable within as well as between species, we tried to select branchlets with similar leaf area (i.e. fewer leaves in the case of species with big leaves such as *Quercus*). On average, 7.4 leaves in angiosperm species were labelled. Branchlets on the same main branch were selected as controls for isotope analysis but within safe distance to prevent any contamination with labelled CO_2.

The labelling treatment was applied between 8:00 and 11:00 on 8 days in summer 2003 between 7 and 16 July (only broad-leaved species) and again in 2004 on 7 days (30 June – 30 July). Additionally, *Fagus* and *Quercus* were labelled in September 2003. Two adjacent branchlets (three branchlets in July 2003) were labelled simultaneously and were considered the same replicate (see below). On average 12 branchlets could be labelled per day. Three to five replicates per species were used which were, whenever possible, on separate trees. However in some cases (*Acer*, *Prunus*, *Tilia*), only one or two trees were available and hence all replicates were on the same tree. The order in which branchlets were labelled was randomly chosen.

Branchlets were enclosed in two liter transparent plastic bags (Minigrip, Seguin, USA), which were sealed tightly around the stem with synthetic modelling clay. After removing the air inside the bag, it was filled with CO_2-free air through a soft silicon tube. At the same time as the CO_2-free air was flushed through the tube, 2 ml highly ^{13}C enriched CO_2 (>98 atom-%, Cambridge Isotope Laboratories, Andover, USA; denoted as $^{13}CO_2$ in the following) was injected into the tube using a syringe resulting in a ca. 1000 ppm $^{13}CO_2$ atmosphere inside the bag. After 15 min 2 ml additional $^{13}CO_2$ were injected with a syringe directly through the plastic bag, resulting in a total injected amount of 2.1 mg ^{13}C. The tiny punched hole in the plastic bag was sealed immediately with sticky tape.

After a total labelling time of 45 min, bags were removed and three small leaf discs (12 mm in diameter) from different leaves were punched immediately to obtain the maximum initial label. Leaf samples were shock-frozen in liquid nitrogen to deactivate enzymes and were kept in a cool box until noon. After approximately two hours (at ca. 12:30) the first set of branchlets was removed and shock-frozen as described above. The branchlets were separated into leaf, xylem, bark and bud tissue before oven-drying at 80°C for two days. Shortly before sunset, roughly nine hours after labelling (at around 19:30) the second set of branchlets was collected and treated as described above. In experiments carried out in July 2004, the axis 5-10 cm below the section of the branchlet that had been enclosed was also collected for analysis.

In July 2003 an additional third set of branchlets was labelled which was sampled in November 2003 after leaves had been shed. Of these branches we collected also tiny (2 mm) wood cores at 50, 80, and 110 cm distance from the tip using an increment puncher (WSL-puncher, Swiss federal research institute WSL, Birmensdorf, Switzerland). After oven-drying, all tissues were weighed.

Carbon isotope analysis of organic samples

Leaf discs were ground using pestle and mortar, bigger samples were ground with a steel ball mill (Mixer Mill, Retsch MM 2000, Haan, Germany), or with a centrifugal mill (ZM 1000, Retsch, Haan, Germany) and 0.6 to 0.8 mg dried powder was packaged in tin capsules for $\delta^{13}C$ analysis. Samples were then combusted in an elemental analyzer (EA-1110, Carlo Erba Thermoquest, Milan, Italy). Via a variable open split interface (Conflo II, Finnigan Mat, Bremen, Germany) gas samples were transferred to the mass spectrometer (Delta S, Thermo Finnigan Mat, Bremen, Germany), which was operated in continuous flow mode. The precision for $\delta^{13}C$ analysis was < 0.1‰. Amounts of assimilated C were calculated according to following formula:

$$C(mg) = \%C * dw * (\delta^{13}C_L - \delta^{13}C_C)$$

where %C is the C concentration of the sample calculated from the calibration line with reference samples of known C concentrations. The area of the voltage signal peak of the mass spectrometer for CO_2 (masses 44, 45, 46) was integrated over time and was proportional to the C concentration of the sample. The dry weight of the sample is annotated as dw and $\delta^{13}C_C$, $\delta^{13}C_L$ refer to the isotope ratio of the control and the labelled plant tissue. ^{13}C signals expressed in mg of label rather than isotope ratios were found to be the ideal units for comparison between species and sampling times, since they do not depend on the weight of the branchlets (supplementary online material). Whenever possible signals are shown in mg.

Since we took samples within a few hours after labelling, we assumed that labelled C was in essence tied to non structural carbohydrates (NSC, mainly starch and sugars) and not to structural components. Using published data of the same trees studied here (Hoch, Richter & Körner, 2003), we estimated the amount of NSC in mg and calculated the time needed to replace NSC pools in leaves by new photoassimilates. We assumed that trees assimilate CO_2 for ten hours a day (of a total day length of 14-15 hours) at the same rate as during the 45 min labelling procedure.

Statistics

We performed two-way ANOVA's with functional type (broad-leaved versus needle-leaved) and tree species as fixed factors. In addition one-way ANOVA's were carried out for each species and time separately. Statistical analysis was carried out using R version 2.0.1, (R Development Core Team, 2004)

Results

After branchlets had been enclosed in bags for 45 min of labelling, the CO_2 concentrations had decreased from the 2 x 1000 ppm injected to 166 ppm on average (only measured in July 2004), indicating that >90% of the applied ^{13}C had been assimilated. The concentration of the remaining $^{13}CO_2$ did not differ significantly between functional types (broad-leaved versus needle leaved).

More than 70% of the calculated C uptake was retrieved in leaves of five out of six broad-leaved species immediately after labelling (t = 0), whereas only 50% of the assimilated C was found in leaves of *Tilia* and 55% in conifers (Tab. 1). Nine hours after labelling we recovered 50% of the calculated C uptake across all tissues (averaged across species), whereof most was still in leaves (45% of the total C assimilated).

Leaf signals

Immediately after labelling at least 1mg labelled C was found in leaves of all nine study species (Fig. 1), with lower signals in conifers compared to broad-leaved species ($P < 0.001$). Differences between these functional types were no longer detectable two hours after labelling. Generally, leaf signals rapidly decreased within the first two hours after labelling (t = 2), and diminished at a somewhat slower rate within the following seven hours (t = 9).

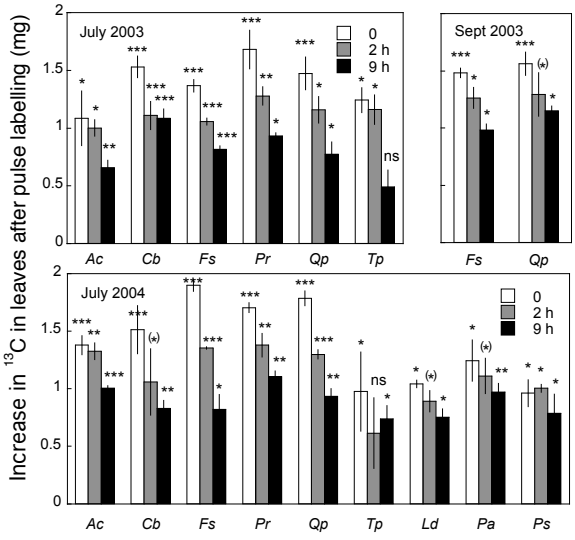

Fig. 1 The net uptake of labelled C in leaves of one year old twigs immediately (0), two (2 h) and nine hours (9 h) after a 45 min pulse labelling. Experiments were carried out in July 2003 (upper left panel), in September 2003 (upper right panel) and in July 2004 (lower panel). Mean and SE are shown (n = 3-5 branches). Ac = *Acer campestre*, Cb = *Carpinus betulus*, Fs = *Fagus sylvatica*, Pr = *Prunus avium*, Qp = *Quercus petraea*, Tp = *Tilia platyphyllos*, Ld = *Larix decidua*, Pa = *Picea abies*, Ps = *Pinus sylvestris*. *P*-values for labelling effects of a one-way ANOVA's are annotated as ns = not significant $^{(*)}$ $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Due to the large branchlet to branchlet variation in conifers, it happened that needle signals at t = 0 were lower in branchlets harvested at t = 9 compared to branchlets harvested at t = 2. Hence, the mean needle signal at t = 0 of branchlets harvested at t = 2 and at t = 9 was lower than the needle signal at t = 2 (Tab. 1). To omit this seemingly increase in the figures, we only show the needle signals at t = 0 for the branchlets harvested at t = 2 (Fig. 1).

The calculated time needed to replace NSC pools in leaves by new photoassimilates was nine days in deciduous species (Tab. 2) whereas in evergreen conifers, replacement times reached up to 63 days in *Picea*.

Table 1 Recovered ^{13}C after labelling in percent of total ^{13}C which had been assimilated from bags (1.95 mg). Results of leaves are shown for all three sampling times (t = 0, t = 2h, t = 9h). In contrast, results of all tissues which had been enclosed in the bag, in addition to bark and xylem approximately 0-10 cm below the enclosed section (annotated as 'below') are presented for t = 9h only. 'Total' refers to the sum of the recovered ^{13}C nine hours after labelling. n = 3-5 branchlets.

	t = 0	t = 2 h	t = 9 h							
Species	Leaves	Leaves	Leaves	Petiole	Bark	Wood	Buds	Bark (below)	Wood (below)	Total
Acer	71 ± 4.2	68 ± 3.8	52 ± 1.1	1.31 ± 0.09	1.02 ± 0.2	0.25 ± 0.1	0.10 ± 0.04	1.35 ± 0.1	0.31 ± 0.02	55.86
Carpinus	78 ± 10.8	54 ± 14.8	43 ± 3.6	0.37 ± 0.12	0.72 ± 0.2	0.32 ± 0.1	0.05 ± 0.02	0.82 ± 0.1	0.25 ± 0.1	45.02
Fagus	97 ± 2.8	69 ± 0.6	42 ± 6.8	0.98 ± 0.18	1.17 ± 0.4	0.54 ± 0.2	0.15 ± 0.07	0.99 ± 0.04	0.41 ± 0.1	46.23
Prunus	87 ± 2.2	71 ± 5.3	57± 2.7	0.87 ± 0.06	0.67 ± 0.2	0.19 ± 0.1	0.17 ± 0.01	0.86 ± 0.4	0.30 ± 0.04	59.59
Quercus	92 ± 3.4	67 ± 2.1	48 ± 3.4	0.40 ± 0.02	1.11 ± 0.1	0.37 ± 0.1	0.21 ± 0.06	1.27 ± 0.4	0.24 ± 0.1	51.50
Tilia	50 ± 17.7	31 ± 15.8	38 ± 6.0	0.65 ± 0.09	2.74 ± 0.7	0.37 ± 0.1	0.31 ± 0.15	2.27 ± 0.4	0.46 ± 0.2	44.59
Larix	37 ± 10.9	46 ± 4.8	39 ± 3.9		6.51 ± 0.9	1.13 ± 0.3	0.13 ± 0.03	1.59 ± 0.4	0.18 ± 0.1	48.02
Picea	43 ± 13.0	57 ± 7.9	50 ± 4.0		4.46 ± 0.7	0.65 ± 0.1	0.39 ± 0.16	0.31 ± 0.05	0.05 ± 0.0	55.52
Pinus	36 ± 9.9	51 ± 1.8	40 ± 8.8		1.43 ± 0.4	0.56 ± 0.1	0.03 ± 0.02	0.52 ± 0.3	0.18 ± 0.1	42.91

Table 2 The total estimated time (days) required to replace the entire non structural carbohydrate (NSC) pool by new photoassimilates. Replacement times were calculated based on the amounts of labelled C assimilated during pulse labelling relative to published NSC concentrations of the corresponding trees (Hoch et al. 2003). We assumed that leaves assimilated CO_2 for ten hours a day.

Species	Leaves (t = 0)	Wood (t = 9)
Acer	9	119
Carpinus	6	99
Fagus	6	137
Prunus	8	153
Quercus	15	419
Tilia	8	155
Larix	14	105
Picea	63	201
Pinus	39	134

Transport to woody tissue and bark

Already two hours after labelling was completed, statistically significant ^{13}C labels were detected in woody tissue and bark of about half of the labelled branchlets (Fig. 2). From two to nine hours after labelling, the signals in bark and woody tissue increased on average four and five fold respectively and became more expressed in conifers compared to broad-leaved species (woody tissue: $P = 0.01$, bark: $P < 0.001$). Signals in bark were in general higher than in woody tissue, which was most expressed nine hours after labelling. In most broad-leaved species, but not in evergreen conifers, labelled C had entered the branch wood 5-10 cm below the branch section which had been labelled (bagged) nine hours earlier (data not shown). In all species (except *Fagus*), no labelled C had been invested beyond 110 cm down the branch as shown by mini cores collected along the branch (Fig. 3). We did not sample any control branches but refer to published data for branch wood $d^{13}C$ of *Fagus* after leaf fall (Damesin & Lelarge, 2003).

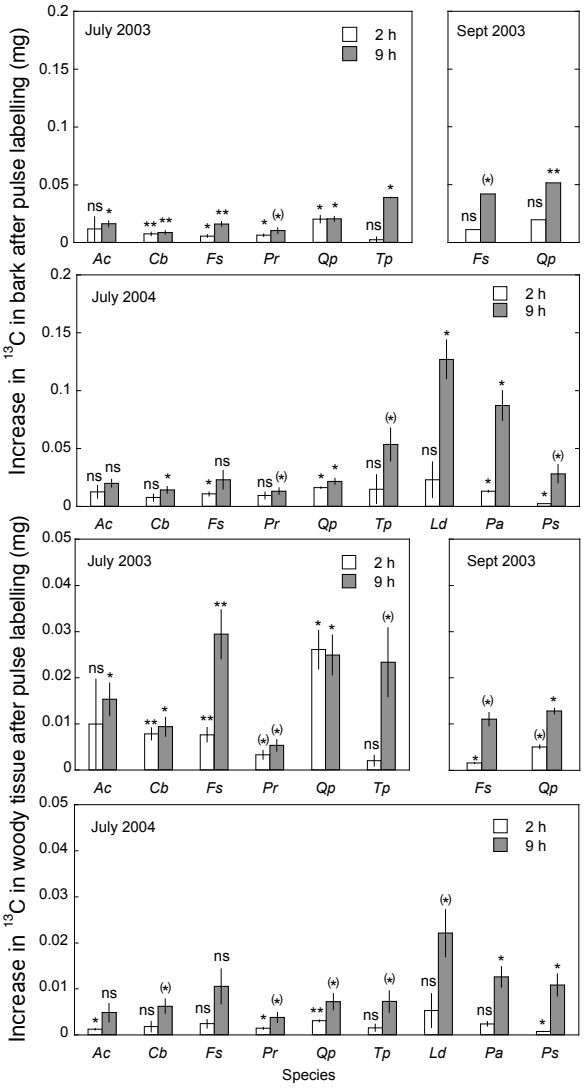

Fig. 2 The net uptake of labelled C in bark (upper three panels) and wood (lower three panels) of one year old twigs two (2 h) and nine hours (9 h) after a 45 min pulse labelling with $^{13}CO_2$. Note the different scales for wood and bark tissue. For further details see Fig. 1. *P*-values for labelling effects of one-way ANOVA's are annotated as ns = not significant; $^{(*)}$ P <0.1; * P <0.05; ** P <0.01; *** P <0.001.

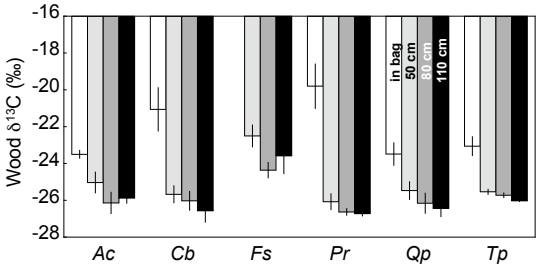

Fig. 3 Wood $\delta^{13}C$ of "mini-cores" in November 2003 four months after labelling (July 2003). Samples were collected within the branch section which had been enclosed during labelling (referred to as 'in bag') and along the branch at different distances from the tip (indicated as 50, 80 and 110 cm). As a reference we refer to published branch wood $\delta^{13}C$ of *Fagus* after leaf fall represented by the dotted line (Damesin & Lelarge, 2003).

Remaining wood signals were species-specific (P = 0.003). Only 14% labelled C relative to the amount found nine hours after labelling was retrieved in wood and bark of *Tilia* whereas in *Acer* and *Carpinus* approximately 55% were detected (Fig. 4). Wood and bark signals in *Quercus* were equal on the day of labelling and four months later, but roughly doubled in *Fagus* and *Prunus*.

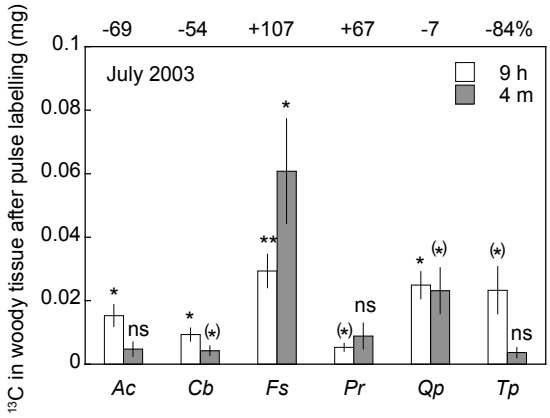

Fig. 4 Remaining ^{13}C signals in woody tissue four months after labelling (t = 4 m) compared to nine hours after pulse labelling with $^{13}CO_2$ in July 2003 (t = 9 h). Numbers above graph indicate the % change in signal size. *P*-values for labelling effects of a one-way ANOVA's are annotated as ns = not significant; $^{(*)}$ P <0.1; * P <0.05; ** P <0.01; *** P <0.001.

NSC pools in wood would be replaced in 138 days averaged over all species excluding *Quercus*, which showed almost four times longer turnover times in both July 2003 and July 2004 (Tab. 2) most likely due to higher NSC concentrations in this species (Hoch et al., 2003). Note that in wood, these replacement times reflect the 'lateral' net allocation of assimilates transported in the phloem. If no lateral exchange of labelled C had occurred, the time needed to replace the pool could become infinite, irrespective of the actual rate of longitudinal phloem transport.

Discussion

Using *in situ* pulse labelling, we showed that recently assimilated C enters woody tissue of one year old twigs in mature trees within two to nine hours after labelling. Since labelled C had not only been recovered within but also below the section which had been labelled (bagged), we have strong evidence for rapid basipetal translocation. Most likely, direct assimilation of labelled C through bark chlorenchym, contributed little to wood signals (Wiebe, 1975; Foote & Schaedle, 1976; Aschan, Wittmann & Pfanz, 2001; Pfanz *et al*., 2002). Our results confirm findings of earlier C allocation studies performed with seedlings or young trees which had found labelled C in the axial tissue shortly after labelling (Balatinecz *et al*., 1966; Hansen & Beck, 1994; Hansen, 1967).

Given the evolutionary older and supposedly less efficient phloem in conifers, we were surprised to find a more rapid propagation of the wood and bark signals in conifers than in broad-leaved species. Based on the smaller signal in needles, our data suggest that labelled C reaches woody tissue more rapidly, possibly because less recent C is stored in needles and ray tissue is more tightly coupled to the phloem.

Four months after labelling, wood signals in *Tilia* were strongly reduced, whereas in *Fagus* and *Quercus*, wood signals remained constant or had even increased compared to the day of labelling suggesting significant species-specificity in C allocation only in the long term. Our data therefore suggest a low mixing of old with new C in *Tilia*, in line with the rapid (within one season) replacement of old by new C reported for the whole-canopy labelling experiment (Keel *et al*., 2006). On the contrary, the slow replacement of old by new C measured in *Fagus* and *Quercus* in the canopy labelling experiment

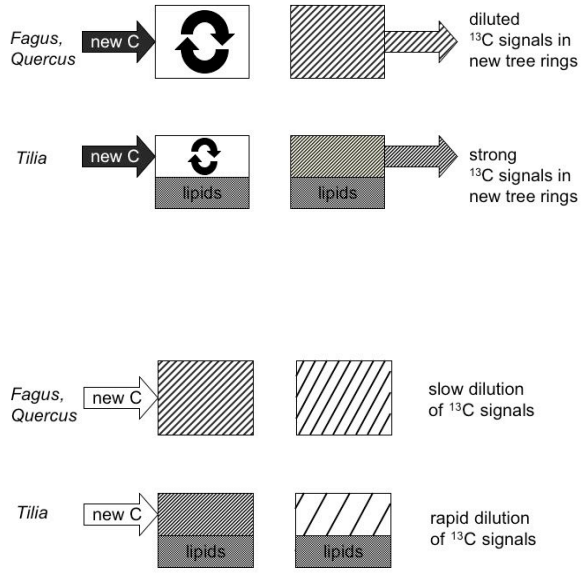

Fig. 5 A schematic representation of carbon uptake (arrows) and mixture with old mobile carbohydrate pools (rectangles) in different tree species. Unlabelled carbon (C) is shown in white, labelled C in black and mixtures of unlabelled and labelled C are hatched. The upper two rows represent an experiment where labelled C (black arrow) is mixed into old C pools (white) resulting in species-specific ^{13}C labels. The two lower rows illustrate the situation after a labelling experiment where unlabelled C (white arrow) is mixed into labelled pools (hatched) resulting in different degrees of signal dilution.

supports the obviously high mixing of old with new C as shown by sustained signals over four months. Among the six broad-leaved species studied, only *Tilia* stores C as lipids (triacylglycerols) in addition to NSC (Hoch et al. 2003). Since the lipid pool most likely undergoes less C-cycling due to more costly synthesis as well as degradation compared to carbohydrates such as starch, the exchangeable C pool is likely to be smaller in *Tilia* than in other species. The occurrence of lipids might therefore explain the lower mixing of old with new C observed (Fig. 5). If this proves to be true, we would expect that low mixing of old with new C also applies for the three conifers studied here, since they store lipids too (Hoch et al. 2003). A three hundred year tree ring chronology showing stronger and more consistent correlations of $\delta^{13}C$ in cellulose with temperature in *Pinus sylvestris* (a 'fat-tree') compared to *Quercus petraea* (a 'starch-tree') strongly supports our findings (CE Reynolds Henne, personal communication).

Carbon or oxygen isotope ratio analysis in tree rings are a widely recognized tool for climate reconstruction (Libby & Pandolfi, 1974; Pearman, Francey & Fraser, 1976; Mazany, Lerman & Long, 1980; Leavitt & Long, 1991) which is based on the assumption that isotope ratios of concurrent assimilation is reflecting mean climatic conditions which in turn leave their one-to-one fingerprint in wood tissue (Schleser *et al*., 1999). However, often correlations of isotope signals have been found between isotope ratios of one or two subsequent years, so called 'autocorrelation' (Monserud & Marshall, 2001). A recent study indeed showed that assimilates from summer and autumn are allocated to earlywood of the following year (Kagawa, Sugimoto & Maximov, 2006). It is not only the plausible transfer of reserves to structural growth, but also intrinsic mixing of all sequentially assimilated carbon within the mobile pool which dampens (flattens) the structure-climate linkages in terms of isotope signals, according to our data.

Most likely, plant respired CO_2 represents a mixture of old and new C as well, which is supported by a recent labelling study with mature *Fagus* trees where dark respiration contained 40% unlabelled C (Nogués *et al*., 2006). In addition, as suggested by the authors, signals were dampened due to respiration of different substrates (carbohydrates and fatty acids, which are not equally labelled due to different turnover times) which is supported by previous studies (Tcherkez *et al*., 2003; Hymus *et al*., 2005).

In our experiment, no signal was detected in branch wood tissue beyond one meter distance from the point of labelling (except for *Fagus*) supporting the theory of spatially constraint branch carbon autonomy (Sprugel, Hinckley & Schaap, 1991; Cregg, Teskey & Dougherty, 1993). Most likely, these continuously turned over (mixed) stores in terminal branches contribute to the spring flush in the following year explaining why our full canopy labelling took so long to produce entirely labelled foliage in branchlets (Keel *et al*. 2006), although new emerging foliage seems to be largely C-autonomous by the time of unfolding (S. Keel, unpublished). Alternatively, we cannot exclude that the rapidly decreasing signals along the branch, resulted from dilution of new C with a basipetally increasing pool of unlabelled (old) C.

The calculated replacement times for NSC pools in woody tissue based on signals measured nine hours after labelling indicated that the pool is replaced nearly twice

during a 200 day growing season. The net transfer of new C from phloem to axial woody tissue within the same day of labelling was therefore considerable, despite shoot elongation had been completed in July and NSC concentrations in branch wood of deciduous species remain rather stable throughout the growing season (Hoch et al. 2003).

The classic view of C allocation where C is directly transferred from leaves to pools that require C for growth, storage or cell maintenance seems too simplistic. Our data suggest that in some species recent C is first mixed with a given pool of mobile carbohydrates before it is invested into new tissue or can passively enter a nearby pool as shown for woody tissue. Hence the occurrence of labelled C in a tissue does not necessarily reflect this tissue's need of C. Although this might be considered self-evident, the data set we present appears to be the first *in situ* proof under natural canopy conditions.

In conclusion we found rapid allocation of recent C to woody tissue of one year old branchlets across nine European forest tree species. Four months after labelling the remaining ^{13}C signals were strongly species-specific reflecting a high degree of mixing of old with new C in *Fagus* and *Quercus* resulting in stronger, persistent signals in branch wood. In contrast, signals were markedly weaker in *Tilia* due to a low mixing of new with existing C. The low mixing in *Tilia* is likely associated with the presence of lipid stores in woody tissue which undergo less C-cycling. The exchangeable carbohydrate pool is therefore smaller, resulting in a lower mixing of C pools. Based on our results, climatic conditions should therefore be more precisely recorded in tree rings of *Tilia* and perhaps other lipid storing species (e.g. *Pinus*), whereas the continuous C pool mixing blurs the structure-climate linkage in tree rings of *Fagus* and *Quercus.*

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Supplementary online material

SOM 1 Dry weight (g) of one year old twigs (mean ± se) which were pulse labelled and separated into leaf, wood and bark tissue after sampling. n = number of branchlets

	July 2003				July 2004			
Species	n	Leaf	Wood	Bark	n	Leaf	Wood	Bark
Acer	11	3.01 ± 0.23	0.34 ± 0.09	0.31 ± 0.09	6	2.02 ± 0.14	0.08 ± 0.02	0.13 ± 0.02
Carpinus	20	1.77 ± 0.09	0.16 ± 0.02	0.15 ± 0.03	6	0.87 ± 0.15	0.06 ± 0.01	0.08 ± 0.01
Fagus	20	1.43 ± 0.08	0.42 ± 0.07	0.29 ± 0.05	6	1.30 ± 0.13	0.10 ± 0.01	0.17 ± 0.01
Prunus	12	3.45 ± 0.23	0.21 ± 0.03	0.38 ± 0.09	6	2.26 ± 0.21	0.06 ± 0.01	0.17 ± 0.03
Quercus	17	2.29 ± 0.30	0.75 ± 0.21	0.49 ± 0.16	6	2.90 ± 0.34	0.22 ± 0.03	0.32 ± 0.04
Tilia	12	2.69 ± 0.27	0.43 ± 0.08	0.71 ± 0.16	6	1.62 ± 0.16	0.11 ± 0.01	0.33 ± 0.03
Larix					6	2.80 ± 0.85	0.59 ± 0.19	1.63 ± 0.39
Picea					6	9.25 ± 0.62	0.69 ± 0.06	4.36 ± 0.39
Pinus					6	5.46 ± 1.54	0.37 ± 0.13	0.77 ± 0.30

7 ^{13}C labelling reveals different contributions of photoassimilates from infructescences for fruiting in two temperate forest tree species

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Research Paper

^{13}C Labelling Reveals Different Contributions of Photoassimilates from Infructescences for Fruiting in Two Temperate Forest Tree Species

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Abstract: **The pathways of currently fixed carbon in fruit bearing branchlets were investigated in two temperate forest tree species *(Carpinus betulus* and *Fagus sylvatica),* which differ in texture of their vegetative infructescence tissues (leaf-like in *Carpinus* vs. woody in *Fagus*). During late spring, ^{13}C pulse-labelling was conducted on girdled, defoliated, girdled plus defoliated and untreated fruiting branchlets of mature trees *in situ,* to assess changes in C relations in response to the introduced C source-sink imbalances. At harvest in early August, 75 – 100 % of the recovered ^{13}C label was bound to infructescences (either fruits or vegetative infructescence tissue), revealing them as the prime C sinks for current photoassimilates. Leaves on girdled branchlets were not stronger labelled than on ungirdled ones in both species, indicating no upregulation of the leaves' photosynthetic capacity in response to the prevention of phloemic transport, which was also supported by measurements of light saturated photosynthesis. In contrast, ^{13}C labels tended to be higher after complete defoliation in the vegetative infructescence tissues of *Carpinus,* suggesting enhanced net photosynthesis of green infructescence parts as compensation for the loss of regular leaves. The total labelling-derived ^{13}C content of whole infructescences was very similar between foliated and defoliated *Carpinus* branchlets. Cupulae of *Fagus,* on the other hand, remained almost unlabelled on defoliated branchlets, indicating the photosynthetic inactivity of this woody infructescence tissue. Consequently, *Carpinus* still produced relatively high fruit masses on girdled plus defoliated branchlets, while in *Fagus* fruit development ceased almost completely at this most severe treatment. Our results highlight that green vegetative infructescence tissue assimilates substantial amounts of C and can partly substitute regular leaves as C sources for successful fruit development.**

Key words: **Defoliation, girdling, carbon isotope, branch autonomy, source-sink balance.**

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Introduction

Can vegetative fruit tissues assimilate enough carbon to replace leaves in branchlets that were defoliated and disconnected from the whole tree's phloem by girdling? While infructescences of some forest trees have vegetative tissue resembling wood (e.g., *Fagus* and *Quercus*), others possess leaf-like structures (e.g., *Carpinus* and *Tilia*), suggesting that vegetative tissues differ in their contribution of photoassimilates to developing fruits. Based on the hypothesis of branch autonomy, single branches, or even smaller branchlets, may function as independent modules in mature tree crowns, at least with respect to carbon acquisition (Watson, 1986). While fruitless branches always resemble carbon sources during the growing period, fruiting branches could be net carbon importers or exporters (Sprugel et al., 1991). Hence, depending on whether the developing infructescences contribute photoassimilates (e.g., via bracts), or depend entirely on the carbon supply from regular leaves, the level of carbon autonomy of fruit-bearing branchlets may differ between tree species (Bazzaz et al., 1979).

Indeed, there is strong evidence for carbon autonomy of fruiting branches in many woody species. For example, experimental and observational evidence for C autonomy of fruit development at the level of single branches was reported for rather different taxa, such as *Alnus* (Hasegawa et al., 2003), *Dryobalanops* (Ichie et al., 2005), *Ilex* (Obeso, 1998), *Prunus* (Agusti et al., 1998), *Styrax* (Miyazaki et al., 2002), and *Vitis* (Candolfi-Vasconcelos et al., 1994). The independence from stored C resources for fruiting within young branchlets may be supported by high levels of bark internal, light-driven refixation of respired CO_2 through photosynthesis of green bark tissue (chlorenchyma, Pfanz et al., 2002) and even functional chloroplasts in wood and pith (van Cleve et al., 1993), which would add strongly to a positive net C balance of branchlets. Although net C uptake is unlikely to occur in stems, the stem internal refixation of CO_2 may make up for 60 – 80 % of the potential respiratory carbon loss (Aschan et al., 2001). A recent study demonstrated complete C autonomy of fruit development at the branchlet level in three deciduous temperate forest tree species, namely *Carpinus, Tilia,* and *Fagus* (Hoch, 2005). By applying all combinations of girdling (i.e., removing a small strip of phloem) and half, as well as complete, defoliation of fruiting branchlets, that study further showed that species possessing a high proportion of photosynthetic infructescence tissue (i.e., bracts of *Carpinus* and hypsophylls of *Tilia*) also possesses a

high degree of C autonomy even at the infructescence level. On the other hand, some studies have indicated that not all carbon needed for successful fruiting derives from current photosynthesis on the same branch. Significant import of stored C into fruiting branches was described for apple trees (Hansen and Christensen, 1974; Palmer et al., 1991) and some peach varieties (Corelli-Grappadelli et al., 1996; Walcroft et al., 2004). However, a clear dependency on stored C reserves for fruiting has, so far, only been reported for single varieties of some orchard species, but not for wild forest trees.

Very useful tools to explore the transport of C assimilates in trees are C isotope tracers. Most tracer studies have investigated seasonal C dynamics and attempted to estimate whole tree carbon relations (e.g., Kuhns and Gjerstad, 1991; Lacointe et al., 1993; Hansen and Beck, 1994; Cerasoli et al., 2004). Regarding the C balance of fruit bearing branches, there were a number of studies using ^{13}C, ^{14}C or both isotopes simultaneously to investigate C pathways and the level of C autonomy. But for operational reasons, these studies were conducted with very young saplings (e.g., Hansen and Christensen, 1974; Candolfi-Vasconcelos et al., 1994; Lacointe et al., 2004) or low stature trees (e.g., Hasegawa et al., 2003). Here, we report on a comparative ^{13}C labelling experiment with fruiting branchlets of two mature temperate forest tree species which aims to identify possible changes in C pathways in response to experimentally modified C relations introduced through girdling and defoliation.

To our knowledge, the current study is the first attempt to use ^{13}C pulse-labelling to investigate the pathways of photoassimilates within fruit-bearing branchlets of mature forest trees in a cross-species comparison. The Swiss Canopy Crane (SCC) site, which is situated in a species-rich mixed temperate forest, enabled easy access to all crown parts, thus allowing synchronous manipulation and labelling of fruiting branchlets *in situ*. In a previous study, the C autonomy of fruiting branchlets of these trees was assessed by quantifying fruit production and mobile carbon pools (Hoch, 2005). Here, we explore species-specific differences in how assimilates are invested within fruiting branchlets, and how assimilate pathways change in response to modified carbon relations by labelling girdled and defoliated fruit-bearing branchlets with $^{13}CO_2$. We hypothesized that infructescences are the main sink for currently fixed C on fruiting branchlets and that photosynthetic activity of infructescences can be identified by the size of the ^{13}C label in fruits on defoliated branchlets. Using ^{13}C signals, we further tested whether C assimilation in green infructescence tissues is enhanced in response to defoliation, compensating for the loss of regular leaves.

Materials and Methods

Site and tree species

The study was performed during summer 2003 in a species-rich, mixed temperate forest at the Swiss Canopy Crane (SCC) site in Hofstetten (47°28′N, 7°30′E, 550 m a.s.l.), 12 km SW of Basel, Switzerland (Körner et al., 2005). The forest stocks on rendzina-type soils above Jurassic calcareous bedrock. The climate is oceanic, with an annual precipitation of close to 1000 mm, 10 °C annual mean air temperature, 19 °C mean air temperature for the warmest month (August) and an approximate 6-month growing season from April to October. By means of a 45 m crane, mature (about 100-year-old and 30 – 35 m tall) trees could be reached *via* a gondola. For this study, two deciduous tree species with different infructescence characteristics were investigated: *Carpinus betulus* L. and *Fagus sylvatica* L. Infructescences of *C. betulus* consist of several (normally 8 – 14) pairs of achenes (fruits), which are equipped with a green, about 3 – 5 cm long, tripartite-lobed bract. Fruiting *F. sylvatica* produces 2 – 4 triangular nuts within a woody cupula, which is green initially but becomes brown in early summer. To simplify matters, the species will be referred to as *Carpinus* and *Fagus,* and achenes of *Carpinus* and nuts of *Fagus* will uniformly be addressed as "fruits". All infructescence tissues apart from fruits will be referred to as "vegetative infructescence tissue" (VIT).

Manipulation of carbon relations

To investigate the carbon relations of fruiting branches, young branchlets were treated with a full factorial set of girdling (i.e., interruption of the phloem transport) and complete (100%) defoliation, as also described in Hoch (2005). All branchlets used for this study were in the uppermost, fully sun-exposed crown parts on dominant branches. While girdling is supposed to prevent the import of C from other branches, the stem and roots, defoliation disables the supply of current photoassimilates from regular leaves on the treated branchlet. Branchlets were assigned to one of the following treatments: (1) ungirdled and undefoliated, ug/ud; (2) ungirdled and defoliated, ug/d; (3) girdled and undefoliated, g/ud; (4) girdled and defoliated, g/d. Each treatment was replicated 5 times for each species. Because the branchlet sizes should have been comparable between species, different age segments were used as the functional branchlet unit. The treatments were thus performed on 2-year-old shoots in *Carpinus* and on 3- to 5-year-old shoots in *Fagus*. Girdling was achieved by removing a small (3 – 5 mm wide) strip of bark and phloem at the base of the branchlet with a razor blade. To avoid desiccation after girdling, the wounds were tightly covered with parafilm. For defoliation, whole leaves were clipped using scissors.

The girdling and defoliation treatments were applied in late spring, shortly after pollination and the onset of seed formation, on 14 and 15 May 2003. For both species, the harvest was done on 5 August; close to fruit maturation but prior to fruit fall (because the summer of 2003 was exceptionally warm, fruit maturation was several days ahead of normal years). Immediately after harvest, the branchlets were cut, separated into xylem, bark (incl. phloem), leaves (on undefoliated branchlets only), fruits and vegetative infructescence tissue. All samples were shock-heated in a microwave oven to deactivate enzymes and oven-dried at 75 °C until weight constancy. The dried samples were weighed, ground to fine powder and stored at 4 °C over silica gel for later analyses.

Leaf gas exchange measurements

CO_2 gas exchange of mature leaves was measured *in situ* on all undefoliated (ug/ud and g/ud) branchlets around midday on 30 July 2003. Net photosynthesis (A_s) was determined with a portable gas exchange system (Li-Cor, Li-6400, Li-Cor, Lincoln, NE, USA) at saturating PFD (1200 µmol m^{-2} s^{-1}, with LED light; Li-Cor 6400-02, Li-Cor), ambient temperature, air humidity

Table 1 Dates, species and replicates, duration of pulse-labelling, air temperature and photon flux density on the three labelling occasions

Date	Species	Replication	Duration of labelling	Air temp.[1] (°C)	PFD[1] (µmol m^{-2} s^{-1})
28 May 2003	*Fagus*	1, 2	60 – 90 min	15.6	716
4 June 2003	*Fagus*	3, 4, 5	30 – 45 min	25.7	1202
4 June 2003	*Carpinus*	1, 2, 3	30 – 45 min	25.7	1202
6 June 2003	*Carpinus*	4, 5	60 – 90 min	20.4	482

[1] Temperatures and PFD are 3 h means between 11:00 and 14:00 h, recorded automatically on the crane-top as 10 min averages of 30 s individual measurements.

and CO_2 concentrations (360 ppm). A_s was recorded immediately after the rate of photosynthesis, and stomatal conductance remained constant (within less than 5 min).

^{13}C pulse-labelling

In order to introduce a substantial pulse of ^{13}C, all treated branchlets were fed with 1000 ppm CO_2 strongly enriched in ^{13}C (99 atom-%, denoted as $^{13}CO_2$ in the following) using a short-term bagging technique for 30 to 90 min around midday between the end of May and early June 2003. Table **1** shows the approximate duration of labelling and the weather conditions for each day on which labelling was performed. Each branchlet selected for the girdling/defoliation treatment was covered with a 1-l transparent plastic bag, which was sealed tightly with modelling clay around the stem. After removing most of the air inside the bag, 1 ml of $^{13}CO_2$ gas was injected into the bag, together with CO_2-free air, resulting in a 1000 ppm $^{13}CO_2$ (i.e., 1.84 mg $^{13}CO_2$ or 0.52 mg ^{13}C) atmosphere inside the branch bags. In order to compensate for differences in photosynthetic activities between days, the duration of labelling was roughly adjusted according to PFD and air temperature conditions for each day. On one sunny day, the branch bags were left on the branchlets for 30 to 45 min (4 June, average PFD 1202 µmol m^{-2} s^{-1} between 11:00 h and 14:00 h), while on two overcast days, the labelling time was extended to 60 to 90 min (28 May and 6 June, average PFD lower than 720 µmol m^{-2} s^{-1} between 11:00 and 14:00 h). For both species, two of the five replicates per treatment were labelled on an overcast and three on a sunny day (Table **1**). A *post hoc* test revealed no significant effect of labelling time on $\delta^{13}C$ of all investigated tissues, except for leaves, where labels were slightly higher after labelling on the sunny day ($p = 0.032$). Thus, the longer labelling duration greatly compensated for the lower light conditions on overcast days.

$\delta^{13}C$ analyses

For carbon isotope analyses, as well as the determination of total C concentration, 0.6 – 0.8 mg plant powder was weighed in tin cups and combusted to CO_2 in an elemental analyzer (EA-1108, Carlo Erba, Italy). The $^{13}C/^{12}C$ ratio was determined in a continuous flow mass spectrometer (DELTA-S, Finnigan MAT, Germany), which was directly connected to the elemental analyzer via a variable open split (Confo II, Finnigan MAT, Germany). The isotope signature of C is expressed in the δ notation, where $\delta^{13}C$ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, and R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the international VPDB carbon standard.

Statistical analyses and calculations

The number of replicates per species was limited in the current study because the forest beneath the crane is species-rich (10 species), and part of the crane area is treated with elevated atmospheric CO_2 concentrations (about 540 ppm) within a long-term experiment (Pepin and Körner, 2002; Körner et al., 2005). Thus, two fruiting individuals of *Carpinus* and three fruiting individuals of *Fagus* were available for this study. Girdling and defoliation treatments were randomly assigned to fully sun-exposed fruiting branchlets, but each treatment (ug/ud, ug/d, g/ud, and n/d) was evenly replicated among the tree individuals per species. For example, each treatment was replicated 3 times on one *Carpinus* and twice on the second tree. The initial replication per species and treatment was n = 5, but due to the loss of some fruits and vegetative infructescence tissues before harvest, the number of replicated infructescences was reduced to n = 3 on girdled and defoliated *Fagus*. Further, one branchlet of *Carpinus* died when girdled and defoliated, reducing the final replication to n = 4 for this treatment combination. Differences in $\delta^{13}C$ between unlabelled controls and labelled branchlets, as well as leaf to fruit ratios between species, were tested for significance with Student's *t*-test. Differences in biomass and $\delta^{13}C$ values between treatments were tested for significance with the Tukey-Kramer HSD test. All statistical tests were performed with JMP 4.0.1. (SAS Institute, Cary, NC, USA).

To quantify the total labelling-derived ^{13}C content at the time of harvest, all $\delta^{13}C$ labels were converted to absolute ^{13}C content for each analyzed tissue by multiplying the ^{13}C concentration by the respective tissue dry biomass. Total labelling-derived ^{13}C content was obtained by subtracting total ^{13}C concentration of controls from that of labelled branchlets. To estimate the proportion of ^{13}C recovered in labelled branchlets, absolute ^{13}C labels are given as % of total applied ^{13}C.

Results

Infructescence biomass

In both species, total fruit mass, as well as total vegetative infructescense tissue (VIT) mass, was not reduced by girdling of foliated branchlets (Table **2**). Defoliation on ungirdled branchlets also had no effect on total fruit and VIT biomasses of *Carpinus* and *Fagus* (Table **2**). When girdling and defoliation were applied simultaneously, both species exhibited marked decreases in their infructescence biomass (Table **2**), which were more expressed in *Fagus* (~ – 80% reduction compared to controls in fruits and ~ – 70% in VIT) than in *Carpinus* (~ – 50% in fruits and ~ – 40% in VIT).

Table 2 Dry biomass of total fruits and vegetative infructescence tissue (VIT) per branchlet in response to the girdling and defoliation treatments. Values are means ± standard errors. n = 5 except for g/d *Carpinus* (n = 4) and g/d *Fagus* (n = 3). Different letters indicate significant differences within one tissue type and species at the $p < 0.05$ level by Tukey-Kramer HSD test. If no letters are given, differences are not significant

		Dry biomass (g)				
		Control[1]	ug/ud[2]	ug/d[2]	g/ud[2]	g/d[2]
Carpinus	Fruits	1.05 ± 0.12	1.00 ± 0.14	0.94 ± 0.14	0.99 ± 0.14	0.51 ± 0.23
	VIT	0.61 ± 0.09	0.59 ± 0.15	0.52 ± 0.11	0.61 ± 0.11	0.38 ± 0.06
Fagus	Fruits	0.25 ± 0.03 [ab]	0.26 ± 0.06 [a]	0.19 ± 0.04 [ab]	0.21 ± 0.01 [ab]	0.05 ± 0.02 [b]
	VIT	0.74 ± 0.02 [a]	0.79 ± 0.06 [a]	0.69 ± 0.03 [a]	0.83 ± 0.01 [a]	0.23 ± 0.12 [b]

[1] Ungirdled and undefoliated, not labelled. [2] ^{13}C labelled branchlets; ug: ungirdled; g: girdled; ud: undefoliated; d: 100% defoliated

Table 3 Leaf area and mass, leaf to fruit ratios and fruit to vegetative infructescence (VIT) ratios on control branchlets (i.e., undefoliated, ungirdled, and unlabelled). Values are means of 5 measurements ± standard errors. Different letters indicate significant differences between species at the $p < 0.05$ level by Student's *t*-test. If no letters are given, differences are not significant

	Carpinus	*Fagus*
Leaf area (cm^2)	69.5 ± 11.6	61.3 ± 6.1
Leaf mass (g)	0.51 ± 0.11	0.49 ± 0.07
Leaf area/fruit mass ($cm^2\ g^{-1}$)	78.7 ± 15.1 [b]	283.99 ± 46.1 [a]
Leaf mass/fruit mass ($g\ g^{-1}$)	0.58 ± 0.14 [b]	2.19 ± 0.34 [a]
Fruit mass/VIT mass ($g\ g^{-1}$)	1.82 ± 0.14 [a]	0.29 ± 0.01 [b]

The level of C autonomy of fruiting branchlets may depend on the absolute leaf area per branchlet, on the ratio of leaf area (or leaf mass) to total fruit mass, as well as on the proportion of vegetative infructescence tissue mass in relation to total fruit mass. Total leaf area and leaf mass were similar in *Carpinus* and *Fagus,* but leaf area/fruit mass and leaf mass/fruit mass was 3.5-times lower in *Carpinus* than in *Fagus* (Table **3**), mainly due to the higher total fruit mass in *Carpinus* (Table **2**). *Fagus* exhibited a very low proportion of fruit mass in relation to vegetative infructescence mass (fruit/VIT ratio of 0.29), while in *Carpinus,* fruit biomass clearly exceeded VIT mass (Table **3**).

Natural ^{13}C abundance

The natural $\delta^{13}C$ values found were characteristic for C3 plants and varied among tissues within a total range of 2.5‰ (from – 25.3‰ in fruits of *Fagus* to – 27.8‰ in leaves of *Carpinus,* Table **4**). Intraspecific $\delta^{13}C$ differences between tissues were mostly consistent between the two species, with fruits showing the highest, i.e., least negative, $\delta^{13}C$ (species mean of – 25.6‰), and leaves representing the lowest $\delta^{13}C$ values (species mean of – 27.8‰). Interestingly, $\delta^{13}C$ of VIT in *Carpinus* was similar to that of leaves, while in *Fagus* VIT, $\delta^{13}C$ was less negative and resembled more that in fruits (Table **4**). Thus, the difference of $\delta^{13}C$ between leaves and VIT increased from 0.1‰ in *Carpinus* to 2.3‰ in *Fagus* (Table **4**).

^{13}C labelling experiment

Even about 2 months after pulse-labelling, leaves were significantly labelled on ungirdled and girdled branchlets of both species (Fig. **1**), with the $\delta^{13}C$ increase ranging from 1.9 to

Table 4 Natural $\delta^{13}C$ values of unlabelled and untreated control branchlet tissues and changes of $\delta^{13}C$ signature in labelled branchlets relative to controls. Values are means ± standard errors. n = 5 except for g/d *Carpinus* (n = 4) and g/d *Fagus* (n = 3). Different letters indicate significant differences among labelled branchlets within one tissue and species at the $p < 0.05$ level by Tukey-Kramer HSD test. If no letters are given, differences are not significant

		Unlabelled	Labelled			
		$\delta^{13}C$ (‰)	Absolute change in $\delta^{13}C$ relative to controls (‰)			
		Control	ug/ud	ug/d	g/ud	g/d
Carpinus	Fruits	– 25.9 ± 0.2	10.9 ± 4.3	4.8 ± 2.2	7.5 ± 1.7	7.0 ± 4.0
	VIT	– 27.7 ± 0.2	10.9 ± 3.7	23.2 ± 11.8	8.3 ± 2.4	13.6 ± 6.5
	Leaves	– 27.8 ± 0.2	3.6 ± 0.9	–	3.4 ± 1.3	–
	Bark	– 26.9 ± 0.2	2.1 ± 0.7	0.0 ± 0.2	0.9 ± 0.5	1.4 ± 0.7
	Xylem	– 26.7 ± 0.1	1.8 ± 0.5 [a]	– 0.1 ± 0.3 [b]	0.9 ± 0.4 [ab]	0.4 ± 0.3 [ab]
Fagus	Fruits	– 25.3 ± 0.2	4.5 ± 2.1	0.2 ± 0.3	4.8 ± 2.8	0.2 ± 0.2
	VIT	– 25.4 ± 0.4	4.9 ± 2.0	1.8 ± 0.9	6.4 ± 2.5	0.7 ± 1.6
	Leaves	– 27.7 ± 0.2	2.7 ± 1.0	–	1.9 ± 0.7	–
	Bark	– 27.4 ± 0.3	1.0 ± 0.5 [ab]	0.3 ± 0.3 [ab]	1.2 ± 0.2 [a]	– 0.2 ± 0.2 [b]
	Xylem	– 26.8 ± 0.2	1.0 ± 0.5 [ab]	0.4 ± 0.2 [ab]	1.5 ± 0.3 [a]	– 0.2 ± 0.1 [b]

ug: ungirdled; g: girdled; ud: undefoliated; d: 100% defoliated.

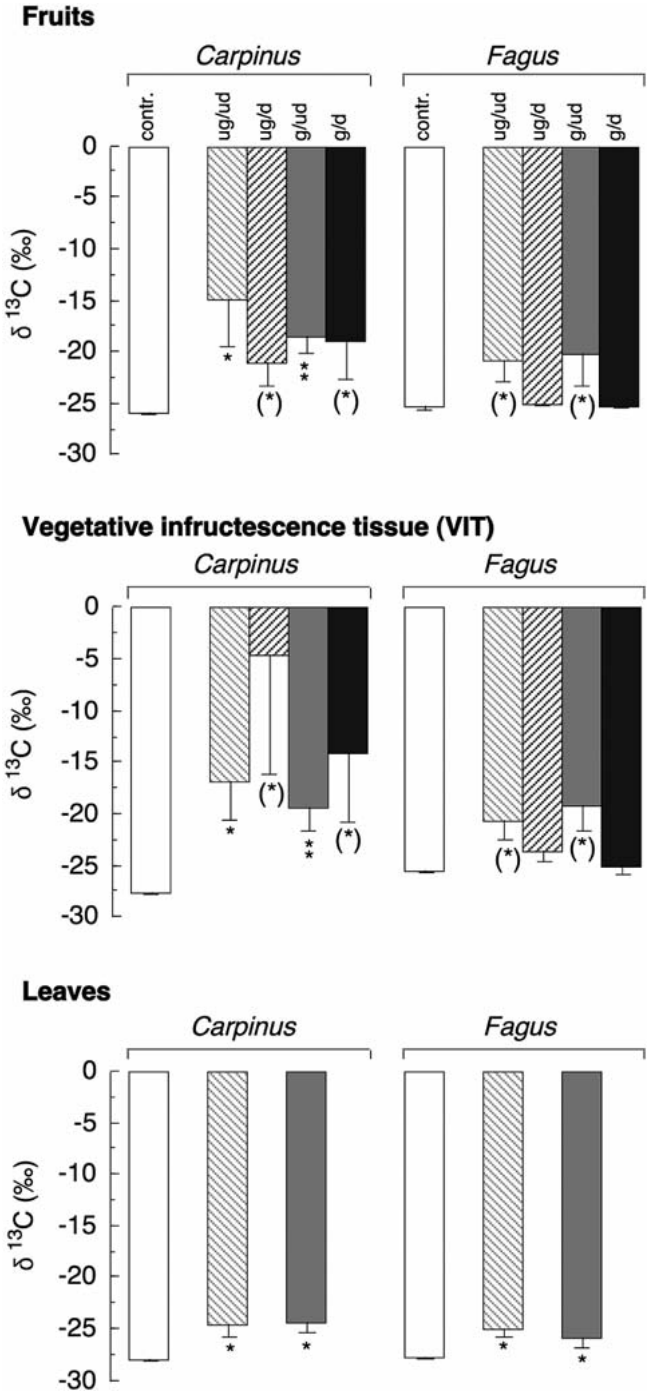

Fig. 1 $\delta^{13}C$ values in fruits, vegetative infructescence tissue (VIT) and leaves of ^{13}C labelled branchlets compared to unlabelled controls (white bars). Treatment abbreviations are: contr.: ungirdled and undefoliated control (unlabelled); ug/ud: ungirdled/undefoliated (labelled); ug/d: ungirdled/defoliated (labelled); g/ud: girdled/undefoliated (labelled); g/d: girdled/defoliated (labelled). Bars represent means of 5 replicates ± standard error, except for g/d *Carpinus* (n = 4) and g/d *Fagus* (n = 3). Significant differences using Student's *t*-test between labelled and control branchlets are indicated (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

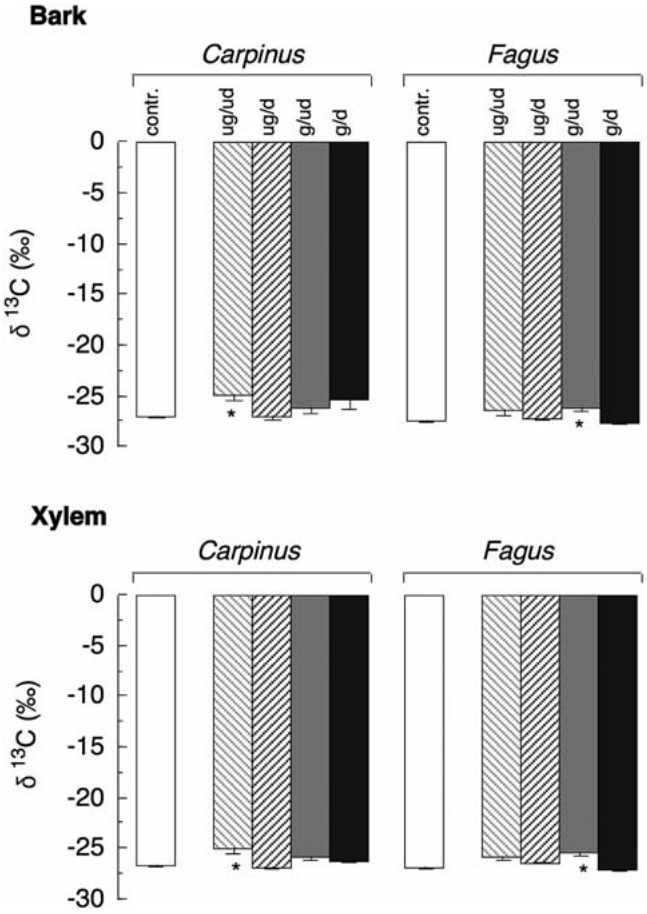

Fig. 2 $\delta^{13}C$ values in bark and xylem of ^{13}C labelled branchlets. For treatment abbreviations and statistics see Fig. **1**.

3.6‰, and no significant effect of girdling (Table **4**). Except for defoliated *Fagus,* all infructescences (fruits and VIT) were labelled markedly, although there was considerable variation between the replicates within a treatment (Fig. **1**). Developing infructescences were clearly the main C sinks within the branchlets, as shown by the generally very strong ^{13}C labels (up to +23‰ $\delta^{13}C$ increase compared to control, Table **4**). Remarkably, ^{13}C labels of VIT on defoliated *Carpinus* branchlets were 112% and 63% higher compared to undefoliated branchlets, on ungirdled and girdled branchlets, respectively (Fig. **1**, Table **4**). Although this trend was not significant due to the high within-treatment variation, this may indicate enhanced C assimilation of the green infructescence tissues as compensation for the loss of regular leaves. In contrast, VIT of *Fagus* was hardly labelled on defoliated branchlets (Fig. **1**), which reflects the low photosynthetic capacity of this species' woody cupulae. Fruits were also labelled significantly in *Carpinus,* but there was no consistent influence of defoliation as found for VIT, while in *Fagus,* only fruits on foliated branchlets were labelled.

Although $\delta^{13}C$ labels were relatively moderate in bark and xylem tissues, some of these signals were still significantly higher compared to controls due to the very small variability among replicates (Fig. **2**). Remarkably, significant ^{13}C label was

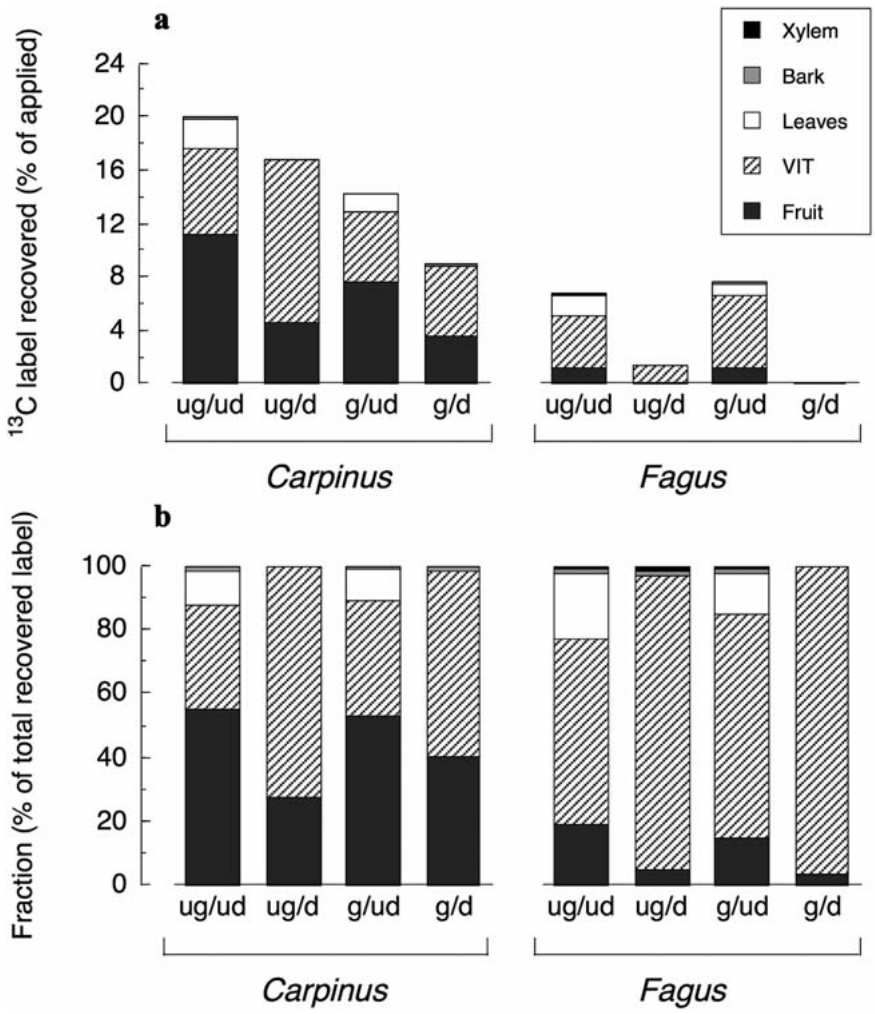

Fig. 3 Total recovered ^{13}C label at harvest. (**a**) Fraction of recovered ^{13}C in treated branchlets. Labelling-derived ^{13}C contents are given relative to the ^{13}C applied originally (0.52 mg). 1% thus equals 5.2 µg ^{13}C. (**b**) Proportional contribution of different branchlet tissues to the total ^{13}C label. Note that the order of organ types within a bar is the same as in the legend.

found only in foliated branchlets (Table **4**). Xylem and bark of *Fagus* was labelled significantly only in girdled and foliated branchlets (Fig. **2**), which may indicate an increased abundance of current assimilates in the branchlet axes caused by the prevention of C export by girdling. The significant label found in xylem and bark of *Carpinus* on ungirdled and undefoliated branchlets suggested that although the assimilate export was not prevented, labelled C remained in the branchlet wood and bark in this species (Fig. **2**).

By considering the biomass of each sampled tissue, the δ^{13}C values were also used to estimate the absolute content of labelling-derived ^{13}C still present at the time of harvest. These calculations allowed assessment of the recovery rate of ^{13}C (fraction of the total applied ^{13}C) about 2 months after labelling, as well as quantification of C sinks within the branchlets. The percentage of pulse-labelling-derived ^{13}C recovered at harvest varied between 0.1% in girdled and defoliated *Fagus* branchlets and 20.1% in ungirdled and foliated *Carpinus* branchlets (Fig. **3a**). For all treatments and species, more than 75% of the label was bound to infructescence tissue (Fig. **3b**), emphasizing the high C sink strength of developing fruits. The low ^{13}C content in leaves of both species suggests that most assimilated C was either exported or respired from these organs between labelling and harvest. Interestingly, the labelling-derived ^{13}C content of whole infructescences (fruits and VIT) was almost identical between foliated and defoliated ungirdled *Carpinus* branchlets (about 17% of total applied ^{13}C, Fig. **3a**), again indicating a high level of C autonomy at the infructescence level for this species. For girdled *Carpinus* branchlets, total ^{13}C content was about 30% lower on defoliated branchlets (Fig. **3a**), mainly due to the significantly lower infructescence biomass of girdled and defoliated branchlets (Table **2**). In *Fagus*, ^{13}C recover rates above 5% were found only on foliated branchlets (Fig. **3a**). On defoliated branchlets, ^{13}C labels were very low and almost exclusively found in vegetative infructescence tissue (cupulae, Fig. **3b**). Due to their weak δ^{13}C labels, as well as their small dry biomass fraction, the proportion of labelling-derived ^{13}C in bark and xylem is almost negligible for the whole branchlet balance of both species (Fig. **3b**).

Leaf gas exchange

Net leaf photosynthesis at saturating PFD (A_s) revealed no significant change in photosynthetic capacity in response to girdling (Fig. **4**). However, while A_s was exactly the same for ungirdled and girdled *Carpinus* branchlets, it was more than 20% lower for leaves on girdled compared to ungirdled branchlets in *Fagus*.

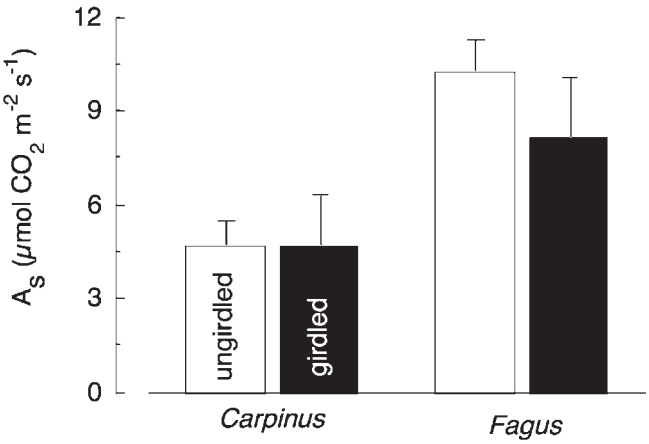

Fig. 4 Light-saturated photosynthesis (A_s) of control leaves on ungirdled and girdled branchlets of *Carpinus* and *Fagus*. Values are means of 5 branchlets ± standard error.

Discussion

The strong ^{13}C labels of infructescences revealed them as the prime sink for currently assimilated C in fruit-bearing branchlets during early summer. High sink strength of developing fruits for C has previously been found in many tree species (e.g., Garriz et al., 1998; Berman and Dejong, 2003; Vaast et al., 2005). However, within the current study, pronounced differences in the ^{13}C signal strength of infructescences were found between species, with *Carpinus* exhibiting stronger labels than *Fagus*. This interspecific difference could not be explained by higher leaf to fruit ratios (i.e., *Carpinus* had a much lower leaf/fruit ratio than *Fagus*), suggesting that the green infructescence tissues themselves are important C sources for fruiting. This was also supported by the high labelling-derived ^{13}C content of *Carpinus* infructescences on defoliated branchlets.

Although developing fruits may incorporate vast amounts of photoassimilates, small branchlets are completely C autonomous units for fruiting in *Carpinus* and *Fagus,* as shown in an earlier study at the same site (Hoch, 2005). Hence, as long as regular leaves were not removed, girdling had no negative effect on fruit and total infructescence biomass. On the other hand, both species were also able to compensate for the loss of regular leaves by importing C from outside the branchlet, as there was no negative effect on infructescence biomass on ungirdled plus defoliated branchlets. Only if girdling and defoliation were applied simultaneously was a reduction of fruit and VIT biomass found, which was stronger in *Fagus* than in *Carpinus*.

C autonomy within fruiting branchlets likely occurs, especially in species which possess a high proportion of green, photosynthetically active infructescence tissue. Accordingly, the proportion of C needed for fruit development, which derives directly from vegetative infructescence tissues (VIT), strongly depends on the species considered. Bazzaz et al. (1979), for example, found that over 60% of C required for fruiting was supplied by *in situ* photosynthesis of infructescence tissues in *Acer platanoides,* while net assimilation of infructescences contributed only 2% of total C in *Quercus macrocarpa* fruits. High levels of C autonomy at the infructescence unit have been reported mainly for fleshy fruits, which are capable of recycling fruit internal respired CO_2 (e.g., Blanke and Lenz, 1989; Aschan and Pfanz, 2003). Within the two species of this study, *Carpinus* develops bracts (lobes), which are photosynthetically active until close to fruit ripening (Hori and Tsuge, 1993). Hence, this species can develop fruits even on defoliated and girdled branchlets. The importance of VIT for the C supply of ripening fruits was obvious on defoliated branchlets, where labelling tended to be stronger, indicating enhanced photosynthesis of VIT in response to the loss of regular leaves. It thus seems that *Carpinus* can effectively adjust the assimilation rate of its infructescences in response to the C supply strength from regular leaves. In contrast, *Fagus* infructescences were quantitatively labelled only on foliated branchlets, suggesting very low photosynthetic activity of VIT in this species. Consequently, the fruit development was almost completely suppressed on girdled and defoliated *Fagus* branchlets.

The $\delta^{13}C$ values of leaves on labelled branchlets also indicated C autonomy at the branchlet level. Leaves on girdled branchlets were not stronger labelled than on ungirdled branchlets, which implies no change in photosynthetic capacity of regular leaves after girdling, in line with the findings of Proietti (2003) and Schaper and Chacko (1993). If fruiting depended on the import of C from sources outside the branchlet, one would expect photosynthetic capacity to increase after girdling to compensate for the loss of these external C sources. Indeed, numerous studies have shown that leaves can modulate their photosynthetic activity to changed sink strengths (e.g., Schaper and Chacko, 1993; Li et al., 2005; Urban and Léchaudel, 2005; Vaast et al., 2005). The ^{13}C labelling results also fit well with the *in situ* measurements of light-saturated leaf photosynthesis (A_s) on the same branchlets, which revealed no significant differences of A_s between ungirdled and girdled branchlets.

The contribution of stem photosynthesis to the whole branchlet C budget could not be directly measured within the current study. Nevertheless, since bark and xylem were labelled significantly on foliated but not on defoliated branchlets, relatively low rates of direct atmospheric CO_2 uptake by young stem tissues are likely for the investigated species. However, both species possess green, sub-peridermal stem tissue, suggesting photosynthetic recycling of stem internally respired CO_2 (Pfanz et al., 2002), which would markedly diminish C costs of branchlets.

Besides the actual labelling experiment, the study documented the natural variation in ^{13}C between different plant tissues and between the two species. The $\delta^{13}C$ signature of plant material reflects the physical and biochemical discrimination against the heavier C isotope during photosynthesis at the leaf level (Farquhar et al., 1989; O'Leary, 1993), but also during all other biochemical processes following assimilation (Gleixner et al., 1998; Jäggi et al., 2002). The interaction of these different discrimination processes finally determines the bulk ^{13}C signature of a specific compound. Thus, for example, lignin and lipids are ^{13}C depleted relative to starch and cellulose (Park and Epstein, 1961; Benner et al., 1987; Brugnoli and Farquhar, 2000; Wanek et al., 2001). Differences between tissues can, therefore, be explained by different proportions and chemical identities of the cell constituents. In accordance with Chevillat et al. (2005), the relative $\delta^{13}C$ differences between tissues were

very similar between species, with leaves showing the lowest and fruits the highest values, indicating similar chemical compositions for a specific tissue type in both species. The only exceptions were the vegetative infructescence tissues, with the cupulae of *Fagus* exhibiting a ^{13}C signature similar to that of xylem and fruits, and the lobes of *Carpinus* resembling $\delta^{13}C$ values of green leaves. This corresponds to the "leaf-like" physiology traits of infructescences of *Carpinus* (i.e., the strong ^{13}C label on defoliated branchlets) and the "wood-like" nature of *Fagus* cupulae (i.e., no ^{13}C label on defoliated branchlets).

Conclusion

The ^{13}C pulse-labelling proved to be a useful tool to investigate the pathways of assimilated C in fruit-bearing branchlets. Thus, even several weeks after the single pulse-labelling event, and respiratory loss of parts of the label, substantial ^{13}C signals were present, especially in infructescences of *Carpinus* and *Fagus*. The strong labels of green vegetative infructescence tissues in *Carpinus,* which even increased after defoliation, revealed them to be important C sources for successful fruiting. Therefore, species with a greater proportion of green infructescence tissue exhibit high levels of C autonomy, even in the infructescence unit. This enables them to develop viable seeds, even after massive foliage loss, for example, following herbivory. Species like *Fagus,* which lack such an assimilatory infructescence organ, depend mostly on C supply from regular leaves for successful fruiting.

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8 Stomatal conductance of mature forest trees exposed to elevated CO_2

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Abstract

Stomatal conductance (g_s) of mature trees exposed to elevated CO_2 concentrations was examined in a diverse deciduous forest stand in NW Switzerland. Measurements of g_s were carried out on upper canopy foliage before noon, over four growing seasons, including an exceptionally dry summer (2003). Across all species reductions in stomatal conductance were smaller than 25% most likely around 10%, with much variation among species and trees. Given the large heterogeneity in light conditions within a tree crown, this signal was not statistically significant, but the responses within species were surprisingly consistent throughout the study period. Except during a severe drought, stomatal conductance was always lower in trees of *Carpinus betulus* exposed to elevated CO_2 compared to *Carpinus* trees in ambient air, but the difference was only statistically significant on two out of fifteen days. In contrast, stomatal responses in *Fagus sylvatica* and *Quercus petraea* varied around zero with no consistent trend in relation to CO_2 treatment. During the 2003 drought in the third treatment year, the CO_2 effect became reversed in *Carpinus*, resulting in higher g_s in trees exposed to elevated CO_2 compared to control trees, most likely due to better water supply because of the previous soil water savings. This was supported by less negative predawn leaf water potential in CO_2 enriched *Carpinus* trees, indicating an improved water status. These findings illustrate (1) smaller than expected CO_2-effects on stomata of mature deciduous forest trees, and (2) the possibility of soil moisture feedback on canopy water relations under elevated CO_2.

Key words: biodiversity, drought, global change, water relations, temperate forest

Introduction

Reduction of stomatal aperture is a common response of plants exposed to elevated CO_2 concentrations (Morison and Gifford 1984). In canopies well-coupled to the atmosphere, such reductions in stomatal conductance (g_s) result in a corresponding decrease in leaf transpiration. Hence, a large number of studies have been carried out during the last few decades to determine the effect of rising atmospheric CO_2 on g_s and water consumption of dominant forest tree species (for reviews see Curtis and Wang 1998; Saxe et al. 1998; Medlyn et al. 2001).

Most of the work examining stomatal responses to CO_2 in tree species has been confined to seedlings and saplings, with little research on mature forest trees. These experiments have demonstrated that g_s in young woody individuals is generally reduced in response to elevated CO_2 (mean reduction of 21%, Medlyn et al. 2001). This has led to the prediction that water use in most forest trees will be reduced as the CO_2 concentration in the atmosphere increases. Similarly to herbaceous species, the stomatal responses of saplings exposed to elevated CO_2 were found to vary among species and to be less pronounced under environmental conditions that reduce maximum g_s (e.g. drought, high temperature, high evaporative demand; Heath 1998; Wullschleger et al. 2002). Furthermore, there is now solid evidence that plant responses to CO_2 may change with experimental conditions such as the duration of exposure and plant age (Medlyn et al. 2001), soil characteristics (Bucher-Wallin et al. 2000) and biotic interactions (Körner 2002). Conifer species seem to make an exception, as stomata appear less responsive to CO_2 enrichment in this group of species (Teskey 1995; Ellsworth 1999). For all these reasons it is unrealistic to predict the long-term water relation responses of mixed forests to rising CO_2 concentrations from data obtained in saplings of certain species grown in artificial substrates for a short period of time. These uncertainties have led to a growing consensus that forest tree research must be carried out on mature individuals under natural forest conditions (Körner 1995).

Nevertheless, exploring the effects of elevated CO_2 on water relations of tall forest trees has always presented a considerable challenge given the large size and complex structure of their canopy. Hence, investigations conducted on trees growing near natural CO_2 springs (Jones et al. 1995; Tognetti et al. 1998; Tognetti et al. 1999) and experiments performed with branch-bags (Dufrêne et al. 1993; Teskey 1995; Roberntz and Stockfors 1998) have provided most of the data currently available on mature trees. Although branch-bags offer a useful alternative to growth chambers for the investigation of mature trees, there is little, if any, possibility of feedback effects from the soil because only a small portion of the whole crown is exposed to elevated CO_2. Based on sap flow measurements carried out in a mature forest in NW Switzerland, Cech et al. (2003) presented evidence that tree responses to elevated CO_2 may be influenced by soil moisture feedback. They reported that during a relatively dry period, CO_2-enriched trees showed increased sap flow density compared to control trees. This reverse CO_2-effect, where an increased rather than a reduced transpirational flux (and hence, g_s) was observed in response to elevated CO_2, suggests that daily water savings by CO_2-enriched trees may have contributed to an improved water status by the time when control trees fell short in soil moisture. These results highlight the importance of large-scale observations, in which coupled plant-soil systems are studied (Wullschleger et al. 2002).

Using free-air CO_2 enrichment (FACE) technology, major advances have been made towards a large-scale approach of studying forest trees under more realistic growth conditions (Ellsworth 1999; Wullschleger et al. 2002; Herrick et al. 2004). Yet for reasons of practicability, long-term field CO_2 experiments with trees have mainly been conducted in relatively young (10–20 years) forest plantations with no (or little) natural interspecific competition for light and water. To our knowledge, no large-scale investigation has examined the effect of CO_2 enrichment on a natural, diverse forest. However, a study conducted on *Liquidambar styraciflua* L. trees emerging through gaps in the Duke *Pinus taeda* L. forest provides support to the significance of species identity (Herrick et al. 2004). While the stomata of *P. taeda* showed no significant CO_2 response (Ellsworth 1999), those of *L. styraciflua* do. Given the evidence that stomatal responses to elevated CO_2 are species-specific, it is essential to account for tree species diversity when investigating the responses of whole forest stands to elevated CO_2.

In this paper, we present stomatal data from the first FACE experiment exposing the canopy of mature broad-leaved trees from six different species in a natural temperate forest ecosystem to elevated CO_2 concentrations (ca. 540 ppm). Stomatal conductance was measured in all species on eleven days over the first full growing season of CO_2 exposure to test the hypothesis that stomatal aperture is reduced under elevated CO_2 and to determine whether CO_2 responses are species-specific. Further measurements were carried out during three additional growing seasons in

the three dominant species only. Our main objective was to provide realistic experimental data on g_s of adult deciduous trees exposed to CO_2 enrichment for model-based predictions on the water consumption of forests in a high CO_2 atmosphere.

Material and methods

Site description

The experiment was conducted in a diverse forest stand located 15 km south of Basel, Switzerland (47° 28' N, 7°30' E; elevation: 550 m a.s.l.). The forest is approximately 100 years old with canopy tree heights between 30 and 35 m. The stand has a stem density of 415 trees ha^{-1} (diameter ≥ 10 cm), a total basal area of 46 m^2 ha^{-1} and a leaf area index of approximately 5 in the experimental area. It is dominated by *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl., with *Carpinus betulus* L., *Tilia platyphyllos* Scop., *Acer campestre* L. and *Prunus avium* L. present as companion species. In addition, the site has a strong presence of conifers (*Abies alba* Mill., *Picea abies* L., *Pinus sylvestris* L. and *Larix decidua* Mill.) outside the CO_2-enriched area. Among the species included in the experiment, *Fagus* and *Quercus* contribute 24% and 18%, respectively, to the total basal area under the crane, whereas the other four species contribute less than 6%.

The climate is a typical humid temperate zone climate, characterized by mild winters and moderately warm summers. During the four study years (2001–2003, 2005), the growing season of deciduous trees lasted from the end of April to the end of October (ca. 180 days). Mean January and July temperatures are 2.1 and 19.1°C. Total annual precipitation for the region averages 990 mm, of which two-thirds fall during the growing season. The soil is a silty-loamy rendzina and is characterized by a 15 cm deep rock-free topsoil and a 15-30 cm deep rocky subsoil (approximately 40% of the subsoil volume are stones) underlain by fragmented limestone bedrock. In the upper 10 cm, the soil has a pH of 5.8 (measured in distilled water extracts).

CO_2 enrichment system (web-FACE)

A 45-m freestanding tower crane equipped with a 30-m jib and a working gondola provided access to 62 dominant trees in an area of about 3000 m^2. A group of 14 adult broad-leaved trees (3 *Fagus*, 4 *Quercus*, 4 *Carpinus*, 1 *Tilia*, 1 *Acer* and 1 *Prunus*), covering a canopy area of roughly 550 m^2 were selected for CO_2 enrichment, whereof one slim individual of *Quercus* died. Control trees (3 *Fagus*, 2 *Quercus*, 2 *Carpinus*, 2 *Tilia*, 2 *Acer* and 1 *Prunus*) were located in the remaining crane area at sufficient distance from the CO_2 release zone (mainly in the SW area of the plot). CO_2-enrichment of the forest canopy was achieved by a free-air, pure CO_2 release system that consisted of a web of 4 mm plastic tubes (approximately 0.5 km per tree) with 0.5 mm laser punched holes (spaced at 30-cm intervals) emitting pure CO_2 into the tree canopy. For a more detailed description, see Pepin and Körner (2002).

Stomatal conductance and meteorological measurements

Stomatal conductance to water vapour (g_s, mmol m^{-2} s^{-1}) was measured on upper canopy foliage of 13 trees in elevated CO_2 (ca. 540 ppm) and 12 trees in ambient CO_2 (ca. 375 ppm) during eleven sunny days in summer 2001 (12 June–25 Aug). In subsequent years measurements were restricted to *Fagus*, *Quercus*, and *Carpinus* (26 June, 8 July and 14 August 2002; 24 June, 22 July [all six species measured], 22 August 2003; 18 August 2005). Measurements of g_s were carried out in the morning (8:00–12:00) on three fully sunlit leaves per tree (1–4 trees per treatment and species) using a transient state diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). The sampling procedure on each measurement day was designed to compare treatments under relatively similar weather conditions. Hence, a tree was randomly selected first, and then an individual of the same species but opposite treatment was randomly chosen. This procedure was subsequently extended to the other trees. In the drought summer 2003 (14 August, 20 August) predawn leaf water potential was measured with a pressure chamber (SKPM 1400, Skye Instruments, Powys, U.K.) from the canopy crane gondola on the same species where stomatal conductance was measured.

Occasional parallel studies with a steady-state photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) recalled consistently lower leaf conductances, a difference for which we found no explanation and which we consider intrinsic to the two devices. Since the LI-6400 system is based on measurements of mass flow and gas concentrations, whereas the AP4's conductance data rely on an indirect calibration procedure with pore plates and does not ventilate leaves, we rather trust the absolute LI-6400 readings. A literature comparison of g_s values by Körner et al. (1979) and results from a study on non-ventilated porometers by Verhoef (1997) point in the same direction. Concurrent measurements performed with the AP4 porometer and the LI-6400 gas exchange system under different environmental conditions indicated that readings from both instruments are linearly related ($g_{s(LI-6400)} = 0.623 * g_{s(AP4)} - 2.09$, $R^2 = 0.972$). Hence, the difference is systematic and the AP4 produces signals proportional to the LI-6400. Subsequent measurements of g_s were, nonetheless, carried out with the AP4 porometer for its far better suitability for such a canopy survey, in which much of the 'true' precision comes from good coverage of the natural variability. Such canopy coverage requires rapid measurement in many leaves across all trees in a daily course. Perhaps even more importantly, the AP4 readings are so fast that they capture the momentary stomatal status in a leaf, whereas the time it takes to achieve readings with the LI-6400 will incure stomatal responses to conditions in the cuvette. Since we are exploring treatment differences rather than absolute values for their own sake, any systematic error would not affect our analysis.

Environmental data

Wind speed, photon flux density, rainfall, air temperature and relative humidity were measured above the tree canopy using a weather station located at the top of the crane (anemometer AN1, quantum sensor QS, tipping bucket rain gauge RG1, shielded temperature and relative humidity probe RHA 1, Delta-T, Cambridge, UK). Measurements were performed every 30 s (except for wind speed which was measured as wind run) and data were recorded as 10-min means using a data logger (DL3000, Delta-T, Cambridge, UK). Vapour pressure deficit (VPD) was calculated from 10-min averages of relative humidity and

air temperature. Soil water content was measured using time domain reflectometry (TDR). Six point probes were buried at approximately 10 cm depth, two of which in the CO_2 enriched area and four in the surrounding control area (ML2x, Delta-T, Cambridge, UK). Three additional probes were installed in 2004 and all probes were recalibrated. In addition we used three profile probes to determine moisture content between 0 and 90 cm depth which provided some indication of relative moisture trends in the sub soil (MP-917 and probes PRB-F, Environmental Sensor Inc., Victoria, BC, Canada).

Data processing and statistical analysis

We analysed mean g_s per tree using a repeated measures analysis of variance (RM-ANOVA) with species and CO_2 treatment as fixed factor effects (type I sums of squares, factors in the same order as listed) and time (measurement day or year) as the repeated factor. Our tree sample consisted of 13 treated trees and 12 controls. The unreplicated species *Tilia*, *Acer* and *Prunus* were pooled and treated as 'other' species, hereafter referred to as 'TAP'. To test the effect of elevated CO_2 on g_s of all six species, a repeated measures ANOVA was carried out for the first year only (2001; in the following years, measurements have been performed on the three dominant species only). To determine whether CO_2-effects on g_s differed among years, a subsample of the three main species (*Carpinus*, *Fagus*, and *Quercus*) which were measured throughout the four study years was analyzed using averages for each tree and year. Furthermore, a RM-ANOVA was computed for each species separately. In the case of dominant species, a seasonal average was calculated for each tree and year, and analysed by a repeated measures ANOVA with treatment as a fixed factor and year as a repeated factor. A similar RM-ANOVA was also carried out for each species and year separately using measurement day as a repeated factor. Additionally, one-way ANOVAs with CO_2 treatment as a fixed factor effect were performed for each species and day separately and for all trees together. CO_2-induced reductions in stomatal conductance (in % of g_s under ambient conditions) were calculated for each species and year. A weighted average reduction was calculated for the years with no exceptional weather conditions (2001, 2002, and 2005) giving the first year a weight of 11/15, the second a weight of 3/15 and the fifth year a weight of 1/15 based on the different number of measurement days (Fig. 2 "All"). In the case of 'TAP', the overall mean is identical with the first year mean, since no additional data were obtained in subsequent years with no exceptional weather conditions. All statistical analyses were computed using R version 2.0.1 with a level of significance of $P < 0.05$.

Results

Weather conditions

The years 2001, 2002 and 2005 were characterized by average weather conditions (Tab. 1), with the exception of a relatively dry period in August 2001 (Fig. 1). In the first three years there were only two TDR probes which produced inconsistent differences between the CO_2 enriched area and the control area, most likely due to the large spatial heterogeneity of soils. After reinstallation and calibration of the probes in 2004, soil moisture was slightly and consistently higher in the CO_2 enriched area. In summer 2003, central Europe experienced a severe drought with precipitation less than half of normal and air temperatures 2 - 4°C higher than the 10-year average (1989-1999). Towards the end of June, soil water content dropped to approximately 10% (no plant available moisture) in the top 30 cm measured at our study site and remained at this level throughout July and August (Fig. 1). Similarly low readings were recorded at 60-90 depth with the profile TDR probes during the peak of the drought in August. Hence, during this period, soils were desiccated down to 90 cm depth of the profile and trees depended on deeper moisture reserves (no ground water table on these slopes).

Table 1 Mean air temperature (T), and vapour pressure deficit (VPD) for each measurement day (8:00-12:00). Due to instrument failure data of 31 July 2001 are missing.

Date	T (ºC)	VPD (hPa)
12 Jun 01	12.2	5.1
13 Jun 01	15.6	6.9
21 Jun 01	18.5	11.0
26 Jun 01	21.5	12.1
27 Jun 01	23.0	12.9
4 Jul 01	19.0	9.1
26 Jul 01	21.3	9.5
28 Jul 01	22.3	7.2
31 Jul 01	-	-
12 Aug 01	14.7	5.8
25 Aug 01	22.2	7.8
26 Jun 02	18.1	7.6
8 Jul 02	20.8	8.9
14 Aug 02	16.8	5.7
24 Jun 03	23.4	12.6
22 Jul 03	22.0	10.4
22 Aug 03	20.5	12.2
18 Aug 05	18.5	2.8

Stomatal conductance

There was, over all six species, a tendency towards lower stomatal conductance in trees exposed to elevated CO_2 compared to trees under ambient CO_2 conditions (-10%, Tab. 2, Fig. 2). Conductances differed significantly among species and between measurement days leading to a lot of noise in the data set. Furthermore, a significant species × day effect indicated that different species responded differently to changing weather conditions, which added to the observed variation. Although we found species-specific reductions in g_s ranging from -4% in *Quercus* to -21% in *Carpinus*, the species × CO_2-treatment factor was clearly not significant (Tab. 2). Only on one single day in 2001 all species showed a slightly reduced g_s in elevated CO_2 (Fig. 2). To eliminate the large differences in g_s among species, the data were standardized with respect to the maximum daily average g_s of each tree. This, however, did not lead to a significant CO_2 effect either. We calculated the reduction of g_s which could still be detected with the given variation using a power t-test with a significance level of 0.05 and a power of 0.8. A one-sided power t-test was used since

Table 2 Results of repeated measures ANOVA for stomatal conductance of six tree species (*Carpinus*, *Fagus*, *Quercus*, 'TAP' species (*Tilia, Acer, Prunus*)) exposed to elevated CO_2 over 11 measurement days from June to August 2001.

Factor	df	*F*	*P*
Species	3	3.8	0.03
CO_2	1	2.4	0.14
Species × CO_2	3	0.2	0.91
Day	10	12.1	<0.001
Species × Day	30	3.0	<0.001
CO_2 × Day	10	1.0	0.47
Species × CO_2 × Day	30	0.5	0.98

Table 3 Results of repeated measures ANOVA for stomatal conductance of trees exposed to elevated CO_2 of three species (*Carpinus*, *Fagus*, and *Quercus*) during four growing seasons (2001, 2002, 2003, 2005).

Factor	df	*F*	*P*
Species	2	6.71	0.014
CO_2	1	0.50	0.49
Species × CO_2	2	0.13	0.88
Year	3	18.38	<0.001
Species × Year	6	2.07	0.09
CO_2 × Year	3	0.93	0.44
Species × CO_2 × Year	6	0.64	0.70

we did not expect an increase in g_s in response to elevated CO_2 under regular weather condition. The power test revealed that given the observed variation, a reduction of 25% in stomatal conductance in CO_2-enriched trees would be detectable across all species.

The dominant tree species (*Carpinus*, *Fagus*, and *Quercus*) were sampled over all four study years to examine whether stomatal responses to elevated CO_2 persisted in the long term. Results were very similar to those obtained in the first year when all six tree species were considered. There were no significant CO_2-effects on g_s, but significant differences in g_s among species and years (Tab. 3), the latter being driven by the lower conductances measured in 2003 during the exceptional drought. These differences in g_s between years were no longer statistically significant after exclusion of the drought year's data.

Among species, *Carpinus* showed the largest and most consistent reduction in stomatal conductance in response to CO_2 enrichment which however was only statistically significant on two single days in the first experimental year (Fig. 2). Over the four year period the CO_2 effect was not significant (Tab. 4). Test results were also not significant when considering the growing seasons with no extraordinary weather conditions only or each year separately. For the 'TAP' species, there were no significant differences between treatments (test results not shown) and *Fagus* and *Quercus* showed very small (to negligible) and inconsistent stomatal responses (Fig. 2).

Stomatal responses to elevated CO_2 during a severe drought

During a prolonged drought period in summer 2003 (Fig. 1), g_s of CO_2-enriched trees and control trees were strongly reduced in most species compared to previous years (Fig. 2). There was, however, considerable variability in stomatal responses to drought among tree species. Mean seasonal g_s at ambient CO_2 during the drought year were only slightly reduced in *Quercus* (6%) compared to 2001, whereas in *Fagus*, the 'TAP' species and *Carpinus,* moderate to pronounced reductions were found (52% - 68%; see 'Year'-effect in Tab. 4).

Towards the end of this severe drought, we observed significantly higher conductances in CO_2-enriched *Carpinus* trees compared to control trees (*P*=0.004, Fig. 2). A trend towards higher g_s values under elevated CO_2 was also observed in the 'TAP' species. At the same time, predawn leaf water potentials tended to be higher in CO_2-enriched *Carpinus* trees (-1.14 MPa in ambient CO_2 vs. -0.91 MPa in elevated CO_2, *P*=0.053) but not in trees belonging to the 'TAP' species). In *Fagus* and *Quercus,* g_s was not altered by CO_2 enrichment under these dry soil conditions, in line with the data for other years with no extraordinary weather conditions when no significant CO_2 effect was detected on g_s.

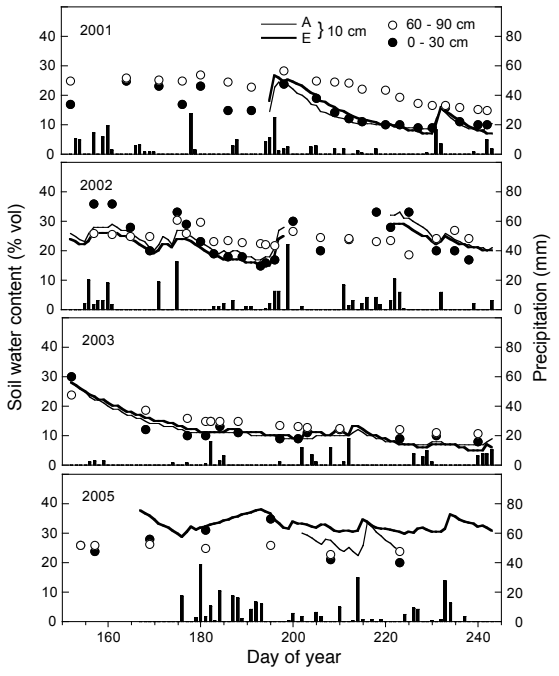

Fig. 1 Soil water content at 10 cm depth in the area exposed to ambient CO_2 (A, thin line, n=2) and elevated CO_2 (E, thick line, n=4-7), at 60-90 cm depth (open symbols) and 0-30 cm depth (closed symbols) both in the ambient CO_2 area (n=3) and precipitation (bars) during four growing seasons.

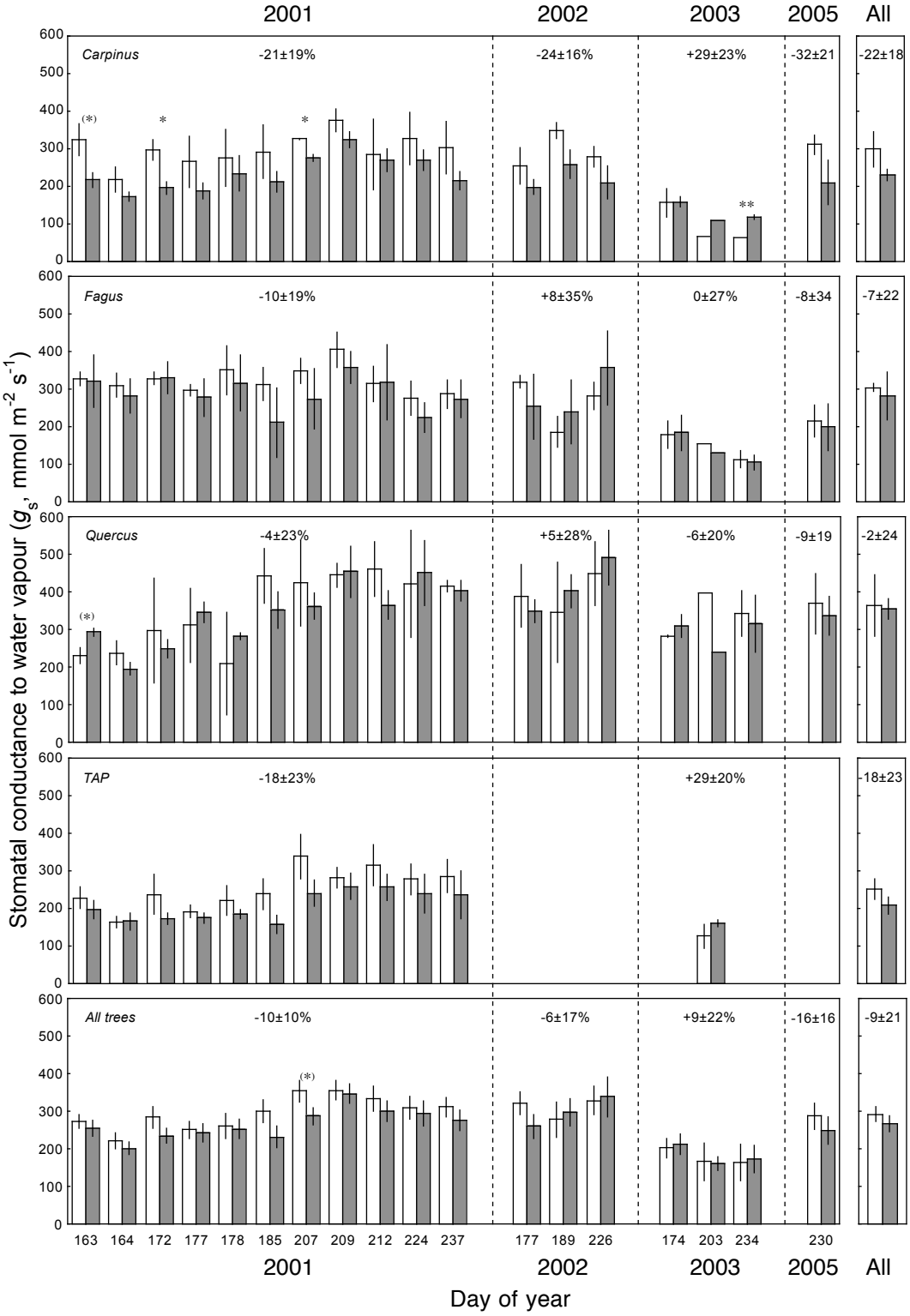

Fig. 2 Stomatal conductance (g_s, mean ± SE) of upper canopy foliage in six deciduous tree species (n = 12 trees) exposed to ambient (A, open bars; ca. 375 ppm) and elevated CO_2 (E, dark bars; ca. 540 ppm) during four growing seasons (8:00–12:00). 'TAP' refers to *Tilia, Acer*, and *Prunus*, three species that were pooled because they were not replicated (E, n=3; A, n=5). The 'All' column refers to average g_s for three years with no exceptional weather conditions (2001, 2002, 2005) weighted by measurement days per year. In the bottom panel, 'All trees' refers to six species in the year 2001 and on day 203 in 2003 (E, n=13; A, n=12) and the three main species (*Carpinus, Fagus, Quercus*) in 2002, 2003 and 2005 (E, n=10; A, n=7). Percent numbers represent mean CO_2 signals ± SE across season. The summer of 2003 was exceptionally dry. Note, the AP4 porometer produces somewhat high absolute g_s values, which however, does not affect differences between CO_2 treatments (see Material and Methods). $^{(*)}$ P <0.1; * P <0.05; ** P <0.01; *** P <0.001

Table 4 Results of repeated measures ANOVA for stomatal conductance of three species exposed to elevated CO_2 over four growing season (2001, 2002, 2003, 2005).

		Carpinus		*Fagus*		*Quercus*	
Factor	df	*F*	*P*	*F*	*P*	*F*	*P*
CO_2	1	3.11	0.18	0.02	0.91	0.02	0.89
Year	3	7.77	0.005	12.7	0.001	2.73	0.11
CO_2 × Year	3	1.58	0.25	0.34	0.80	0.20	0.90

Discussion

This study documents responses of stomatal conductance in a diverse natural forest to elevated CO_2. The four year data reveal that reductions in conductance in response to 540 ppm CO_2 are certainly smaller than 25%. Despite the large number of readings and four years of data collection, we were unable to demonstrate the occurrence of a statistically significant reduction of stomatal conductance and onlyfound a tendency towards reduced g_s of around 10% when averaged across all species and trees. As can be seen in Fig. 2 *Carpinus* and the 'TAP' species (*Tilia*, *Acer* and *Prunus*) consistently show more pronounced reductions of g_s compared to other species which is in full agreement with sap flow data from the same trees in 2001 (Cech et al. 2003). The size of the responses seen in *Carpinus* and the 'TAP' species over the four years, though not significant, are well in line with the mean 21% reduction reported by Medlyn et al. (2001) using a meta analysis for long-term experiments carried out in the field and are also consistent with measurements carried out on *Liquidambar styraciflua* in two FACE experiments (-24%, Gunderson et al. 2002; -28%, Herrick et al. 2004). The responses in *Fagus* and *Quercus* were much smaller and less uniform, hence CO_2 effects at the leaf level are much less likely even if we had more trees. In line with our findings Dufrêne et al. (1993), using branch-bags in mature *Fagus,* also found no CO_2-effect on g_s in this most common European deciduous tree species. A number of full grown tree species, noteworthy conifers, also have shown no stomatal response to elevated CO_2 (Teskey 1995; Ellsworth 1999).

Our data therefore support that stomatal responses to elevated CO_2 are most likely species-specific. The presence or absence of a certain species in a catchment would thus have hydrological consequences. Our data illustrate the risk of drawing general conclusions from a single species' response. In this sense, our results match studies conducted on saplings and potted trees, which also reported great species specificity in stomatal responses to CO_2 (Picon et al. 1996; Heath 1998).

The study of g_s in this natural, highly diverse, mature forest exposed to elevated CO_2 suffered from the common experimental and analytical difficulties when realistic test conditions come into play (Körner 2001). To comply with the height requirement of the study trees (between 30 and 35 m) and the complex structure of the canopy, a new CO_2 enrichment system was designed (web-FACE, Pepin and Körner 2002). However, the need of a canopy crane did not permit randomization of the treatment units (it would require several cranes) and therefore, we employed a detailed investigation of *a priori* differences in physiology and morphology between control trees and those later exposed to CO_2. The analysis performed by Cech et al. (2003) revealed no systematic differences between the two groups of trees, hence the prerequisites for the experiment were fulfilled. Given the stature of the forest, the large size of the CO_2-enriched area exerted a further constraint, namely that not all species could be measured at the same time despite rapid crane operation. On the other hand, the area was still not large enough to permit replication in both treatments to a desirable extent. One way to handle this was to consider the CO_2 response of 'trees' only, irrespective of species (13 treated trees and 12 control trees). We performed such tests, but these revealed no significant CO_2 effect either, possibly due to the large variation in absolute g_s among species (at full stomatal opening g_s in *Quercus* is roughly 2 times that of the other species).

Despite these inevitable problems, it is still remarkable that the trend towards reduced stomatal conductance in these deciduous tree species exposed to elevated CO_2 was sustained throughout the summers of 2001, 2002 and 2005 (with no exceptional weather conditions as opposed to 2003). This is in agreement with earlier studies which have shown small but consistent responses in g_s of *Liquidambar styraciflua* trees over 3 - 4 years of CO_2 enrichment (Gunderson et al. 2002; Herrick et al. 2004). Bearing in mind that trees in this study are of considerable age, height, and size, the consistency of the responses over several years (particularly in *Carpinus*) leads to our confidence in the data, although differences were rarely statistically significant. Very good correspondence between these leaf level stomatal conductance data and sap flow density carried out on the same trees in 2001 adds to this confidence. Reductions in mean daily sap flow density of CO_2-enriched trees averaged approximately 11% across all days of the growing season in 2001 (Cech et al. 2003), a value close to the non significant overall 10% reduction in g_s (mean of all six tree species) reported here for the first year, although the stomatal signal does not include a boundary layer component. Furthermore, the same species were found to be more responsive to elevated CO_2 (*Carpinus* and the 'TAP' species).

In plant systems with high stomatal control over transpiration, CO_2-induced reductions in g_s can lead to a decrease in water consumption and result in higher soil moisture content (Hungate et al. 2002), which was also found at our study site (Leuzinger, 2006). During a period of relatively high evaporative demand and decreasing soil water content in summer 2001, Cech et al. (2003) observed greater sap flow density in CO_2-enriched trees than in control trees. Although the differences in sap flow density between the two groups of trees were not statistically significant, treatment differences increased over time, providing support to the hypothesis that soil moisture savings in the CO_2-enriched area could reverse the effect of elevated CO_2 on stomatal conductance and transpiration. More recent soil moisture data from our site confirm this pattern of a reverse effect of soil drying during prolonged rainless periods (Leuzinger 2006). Based on these findings we expected similar soil moisture feedback to appear during the drought conditions of summer 2003 and, indeed we did observe such a reversal of CO_2 effects on g_s of *Carpinus* and a tendency in this direction in the 'TAP' species. Less negative predawn leaf water potentials in *Carpinus* under elevated CO_2 and drought adds a piece of evidence that water status in this species was improved compared to control trees and indicates that this species was more sensitive to CO_2 enrichment than the other study species.

These results suggest that small, at the leaf level hardly measurable, CO_2-induced decreases in g_s are sufficient to translate into a cumulative soil water enrichment in the area exposed to elevated CO_2. There are insufficient data for the rocky subsoil to verify this for roots at greater depth, but a higher predawn leaf water potential under elevated CO_2 and drought suggests water savings throughout all rooted soil horizons (Leuzinger et al. 2005). During the summer drought of 2003, these water savings must have occurred at much deeper soil layers, because soil moisture dropped to 10% at 60-90 cm depth (corresponding to air dry soil moisture). Manual TDR readings at high spatial frequency in 2002 confirmed that soil water content at approximately 10 cm was significantly increased in the CO_2-enriched area (Cech et al. 2003). Yet, during the extreme summer drought, this method was not applicable since the top soil was completely dry.

During the severe drought of summer 2003, g_s decreased considerably in all investigated species, with the exception of *Quercus* (a deep rooted, drought-tolerant genus which might have access to soil moisture at greater depth, Becker 1990; Epron and Dreyer 1993; Leuschner et al. 2001). Again, the results matched sap flow measurements showing nearly constant sap flow density in *Quercus* throughout the summer months, whereas in *Carpinus* and *Fagus* sap flow density decreased to half of the early summer maxima (Leuzinger et al. 2005).

In conclusion, we showed that stomatal responses to elevated CO_2 in these mature forest trees are certainly smaller than 25%, most likely in the range of about 10% across species. Globally, even small CO_2-driven reductions in stomatal conductance could have a significant impact on the water balance. Using gas exchange theory only, Gedney et al. (2006) speculated that such trends influenced run-off in the twentieth century in a continental specific way. But based on our and other field data (those for conifers in particular) and accounting for atmospheric feedback, we believe that theory based signals are likely to overestimate actual effects substantially. Based on gas exchange theory (Farquhar and Wong 1984) no species specificity would be expected. However, although we were unable to detect significant reductions in any species, the combined trends in leaf conductance and leaf water potential point at a great sensitivity of *Carpinus* to elevated CO_2 and no leaf level response in *Fagus* and *Quercus*. In the long run, such species specific differences may lead to a change in species abundance driven by soil moisture (and nutrient) effects. Our results clearly demonstrate the need to account for biodiversity and both soil moisture and atmospheric humidity feedback on CO_2 responses of stomata in order to arrive at a realistic picture of the hydrological and biological consequences of ongoing atmospheric CO_2-enrichment.

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General summary

This thesis presents the fate of recently assimilated carbon (C) in a mature deciduous forest. Continuous canopy labelling permitted to study C allocation on the whole forest scale, whereas short term branch labelling allowed to trace recent C within the crowns of 35 m tall trees. In addition stomatal conductance was measured to assess the potential uptake of labelled CO_2. The following paragraphs summarize the different aspects of carbon transfer in the same order as the chapters appear.

The fate of recently assimilated carbon in a mature deciduous forest

Exposing the crowns of mature trees to labelled CO_2 permitted to trace the fate of recent C into various forest compartments. Potted C4 grasses grown in the canopy served as a reference to calculate the fraction of labelled C in each compartment studied and allowed to estimate turnover times of the C pools.

In the first year of canopy labelling 70% new C was found in leaves and tree rings averaged over all species (**chapter 2**). Only after four growing seasons of labelling, C signals had reached nearly 100% in new leaf tissue, whereas in new tree rings still 10% unlabelled C was observed. These longer than expected replacement times of old by new C were attributed to a dilution of isotope signals rather than to a dependence of new structures on old C. Most likely labelled C is filled into existing unlabelled mobile carbohydrate pools (in woody tissue), gets mixed with these old pools and only then is invested into new structures, resulting in dampened signals. Interestingly these diluted signals were observed in five of the six broad-leaved species (*Acer, Carpinus, Fagus, Prunus, Quercus*) but not in *Tilia*, where 100% signals were attained in leaves as well as new tree rings already in the first year of labelling.

Leaf litter sampled in the crowns shortly before leaf fall had nearly identical signals with fresh leaves. In contrast, litter collected near the ground with traps showed much weaker signals which results from strong wind-mixing of labelled litter with litter from surrounding, unlabelled trees explaining the observed signal dilution.

In late summer of the fourth labelling year fine roots contained 40% new C. As for leaves, fine roots could be built from a mixture of old and new C which accounts for the rather weak signals. Alternatively, these results might indicate a greater than expected fine root longevity with a ten year turnover if assuming a linear increase.

In sporocarps of mycorrhizal fungi, 60% new C was found from the first year on and signals were sustained throughout the study years (except for the year 2004, when signals dropped to 40% but resumed to 60% in the following year; **chapter 3**). Since sporocarps have a high turnover, the pronounced signals in mycorrhizal fungi indicate that recent C is rapidly returned to the atmosphere. Signals in sporocarps of mycorrhizal fungi were confined to the labelled area and hardly spread beyond the 6 m periphery of the labelled zone.

Surprisingly no labels were found in saprophytic fungi even after five years of labelling, suggesting that C older than five years was decomposed. We found clear evidence for a distinct differentiation in the path and speed of carbon transfer from the forest canopy to mycorrhizal and saprophytic fungi.

In line with the lack of signals in saprophytic fungi, we did not detect labelled C in bulk soil shortly before the fifth labelling season started (**chapter 2**). However, in the vicinity of fine roots (rhizosphere), 9% of the soil was labelled after four treatment years, indicating that new C enters the soil mainly via fine roots or associated mycorrhizal fungi.

Depending on climatic conditions soil-air CO_2 at 3-11cm depth contained 35-50% new C, with highest signals during an exceptionally dry summer. The new C signals measured in the first years reflect the fraction of soil respiration attributed to CO_2 released by roots, mycorrhizal fungi and root-associated microbes, together referred to as autotrophic respiration. These results suggest that considerable amounts of recent C are invested into C pools with high turnover rates.

Carbon flux and growth of mature trees exposed to elevated CO_2

Canopy labelling was achieved by means of a free air CO_2 enrichment (FACE) system which uses ^{13}C depleted CO_2. The FACE experiment was set up to test whether forests will grow faster and store more C under future atmospheric CO_2 concentrations. During four years of exposure to roughly twice the pre-industrial CO_2 concentrations (ca. 540 ppm) no consistent overall stimulation in stem growth was observed (**chapter 4**). *Fagus* was the only species showing a transient growth response (in the first as well as the third treatment year) which was absent in the other species. Annual litter production did not respond to CO_2 and we found no downward adjustment of photosynthetic capacity in CO_2 enriched trees. Leaf chemistry changes were minor and tree species differed in their responses. Only *Carpinus* showed a reduction in leaf nitrogen whereas an increase in non structural carbohydrates (NSC) was only observed in *Quercus* and subdominant species (*Acer*, *Prunus*, *Tilia*). In leaf litter, NSC concentrations were increased across all species indicating an overflow of photoassimilates channelled to the decomposer pathway. In contrast, lignin concentrations were reduced suggesting a shift in C fractions from recalcitrant to more labile compounds. Carbon flux through these 35 m tall temperate forest trees was enhanced already in early summer of the first full treatment year. Throughout the four study years soil CO_2 concentrations remained mostly higher in the CO_2 enriched area (except for a severe drought period in summer 2003), suggesting enhanced metabolic activity in soils under trees exposed to elevated CO_2. This was supported by strongly labelled soil CO_2 which indicated that much of the respired CO_2 had been recently assimilated. Highly pronounced new C signals in mycorrhizal fungi are well in line with these results, since hyphae are known to turnover rapidly. In summary these trees did not accrete more biomass carbon in response to elevated CO_2 but rather 'pumped' more C through their body.

Arthropod movement in the canopy

The canopy labelling produces food with a different isotope signature for arthropods living in the treated crowns and allowed to study their movement patterns (**chapter 5**). For arthropods of limited mobility there should be a close correlation between the isotope signature of their body tissue and the signatures of leaves

on which they feed. For highly mobile insects, we expect that $\delta^{13}C$ of arthropods would not correlate with host plant tissue values since they can change trees and feed on labelled as well as unlabelled plant tissue. In the studied phytophagous groups (feeding on leaves or phloem) we found a significant correlation between $\delta^{13}C$ of leaf tissue with $\delta^{13}C$ of aphids, lepidopteran caterpillars, bush-cricket nymphs, true bugs (nymphs) and leafhoppers (nymphs and adults) suggesting that these groups display little spatial shifts between the CO_2 enriched area and the control area. In contrast, this correlation vanished in fully winged adults of bush-crickets and true bugs, suggesting a higher degree of mobility in the canopy. Omniphagous earwigs with a wide range of food revealed a marginally significant correlation with $\delta^{13}C$ of body tissue and $\delta^{13}C$ of leaf tissue, whereas no correlation was found for predators (zoophagous), which is likely to be due to a wide prey spectrum, which dilutes the $\delta^{13}C$ signature.

Carbon allocation within tree crowns

To test whether mature branchlets are neutral transport pathways or whether there is an exchange of recent C between the phloem and the adjacent woody tissue along the branch, we pulse labelled one year old branchlets in summer (**chapter 6**). These experiments were carried out on trees not exposed to elevated CO_2 but which were still accessible from the crane. Recently assimilated C entered branch wood of nine European forest tree species (6 broad-leaved species, 3 conifers) within two to nine hours after pulse labelling. Four months after labelling we found noticeable differences in the amount of labelled C which had remained in branch wood of the six broad-leaved species (not examined for the three conifers). In *Fagus*, *Prunus* and *Quercus* signals were either unchanged or had increased since the day of labelling, whereas signals had nearly disappeared in *Tilia*. Since the branch labelling experiments were carried out after shoot growth had been completed, the species-specific signals do not reflect different degrees of allocation into structural growth, but rather represent different degrees of mixing of new with old mobile C in branchlet wood. Low remaining signals after branch labelling in *Tilia* thus indicate a low degree of mixing which is in line with the rapid replacement of old by new C observed in the canopy labelling experiment (chapter 2). This suggests a direct investment of new C only in this species, as opposed to the five others. Among the study species *Tilia* is the only one which stores considerable amounts of lipids (Hoch et al., 2003). Since lipid stores are likely to undergo less C-cycling, the exchangeable C pool is smaller, offering a plausible explanation for the lower mixing of new with old C observed in this species.

Based on these results we suggest that lipid storing species should be most useful for climate reconstruction using carbon isotope ratios in tree rings as proxies for actual climatic conditions. We expect a tighter linkage between isotope ratios of tree ring wood and climatic conditions. In contrast, the constant mixing of new with old C in other species could explain the often observed autocorrelation; a correlation of isotope ratios in subsequent years. A recent study showing stronger and more consistent correlations of $\delta^{13}C$ in cellulose with temperature in *Pinus sylvestris* (a 'fat-tree') compared to *Quercus petraea* (a 'starch-tree') strongly supports our findings (C.E. Reynolds Henne, personal communication).

Contribution of photoassimilates from infructescences for fruiting in two deciduous forest tree species

Applying the same pulse labelling method in combination with a girdling and defoliation experiment, the pathways of currently fixed carbon in fruit bearing branchlets were investigated in two temperate forest tree species (**chapter 7**). The two species differ in texture of their vegetative infructescence tissues (leaf-like in *Carpinus betulus* vs. woody in *Fagus sylvatica*). During late spring, ^{13}C pulse-labelling was conducted on girdled, defoliated, girdled plus defoliated and untreated fruiting branchlets of mature trees *in situ*, to assess changes in C-relations in response to the introduced C-source-sink imbalances. At harvest in early August, 75 - 100 % of the recovered ^{13}C label was bound to infructescences (either fruits or vegetative infructescence tissue), revealing them as the prime C-sinks for current photoassimilates. Leaves on girdled branchlets were not stronger labelled than on ungirdled ones in both species, indicating no upregulation of the leaves' photosynthetic capacity in response to the prevention of phloemic transport, which was also supported by measurements of light saturated photosynthesis. In contrast, ^{13}C labels tended to be higher after complete defoliation in the vegetative infructescence tissues of *Carpinus*, suggesting enhanced net photosynthesis of green infructescence parts as compensation for the loss of regular leaves. The total labelling-derived ^{13}C content of whole infructescences was very similar between foliated and defoliated *Carpinus* branchlets. Cupulae of *Fagus*, on the other hand, remained almost unlabelled on defoliated branchlets, indicating the photosynthetic inactivity of this woody infructescence tissue. Consequently, *Carpinus* still produced relative high fruit masses on girdled plus defoliated branchlets, while in *Fagus* fruit development ceased almost completely at this most severe treatment. These results highlight that green vegetative infructescence tissue assimilates substantial amounts of C and can partly substitute regular leaves as C sources for successful fruit development.

Stomatal conductance in mature trees exposed to elevated CO_2

To test whether elevated CO_2 reduces stomatal conductance in mature, field-grown trees measurements were carried out on upper canopy foliage over four growing seasons (**chapter 8**). In response to elevated CO_2, we observed a slight but insignificant reduction of stomatal conductance across all species, which was certainly less than 25%, most likely in the range of 10%. Stomatal responses at the leaf level were thus similar in magnitude to signals in whole-tree transpiration measured in the same trees (Cech et al., 2003; Leuzinger & Körner, in revision), suggesting that tree foliage in this canopy was well coupled to the atmosphere. We found evidence for a species-specificity in the stomatal response to CO_2, with *Carpinus* exhibiting consistently lower stomatal conductance in CO_2 enriched trees compared to control trees in contrast to *Fagus* and *Quercus* where treatment differences varied around zero. Although the CO_2 induced reductions were rarely significant in

Carpinus, they were large enough to lead to a reversed CO_2 effect (higher conductance and less negative predawn leaf water potential in trees exposed to elevated CO_2) during a severe drought, resulting from an improved water status as a consequence of previous soil water savings. As atmospheric CO_2 concentrations will continue to rise and drought events become more frequent, these species-specific responses might alter species abundance driven by soil moisture and related nutrient effects.

Isotope ratios of plant tissue reflect climatic conditions during assimilation and the isotope ratio of source air (Farquhar et al., 1989). Conditions leading to lower stomatal conductance result in less negative plant $\delta^{13}C$. Hence, the observed reduction in stomatal conductance in response to elevated CO_2 (chapter 8) could result in less negative $\delta^{13}C$ and have an opposing effect on the ^{13}C signals in plant tissue (chapter 2). ^{13}C labels should thus be most pronounced in species showing no reduction in stomatal conductance (*Fagus*, *Quercus*) when exposed to elevated CO_2 and weakest ^{13}C labels would be expected in species reflecting a reduction of stomatal conductance (*Carpinus*) which however, was not the case. The direct effect of elevated CO_2 on ^{13}C signals can therefore assumed to be negligible and does not interfere with the ^{13}C labelling study.

In conclusion this large scale carbon isotope labelling experiment in a mature temperate forest showed that recently assimilated carbon is mostly allocated to short turnover pools (<10 years). Additional carbon storage, which relies on an enhanced carbon transfer to long-lived pools such as stem wood and soil is therefore rather unlikely. The canopy labelling revealed a slow and highly species-specific dilution of old by new carbon in trees, which could be confirmed by branch labelling experiments. Based on our results climate reconstruction using carbon isotope ratios as proxies for climatic conditions could be improved by choosing lipid storing species.

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