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Central European hardwood trees in a high-CO_2 future: synthesis of an 8-year forest canopy CO_2 enrichment project

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Summary

1. Rapidly increasing atmospheric CO_2 is not only changing the climate system but may also affect the biosphere directly through stimulation of plant growth and ecosystem carbon and nutrient cycling. Although forest ecosystems play a critical role in the global carbon cycle, experimental information on forest responses to rising CO_2 is scarce, due to the sheer size of trees.

2. Here, we present a synthesis of the only study world-wide where a diverse set of mature broadleaved trees growing in a natural forest has been exposed to future atmospheric CO_2 levels (*c.* 550 ppm) by free-air CO_2 enrichment (FACE). We show that litter production, leaf traits and radial growth across the studied hardwood species remained unaffected by elevated CO_2 over 8 years.

3. CO_2 enrichment reduced tree water consumption resulting in detectable soil moisture savings. Soil air CO_2 and dissolved inorganic carbon both increased suggesting enhanced below-ground activity. Carbon release to the rhizosphere and/or higher soil moisture primed nitrification and nitrate leaching under elevated CO_2; however, the export of dissolved organic carbon remained unaltered.

4. *Synthesis.* Our findings provide no evidence for carbon-limitation in five central European hardwood trees at current ambient CO_2 concentrations. The results of this long-term study challenge the idea of a universal CO_2 fertilization effect on forests, as commonly assumed in climate–carbon cycle models.

Key-words: CO_2 fertilization, coupled climate–carbon cycle model, ecosystem carbon cycling, elevated CO_2, free-air CO_2 enrichment (FACE), global carbon cycle

Introduction

At present, the Earth's vegetation is exposed to atmospheric CO_2 concentrations that have been unprecedented over the past 15 million years (Tripati, Roberts & Eagle 2009). By default, land ecosystems are probably absorbing *c.* 25–30% of the annual anthropogenic CO_2 emissions, which have dramatically increased over the last decade and are now tracking the worst-case IPCC scenario (A1F1, Meehl *et al.* 2007; LeQuéré *et al.* 2009). Forests are major potential contributors to this terrestrial carbon (C) sink as they account for nearly 90% of the terrestrial biomass and about half of terrestrial net primary productivity (NPP; Roy, Saugier & Mooney 2001). Including soils, forest ecosystems accommodate roughly twice the amount of C held in the atmosphere (Jobbágy & Jackson 2000; Roy, Saugier & Mooney 2001). Given their prominent role in the global C cycle, the responses of trees and forests to rising atmospheric CO_2 are essential to understand how C cycling will change in a future high-CO_2 world and how these changes might feedback to other ecosystem processes and the climate system. Future climate predictions are highly sensitive to changes in the global C cycle in response to increasing atmospheric CO_2 (Friedlingstein *et al.* 2006). Coupled climate–carbon models commonly assume a CO_2 fer-

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tilization effect on plant growth that is further assumed to eventually result in enhanced C storage in organic matter, a step that lacks a theoretical as well as an empirical foundation (a flux vs. pool confusion, see Körner 2006). Any increase in terrestrial C storage would reduce the rate of CO_2 accumulation in the Earth's atmosphere and thus potentially mitigate the trajectory of climate change. However, the magnitude of the CO_2 fertilization effect and its continuity into the future are a matter of debate (Matthews 2007). There is plenty of evidence that elevated atmospheric CO_2 can stimulate plant growth when nutrients and other resources are not limiting, as in many agricultural and horticultural environments. Growth responses to CO_2 enrichment in near-natural systems, where growth is often water- or nutrient-limited, vary widely but are certainly smaller than anticipated and tend to decrease over time, especially in forests (Leuzinger *et al.* 2011; Norby & Zak 2011). Even under favourable soil conditions, growth in elevated CO_2 may eventually lead to progressive nitrogen (N) limitation through accelerated fixation of N in organic matter and thus cause downregulation of forest growth responses (Luo *et al.* 2004; Norby & Zak 2011). Such interactions of elevated CO_2 with biogeochemical processes that operate on longer time scales cannot be revealed in short-term studies in decoupled systems and highlight the importance of long-term CO_2 enrichment experiments in natural forest settings. CO_2-induced growth stimulation may only translate into enhanced forest biomass C sequestration if trees grow larger by maturing more rapidly and also show a prolongation of their life span (Körner 2009). However, any CO_2 fertilization effect may be offset by effects on tree longevity (Bugmann & Bigler 2011), because carbon storage in forest biomass is, in essence, an issue of tree demography that cannot be explored in CO_2 enrichment experiments.

Owing to the size of trees, forest data are critically underrepresented in the experimental CO_2 research literature, with most evidence originating from young trees and premature stands. Further, tree size restricts the tree species diversity that can be accommodated in a given test plot, which conflicts with the well-known species specificity of tree CO_2 responses (Keel *et al.* 2007; Leuzinger & Körner 2007; Dawes *et al.* 2011; Smith *et al.* 2013a). Here, we present a synthesis of the effects of 8 years of CO_2 enrichment in the so far only natural, diverse forest tested, including novel, previously unpublished data on tree-ring increments, stable C isotope signatures in wood, dissolved C fluxes in soil water and N availability (foliar $\delta^{15}N$). In addition, we extended the published 2001–2004 time series for leaf litter production, leaf traits and soil CO_2 dynamics (Körner *et al.* 2005; Keel, Siegwolf & Körner 2006) with more recent data from 2005–2008 and report the results of the new multiyear analyses.

This long-term free-air CO_2 enrichment (FACE) experiment was established in north-west Switzerland to test whether rising atmospheric CO_2 concentrations will stimulate tree growth and the forest C cycle and alter N availability. With the aid of a construction crane, we installed a fine web of perforated tubes releasing pure CO_2 into the crowns of 11 35-m-tall deciduous trees to simulate a future atmosphere containing *c.* 550 ppm CO_2 (Pepin & Körner 2002). The study comprised *Fagus sylvatica* and *Quercus petraea* – two dominant forest trees in Central Europe – as well as the subdominant *Carpinus betulus, Tilia platyphyllos* and *Acer campestre*.

Materials and methods

STUDY SITE

The Swiss Canopy Crane (SCC) FACE site was established in 1999 in an *c.* 100-year-old mixed forest, situated 15 km south of Basel, Switzerland (47°28′N, 7°30′E, 550 m.a.s.l.). The closed-canopy stand is about 30–35 m tall and has a tree density of 415 trees ha^{-1} (breast height diameter ≥ 0.1 m), a peak leaf area index of 5 (without counting the largely herbaceous forest understorey) and a basal area of 46 m^2 ha^{-1} (Pepin & Körner 2002). *Fagus sylvatica* L. (European beech) and *Qercus petraea* (Matt.) Liebl. (sessile oak) dominate the forest canopy, accompanied by subdominant tree species such as *Carpinus betulus* L. (hornbeam), *Tilia platyphyllos* Scop. (large-leaf linden), *Acer campestre* L. (field maple), *Prunus avium* L. (cherry) and four species of conifers [*Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.]. With the help of a 45-m-tall construction crane, 12 deciduous trees were initially subjected to canopy CO_2 enrichment, beginning in autumn 2000 (three *F. sylvatica*, three *Q. petraea*, three *C. betulus*, one *T. platyphyllos*, one *A. campestre* and one *P. avium*). Due to its near to off-site position under the crane's reach and some storm damage, the single *P. avium* tree had to be excluded from the CO_2 treatment in 2006, and a maple control tree was felled by a winter storm. The crowns of the study trees were exposed to an elevated concentration of atmospheric CO_2 (*c.* 550 ppm) during daylight hours throughout the growing season using a canopy FACE system (Pepin & Körner 2002). Intentionally, the understorey vegetation did not receive CO_2 enrichment, and canopy CO_2 enrichment 25–35 m above-ground did not leak to the ground, hence offering unbiased reference conditions for obtaining soil ^{13}C isotope signals (Keel, Siegwolf & Körner 2006). A larger number of control trees were selected at sufficient distance to the CO_2-enriched area. The applied CO_2 was of fossil origin and thus depleted in ^{13}C ($-29.33 \pm 0.23‰$, 8-year mean ± SE, i.e. delivered at a constant quality over the study years), which allowed us to trace the fate of the newly assimilated C. The experiment was terminated at the end of the 2008 growing season.

LEAF LITTER PRODUCTION, LEAF TRAITS AND FOLIAR ^{15}N ANALYSIS

Leaf litter was collected from 44 litter traps (0.5 m^2) arranged in a 6-m grid within the study area. In autumn, traps were emptied biweekly, and the litter was oven-dried at 80 °C for at least 48 h and then weighed. Litter fall in the year 2001 was considered to still represent foliage produced under pre-treatment conditions. Hence, litter fall of 2001 was averaged with data from 1999 and 2000 to account for natural spatial variability of litter fall over 3 years before the start of CO_2 enrichment.

In summer (June-July), 10 leaf discs (1.2 cm^2) per tree were collected from the crane gondola for determination of specific leaf area (SLA), non-structural carbohydrates (NSC), nitrogen concentrations and the $^{15}N/^{14}N$ isotope ratio ($\delta^{15}N$) in the top of the canopy. Leaf discs were dried at 80 °C for at least 48 h and then weighed for biomass quantification. After grinding, all samples from one tree were

merged for leaf N analysis using a CHN-analyser (Vario EL *III*, Elementar Analysensysteme GmbH, Hanau, Germany) and for NSC analysis based on an enzymatic starch digestion followed by a spectrophotometric glucose test after invertase and isomerase addition. Foliar $\delta^{15}N$ was analysed with an isotope ratio mass spectrometer (Delta S, Thermo Finnigan Mat), which was linked via a variable open split interface (CONFLO II) to an elemental analyser (EA 1108, Carlo Erba, Milano, Italy). Natural ^{15}N abundance was expressed in the δ-notation: $\delta^{15}N = R_{sample}/R_{standard} - 1$, where R is the molar ratio of ^{15}N to ^{14}N for the sample and the standard, respectively.

RADIAL INCREMENT AND TREE-RING $\delta^{13}C$

We sampled 2–3 wood cores per tree using a stainless steel wood corer to assess radial increment and $\delta^{13}C$ in growth rings. Tree-ring widths were measured using an automated bench with a stereomicroscope (Lintab/TSAP, Rinntech, Heidelberg, Germany). Dendrochronological analyses were performed using the dplR library (Bunn 2008) within the R environment (R 2.12.0, R Development Core Team 2011). From the raw tree-ring series, we created a mean value chronology for each of the sampled tree species by averaging each year's radial increment using Tukey's biweight robust mean. One of the advantages of working with mature trees is that their individual pre-treatment history of growth is conserved in the tree-ring chronology. This is of particular significance in a case, where large spatial replication finds practical limitation (would require several crane sites). We used pre-treatment growth of each individual to standardize its growth rate during the 8 years of experimentation. However, we also show the non-standardized data, illustrating that the results are robust, irrespective of standardization. Wood samples of yearly growth rings for $\delta^{13}C$ analysis were collected from wood cores with a scalpel under a stereomicroscope. Subsequently, the sample material was oven-dried at 80 °C for 48 h and then ground in a steel ball mill (Retsch MM 2000, Haan, Germany). 0.6–0.8 mg of the dried powder was filled into tin capsules and then combusted in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy). Continuous-flow isotope ratio mass spectrometry was employed for ^{13}C isotopic analysis as described above. The precision of $\delta^{13}C$ analyses was $< 0.1‰$. The isotope values are expressed in the δ-notation: $\delta^{13}C = R_{sample}/R_{standard} - 1$, where R is the molar ratio of ^{13}C to ^{12}C for the sample and standard (VDBD), respectively.

VOLUMETRIC SOIL WATER CONTENT AND SOIL WATER ANALYSES

Soil water content at 0–10 cm depth was logged at 6-hourly intervals using 'ECH$_2$O Probes' (EC-10, Decagon Devices Ltd., Pullman, Washington, DC; ambient CO_2: $n = 20$, elevated CO_2: $n = 15$). Individual sensor readings were standardized to their maximum value.

Ceramic suction cups were installed along three transects through the experimental area, each running under individuals of each of the main tree species, *F. sylvatica*, *Q. petraea* and *C. betulus* (Schleppi *et al.* 2012). At 10–12 locations along each transect, two ceramic cups were installed at a depth of 15 cm and 1 or 2 at 5 cm. All suction cups were connected via a sampling bottle to vacuum systems reaching approximately −400 hPa. A soil solution sample from each location and depth was collected on average every 3 weeks, except when temperatures were below freezing point in winter. The samples were immediately refrigerated, then filtered (0.45 μm) and analysed for nitrate (NO_3^-), ammonium (NH_4^+), dissolved organic nitrogen (DON), organic carbon (DOC) and inorganic C (DIC). At 17 different dates during the experiment, the abundance of ^{13}C in DIC was measured by releasing the CO_2 through acidification with phosphoric acid (H_3PO_4) in gas-tight vials (see Schleppi *et al.* 2012 for details). During summer, ion-exchange resin bags were buried near the suction cups to provide integrated measurements of available N. After 6 months in the soil, they were retrieved and analysed for NH_4^+ as well as for NO_3^- and ^{15}N abundance (Schleppi *et al.* 2012).

Vertical fluxes of C and N below the litter layer and at depths of 5 and 20 (15) cm were estimated by multiplying the DOC, DON and NO_3^- concentrations with water fluxes obtained with the COUP simulation model (Jansson & Karlberg 2004). The model was parameterized using the organic C content, density and texture of different soil layers, as well as the height and leaf area index of the forest stand. The input variables were air temperature, precipitation, vapour pressure deficit, wind speed and net radiation.

STABLE CARBON ISOTOPE ANALYSIS IN SOIL AIR CO_2

The stable carbon isotope ratio ($\delta^{13}C$) and CO_2 concentration in soil air were monitored monthly during growing seasons using a grid of 86 PVC gas wells whose tops were sealed with a silicone rubber septum ($L \times D = 12 \times 2$ cm; 3×3 m grid spacing in the FACE area, 6×6 m in the control area). Soil air $\delta^{13}C$ analyses were performed using a mass spectrometer (Delta Plus XL) linked to a Gasbench-II periphery (both Termo Finnigan, Bremen, Germany). Soil air CO_2 concentration was estimated from instrument calibration curves based on CO_2 reference gases (340 and 4995 ppm). For each sampling date and CO_2 treatment, the Keeling plot approach was applied to ascertain the $\delta^{13}C$ of soil air CO_2, that is, we applied linear regression models using measured $\delta^{13}C$ values as response and the inverse soil air CO_2 concentrations as explanatory variable. The intercept of these models corresponded to the mean soil air $\delta^{13}C$ and the standard error of the intercept served as a measure of spread. Isotope fractionation due to slower diffusion of the heavier $^{13}CO_2$ was accounted for by subtracting 4.4‰ (cf. Steinmann *et al.* 2004; Keel, Siegwolf & Körner 2006).

STATISTICAL ANALYSIS

All statistical analyses and graphics were performed using R version 2.12.0 (R Development Core Team 2011). Linear mixed effects models (package *nlme*) fitted by restricted maximum likelihood (REML) were applied to assess the effects of elevated atmospheric CO_2 on radial increment, litter fall, leaf traits, foliar $\delta^{15}N$, soil air $\delta^{13}C$ and soil air CO_2 concentration. These models contained the factors 'species', 'CO_2', 'year' and their interactions as fixed term and the factor 'month' nested within 'year' or simply 'tree' (in the case of radial increment and foliar $\delta^{15}N$) as repeated measure effects (random term). Homogeneity of variance was examined by inspection of the residuals after model fitting (plots of standardized residuals vs. fitted values and explanatory variables). Heterogeneous within-group variances were modelled either using a constant variance function (varIdent function, package *nlme*), a power variance function (varPower, package *nlme*), an exponential variance function (varExp, package *nlme*) or a combination thereof. Quantile–quantile plots were used to test for normally distributed model residuals and random effects. An autocorrelation function was used to check model residuals for temporal autocorrelation, and an autoregressive moving average (ARMA) correlation structure was employed to model dependence among observations. Backwards model selection to determine the optimal fixed term was based on comparing nested models using the Akaike Information

Criterion (AIC) and likelihood ratio tests. For the analysis of tree-ring $\delta^{13}C$ in CO_2-enriched trees, we applied a generalized additive mixed model (GAMM) treating 'tree individual' as random effect to account for repeated measures. Temporal autocorrelation was modelled using a first-order autoregressive structure (AR1), and variance heterogeneity was modelled using an exponential variance function (varExp). The standardized soil moisture data were also analysed using GAMMs. The specific sensor locations within the control and CO_2-enriched area were modelled as a random effect, and temporal autocorrelation was modelled using an autoregressive model of order 1 (AR-1 autocorrelation structure). The significance of the effect of CO_2 enrichment was tested using a likelihood ratio test comparing two nested GAMMs; a first model with a common smoother term for ambient and elevated CO_2 vs. a second model employing individual smoothers for each treatment.

The local effect of the CO_2 treatment in the soil around suction cups and resin bags was quantified by the seasonal depletion of their DIC in ^{13}C (Schleppi *et al.* 2012). Concentrations in the soil solution were then interpreted based on a generalized linear model including this ^{13}C-derived index and the duration of the treatment. The interaction between both was tested statistically to show whether the effect of soil locations changed over time as related to the CO_2 enrichment. Due to their very high variability, NO_3^- concentrations in the soil solution were log-transformed prior to analysis, which yielded homoscedastic residuals with a distribution not significantly departing from normality.

Results

LEAF LITTER PRODUCTION AND LEAF TRAITS

Annual leaf litter fall collected in traps did not change in response to canopy CO_2 enrichment (Fig. 1). Foliar non-structural carbohydrates (NSC) in *F. sylvatica*, *C. betulus* or any of the two unreplicated species (*T. platyphyllos*, *A. campestre*) were unaffected by elevated atmospheric CO_2. In CO_2-enriched *Q. petraea* leaves however, NSC accumulated significantly and consistently throughout the eight experimental years (on average + 22%, CO_2 × species, $P < 0.0001$), a signal that disappeared in the first post-treatment year. Specific leaf area in the upper canopy showed significant interspecific variation ($P < 0.001$) but was not consistently affected by elevated CO_2 (Fig. 2). Leaf N in the replicated *F. sylvatica, Q. petraea* and *C. betulus* trees remained unaffected by elevated CO_2 (Fig. 2). In the second half of the CO_2-enrichment period (2005–2008), the two unreplicated species, *A. campestre* and *T. platyphyllos*, showed reductions in leaf N concentration under elevated CO_2 (on a NSC-free dry matter basis, species × CO_2, $P < 0.001$), but already in the first post-treatment year, foliar N had returned to control levels.

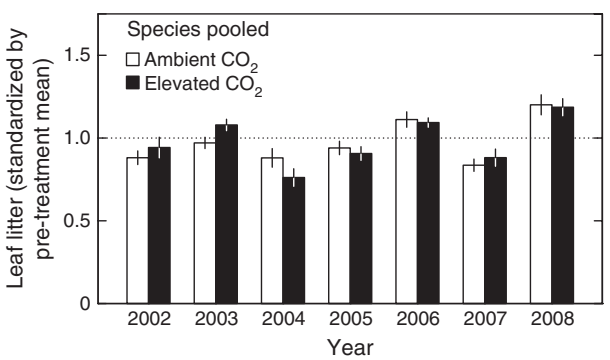

Fig. 1. Leaf litter production of CO_2-enriched and control trees (standardized by the 3-year-pre-treatment mean for each 1 m^2 litter trap 1999–2001). Means ± SE, ambient CO_2 $n = 32$, elevated CO_2 $n = 12$.

RADIAL INCREMENT AND TREE-RING $\delta^{13}C$

CO_2 enrichment had no significant effect on radial increment growth (expressed as raw tree-ring width and standardized by the 10-yr pre-treatment mean) in the dominant *F. sylvatica* and *Q. petraea* trees and the subdominant *Tilia* tree ($P = 0.52$, Fig. 3). However, not surprisingly, in some study years, there was significant interspecific variation in radial growth (species × year interaction, $P < 0.01$). Tree-ring series obtained from *C. betulus* and *A. campestre* were not reliably dateable and therefore had to be excluded from the analysis. The incorporation of recently assimilated (isotopically labelled) C into newly formed wood occurred remarkably fast in our study trees (Fig. 4). Already after the first year of canopy enrichment with ^{13}C-depleted CO_2, the stable C isotope ratio in growth rings of CO_2-treated trees had dropped by *c.* 4‰ and remained at a significantly lower level throughout the experimental period ($P < 0.001$, Fig. 4). In the first tree-ring produced after the termination of CO_2 enrichment (i.e. cessation of stable carbon isotope labelling), the $\delta^{13}C$ signal had already become less negative, and after the third post-treatment year, the tree-ring $\delta^{13}C$ signature had returned to pre-treatment values (Fig. 4).

SOIL WATER SAVINGS

During growing seasons, volumetric water content was significantly higher in soil under trees receiving CO_2 enrichment compared to soil surrounding control trees resulting from reduced transpiration under elevated CO_2 (Fig. 5, L = 16.74, $P < 0.001$, ΔAIC = 12.75). For example, in 2007, before the spring leaf flush, soil moisture was similar under all trees regardless of treatment. However, with advancing leaf maturation and thus rising canopy transpiration, soil water content became progressively lower around control trees compared to trees exposed to elevated CO_2. Replenishment of soil water resources by a number of consecutive rainy days or heavy rainfall events transiently offset treatment-related differences in soil moisture (Fig. 5). After leaf fall, soil water content under control trees and CO_2-enriched trees gradually converged again.

SOIL WATER CHEMISTRY

The depletion of ^{13}C in the dissolved inorganic carbon sampled in suction cups was on average 2.2 ± 1.4‰, ranging

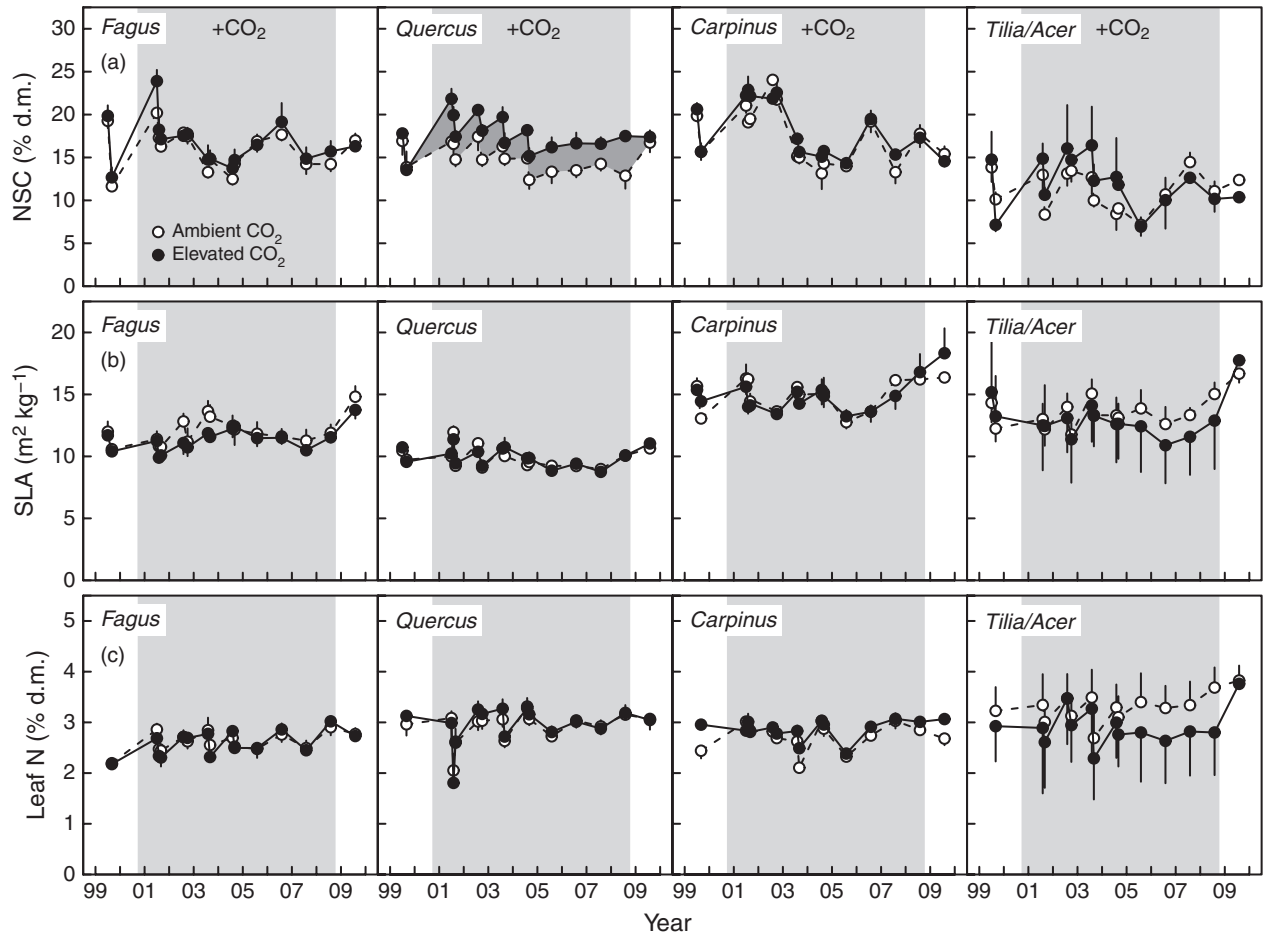

Fig. 2. Traits of fully expanded upper-canopy leaves produced under ambient (white) and elevated (black) atmospheric CO_2. Non-structural carbohydrate (NSC) content (a), specific leaf area (SLA) (b) and leaf nitrogen (N) concentration (c). SLA and leaf N are given on an NSC-free basis. Means ± SE, ambient CO_2 n = 3–5, elevated CO_2 n = 3. For the single *Tilia platyphyllos* and *Acer campestre* trees, the pooled response is shown (n = 2 trees). The grey-shaded area indicates the CO_2-enrichment period. The dark grey area highlights the pronounced NSC response in CO_2-enriched *Quercus petraea* trees.

from 4.9 to 1.3‰. These values were used as an indicator of the degree of local exposure of soil pockets to the effects of canopy CO_2 enrichment across the treatment area. Their interaction with the duration of the experiment had a positive effect on nitrate concentrations ($P < 0.01$; Schleppi *et al.* 2012). Multiplying the measured concentrations at 15 cm depth by drainage water fluxes obtained with the COUP model resulted in an annual NO_3^--N leaching of 2.3 kg ha^{-1} at control locations. At corresponding locations along the soil transects, the CO_2 enrichment increased this leaching by an additional 1.2 kg ha^{-1}. Amounts of nitrate captured by the resin bags confirmed this trend, showing a clear increase in free nitrate due to the CO_2 enrichment ($P < 0.0001$). In parallel, the natural abundance of ^{15}N in this nitrate strongly increased (Schleppi *et al.* 2012).

In the topsoil at 5 cm depth, concentrations of DIC were increased by 50% under elevated CO_2 (time × CO_2, $P = 0.0012$), while DOC concentrations declined by 20% during the eight treatment years (time × CO_2, $P < 0.0001$). The corresponding effects on the fluxes of dissolved C cancelled each other, with CO_2 enrichment increasing fluxes of DIC by 2.8 g C m^{-2} a^{-1}, but decreasing DOC fluxes by 2 g C m^{-2} a^{-1} (Fig. 6). At 15-cm-soil depth, concentrations and fluxes of DIC and DOC were not affected by elevated CO_2; hence, the signal disappeared within the soil profile.

SOIL AIR $\delta^{13}C$ AND CO_2 CONCENTRATION

Throughout the eight-year study period, respiratory air in the soil pore space under CO_2-enriched trees remained isotopically labelled, also during the dormant seasons, when CO_2 enrichment was discontinued (treatment-related difference in $\delta^{13}C$ including the dormant seasons: 1.7 ± 0.12 ‰, 8-yr mean ± SE, Fig. 7a), with the remainder of CO_2 release apparently resulting from older (pre-treatment) carbon. Relating this isotopic signature to the isometer reference $\delta^{13}C$ (obtained from C_4 grasses installed in the tree canopies) suggested that soil respiratory CO_2 under CO_2-treated trees consisted on average of *c.* 30% labelled (novel) C; however, the proportion of this recent C varied significantly between study years (CO_2 × year interaction, $P < 0.01$).

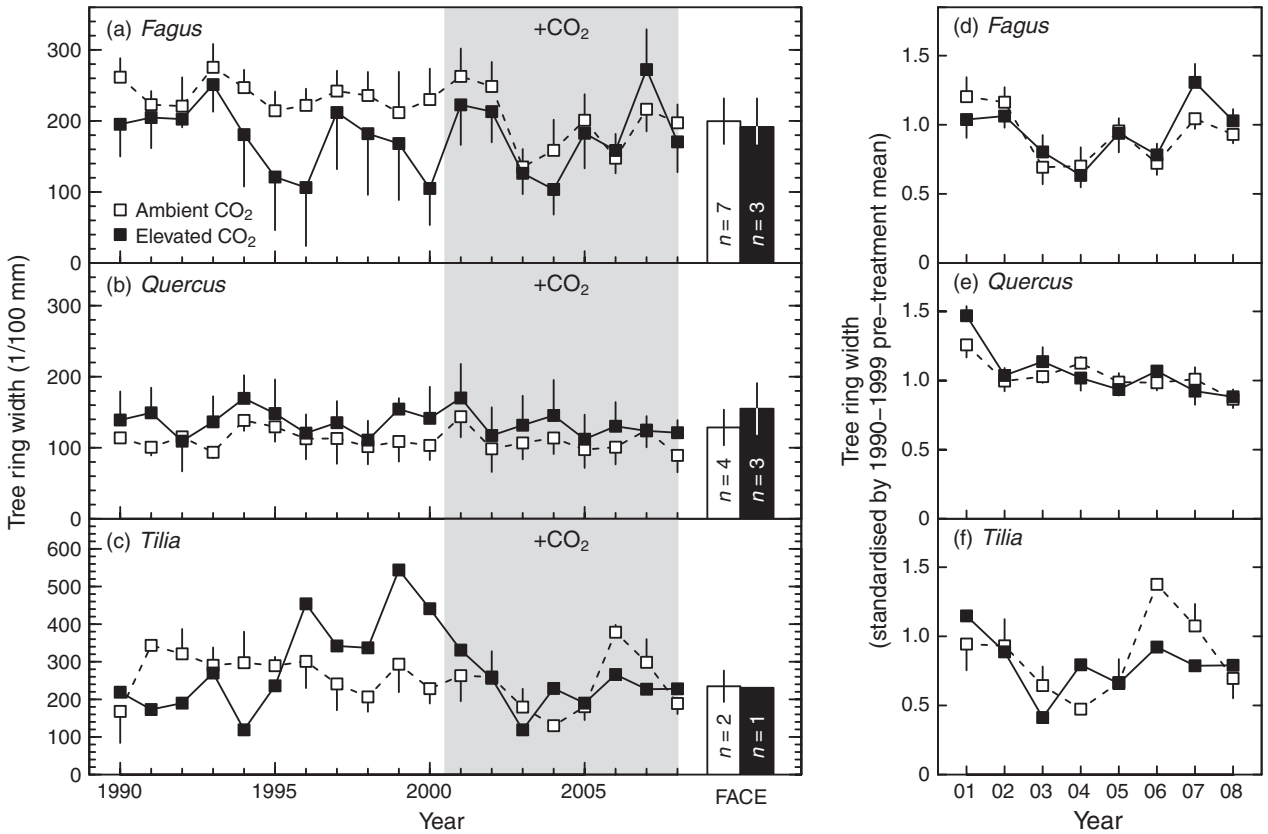

Fig. 3. Annual radial stem growth given as raw ring width (left panels) and standardized ring width (right panels, divided by the 1990–1999 pre-treatment mean) of dominant *Fagus sylvatica* (a, d), *Quercus petraea* (b, e) and subdominant *Tilia platyphyllos* trees (c, f) growing under ambient (dashed line, white symbols) and elevated (solid line, black symbols) atmospheric CO_2 in a closed-canopy temperate forest in northern Switzerland (ring widths of the remaining tree species were not dateable). The grey-shaded area indicates the CO_2-enrichment period (8 years). Bar chart inserts give the average ring width increment during the free-air CO_2-enrichment period (FACE).

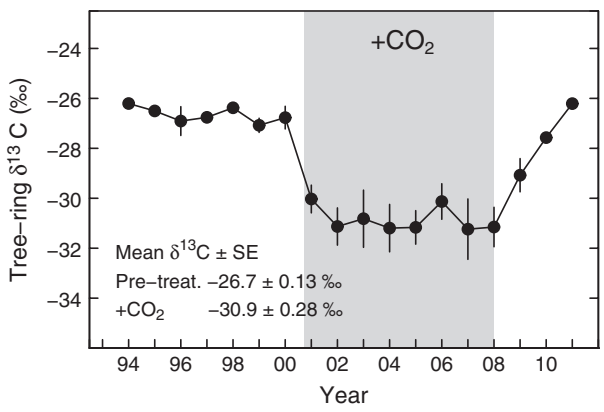

Fig. 4. Tree-ring $\delta^{13}C$ signals derived from CO_2-enriched *Fagus sylvatica*, *Quercus petraea* and *Tilia platyphyllos* trees. Means ± SE, n = 1–3.

During growing seasons, CO_2 accumulated more strongly in the soil pore space under CO_2-enriched trees compared to controls, on average by 35% ($P < 0.001$, Fig. 7b). When sampling had been extended into the dormant season (in the years 2001–2003), treatment-related differences in soil air CO_2 concentrations disappeared and values were similar under all trees (Fig. 7b).

FOLIAR $\delta^{15}N$

We monitored foliar ^{15}N natural abundance (expressed as $\delta^{15}N$) in archived leaf samples of our tall study trees as a proxy for ecosystem N availability. Prior to the start of CO_2 enrichment, leaf $\delta^{15}N$ signatures were similar among all trees (Fig. 8). Throughout the entire CO_2-enrichment period, foliar $\delta^{15}N$ remained extraordinarily steady under elevated CO_2, while the $\delta^{15}N$ values of control foliage showed more variation and were overall slightly but significantly more negative (2001–2008 average: elevated CO_2 −2.35 ± 0.15‰, ambient CO_2 −2.82 ± 0.18‰, $P < 0.01$, Fig. 8). Of particular note was a pronounced drop in the $\delta^{15}N$ signal by roughly 0.7‰ in leaves of control trees in 2004, following a centennial heat wave in 2003.

Discussion

Combining novel data that became available at or after the end of canopy CO_2 enrichment, with data that had been collected during the entire 8-year experiment, permitted us to consolidate an overall message of this unique experiment. Using pre-treatment and post-treatment signals, those of ^{13}C in particular, illuminates the C dynamics in such tall trees.

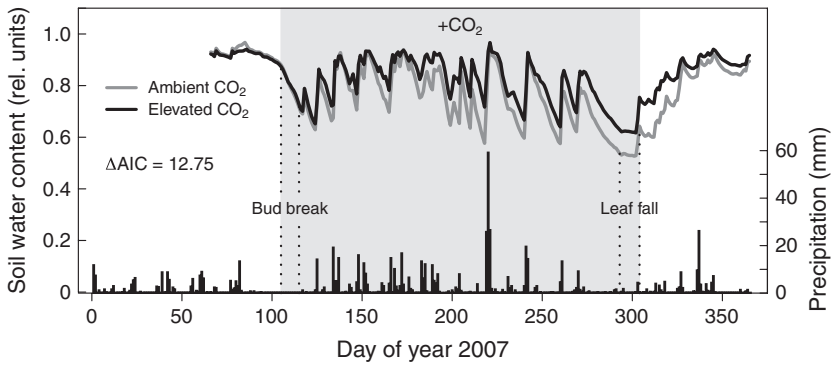

Fig. 5. Precipitation and volumetric soil water content under CO_2-enriched and control trees in 2007. The grey-shaded area designates the CO_2-enrichment period, and the dotted lines indicate the bud break and leaf fall period, respectively. The ΔAIC value is derived from a model comparison between a generalised additive mixed model (GAMM) with a common smoother term and a GAMM with individual smoother terms for ambient and elevated CO_2 (the large ΔAIC value lends strong support to the model with individual smoothers suggesting treatment-related differences, see Materials and Methods for details).

LITTER PRODUCTION AND LEAF-LEVEL C UPTAKE UNDER CO_2 ENRICHMENT

Based on leaf duration data (Asshoff, Zotz & Körner 2006) and annual leaf litter production, which both remained unaffected by high CO_2, we can conclude that canopy CO_2 enrichment did not alter the steady leaf area index of five in this mature forest, similar to what has been reported for a deciduous plantation forest in Tenessee (Oak Ridge FACE; Norby & Zak 2011). The strong sugar signal observed in *Q. petraea* foliage did not feedback negatively on photosynthetic performance, which is also in line with the findings for *Liquidambar styraciflua* at the Oak Ridge FACE site (Sholtis *et al.* 2004). In the eighth and final year of CO_2 enrichment, photosynthetic C uptake in the upper canopy was on average 42–48% higher in CO_2-enriched trees and showed no signs of downregulation (Bader, Siegwolf & Körner 2010), consistent with earlier findings from this and other forest FACE sites (Zotz, Pepin & Körner 2005; Liberloo *et al.* 2007; Darbah *et al.* 2010; Norby *et al.* 2010; Ellsworth *et al.* 2012). However, at the Oak Ridge FACE site, progressive nitrogen limitation has eventually caused a complete loss of the photosynthetic response to elevated CO_2 (Norby *et al.* 2010).

RADIAL GROWTH AND STABLE C ISOTOPE SIGNATURES IN TREE-RINGS UNDER ELEVATED CO_2

Elevated atmospheric CO_2 has often been reported to stimulate radial stem growth in young trees, at least temporarily, but it has also become evident that potential growth responses are highly species-specific and contingent on tree age, stand demography and the availability of resources other than CO_2 (Körner 2006; Norby & Zak 2011; Smith *et al.* 2013a). The forest soil at our SCC FACE site is nutrient-rich and according to accepted forestry standards the currently *c.* 110-year-old stand grows vigorously, yet our study trees showed no persistent growth stimulation in response to CO_2 enrichment, suggesting alternative pathways for the extra C assimilated under elevated CO_2. By contrast, *Pinus taeda* growing in a temperate plantation forest (Duke FACE) and young poplar saplings in bio-energy plantations on fertile ground (POP-FACE) showed sustained enhancement of radial stem growth under CO_2 enrichment (pine: 13–27% across study years, poplar: 20–29%, Moore *et al.* 2006; Liberloo *et al.* 2006). However, at the Oak Ridge FACE site, the initial stimulation in radial stem growth of *Liquidambar styraciflua* under elevated CO_2 rapidly shifted below-ground to increased fine root

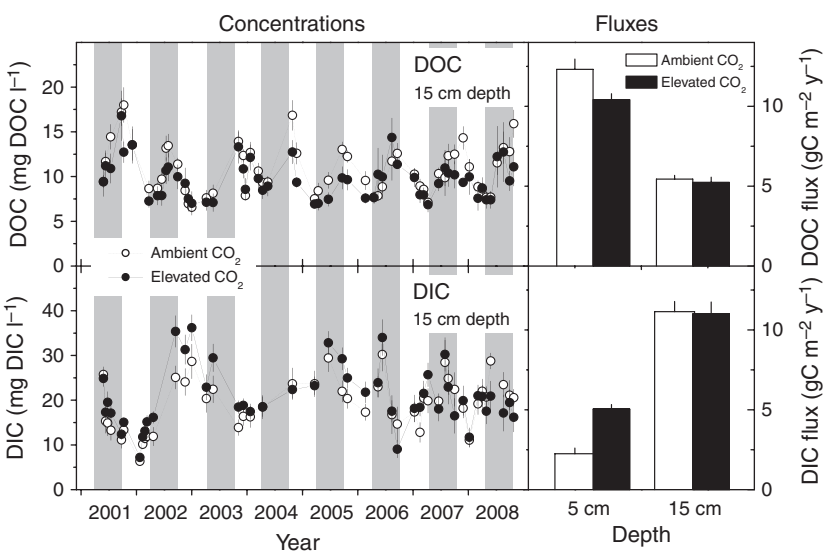

Fig. 6. Inter-annual and intra-annual variation in dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) concentrations in the upper soil horizon (at 15 cm depth) under CO_2-enriched and control trees (*left panels*). Corresponding DOC and DIC fluxes at 5 and 15 cm depth (*right panels*). The grey-shaded rectangles indicate the CO_2-enrichment periods that correspond to the 6-month growing seasons.

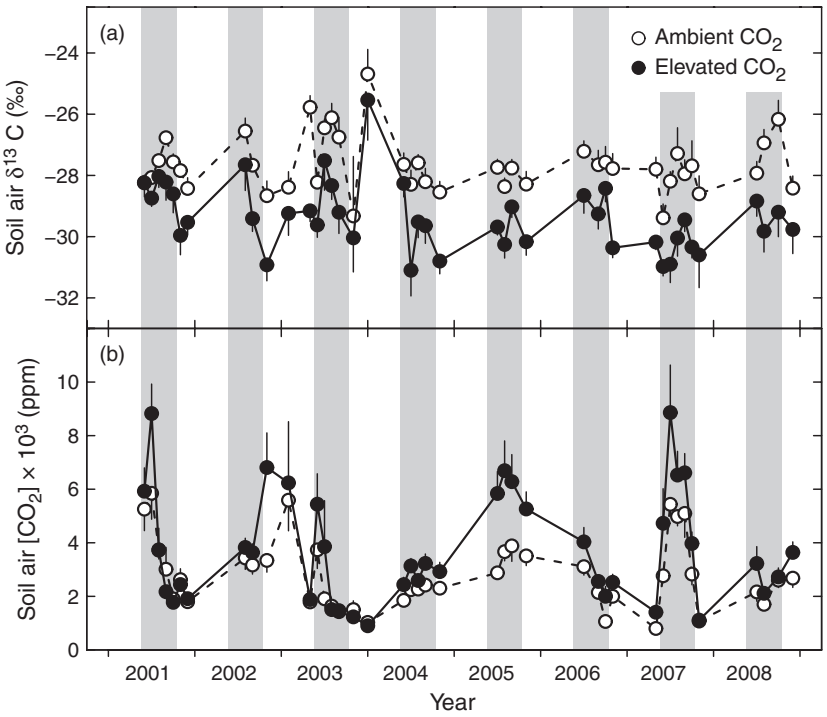

Fig. 7. Interannual and intra-annual variation in the stable carbon isotope signature (δ^{13}C) (a) and CO_2 concentration (b) in the soil pore space at 3–11 cm depth under CO_2-enriched and control trees. Means ± SE, ambient CO_2 n = 43–58, elevated CO_2 n = 17–28. The grey-shaded rectangles indicate the CO_2-enrichment periods that correspond to the 6-month growing seasons.

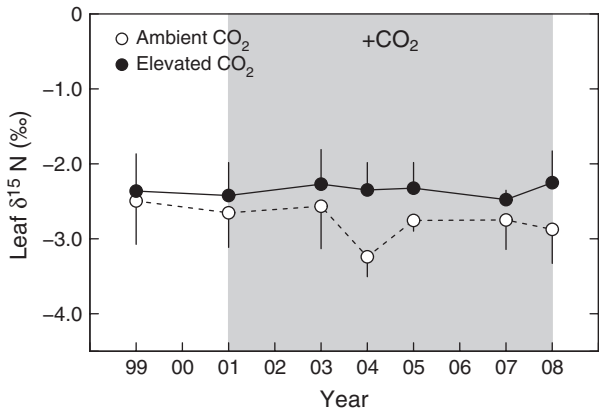

Fig. 8. Nitrogen availability given as δ^{15}N of freshly harvested foliage. Symbols indicate composite sample means of the replicated *Fagus sylvatica*, *Quercus petraea* and *Carpinus betulus* trees. Means ± SE, ambient CO_2 n = 3–5, elevated CO_2 n = 3.

production, a signal that also became insignificant towards the end of the experiment (Norby *et al.* 2010). Mediterranean *Quercus ilex* trees growing around two natural CO_2-springs as well as CO_2-enriched *Populus tremuloides* clones at the ASPEN FACE site both showed initial growth enhancement that disappeared with time (Hättenschwiler *et al.* 1997; Kubiske *et al.* 2006). Species-specific growth responses became strikingly apparent at the Swiss tree line FACE site where tree-ring increments in the late-successional *Pinus uncinata* remained completely unaffected by elevated CO_2 over 9 years, while growth in early successional *Larix decidua* trees was stimulated (though diminishing with time), depending on summer temperature (Dawes *et al.* 2011, 2013). At the Bangor FACE site, above-ground woody biomass of CO_2-enriched *Fagus sylvatica* saplings increased only in monoculture (+22%) but showed no response when saplings were grown in polyculture (Smith *et al.* 2013a). Although there was no sustained growth response in CO_2-enriched trees at our SCC FACE site, the δ^{13}C signal in newly formed tree-rings declined markedly and very rapidly following the start of CO_2 enrichment and quickly returned to pre-treatment levels after the end of the experiment, highlighting the tight coupling between recent photosynthates and wood formation. Yet, the data also illustrate that C ending up in tree-rings has been assimilated over a period of 2–3 years, implying that the current year only contributes a certain percentage to the total bulk signal. The post-treatment return path shown in Fig. 4 depicts this important finding most clearly and suggests that climate correlates of seasonal isotope signals in tree-rings should be roughly halved in strength due to such mixing of photo-assimilates.

ELEVATED CO_2 EFFECTS ON TREE WATER RELATIONS AND SOIL MOISTURE

Many plants reduce stomatal conductance and thus leaf transpiration in response to elevated CO_2 (Medlyn *et al.* 2001; Holtum & Winter 2010). In our study trees, stomatal conductance was slightly diminished by CO_2 enrichment on bright days, with species-specific responses between zero in *Q. petraea* and 22% in *C. betulus* (Keel *et al.* 2007). Similarly, whole-tree transpiration estimated from sap flow measurements was on average 10–15% reduced under elevated CO_2, resulting in measurable soil water savings (Fig. 5; Leuzinger & Körner 2007; Bader, Hiltbrunner & Körner 2009). These findings are corroborated by other closed-canopy forest FACE experiments and modelling approaches (Warren *et al.* 2011). Water savings on a full year/all weather basis are much

smaller, however. Model simulations based on historical rainfall patterns showed that rainfall distribution by far outweighs the remaining, small (< 3%) effects of CO_2-related soil water savings on runoff in our study forest (Leuzinger & Körner 2010). The main effects of CO_2-driven water savings are below-ground processes discussed below.

BELOW-GROUND RESPONSES TO CO_2 ENRICHMENT

Because of the largely lacking above-ground growth stimulation in our study trees, we assumed greater C investments below-ground. Indeed, proxy data such as higher soil CO_2 concentrations suggested such a response during early experimental years (Steinmann *et al.* 2004; Keel, Siegwolf & Körner 2006). However, we found a 20–30% reduction in fine root biomass and new fine root growth into root-free soil cores under CO_2-enriched trees in year five and six of the study and no significant treatment-related differences in soil cores taken in the following year (Bader, Hiltbrunner & Körner 2009). Similarly, elevated CO_2 failed to stimulate fine root investments in larch and pine trees receiving CO_2 enrichment at the tree line in the Swiss Central Alps and also in a Florida scrub oak system (Brown *et al.* 2007; Handa, Hagedorn & Hättenschwiler 2008). At the Bangor FACE site, CO_2 enrichment merely produced a transient stimulation in fine root biomass of *Fagus sylvatica* saplings and even caused a decline in their coarse root biomass (Smith *et al.* 2013b). Fine root production dominated the CO_2-driven stimulation of NPP over several years of CO_2 enrichment at the Oak Ridge *Liquidambar styraciflua* plantation, particularly at greater soil depth. But this response ceased during the last years of the study and might therefore reflect a transitory response to a step change in CO_2 supply (Norby *et al.* 2010).

The fossil CO_2 used for canopy enrichment was consistently depleted in ^{13}C relative to ambient atmospheric CO_2 and thus allowed us to trace the flow of newly assimilated (labelled) C from the crowns to the rhizosphere of the treated trees (Steinmann *et al.* 2004; Keel, Siegwolf & Körner 2006). Only 11 days after the start of CO_2 enrichment, the isotopic signal of the fumigation gas became detectable in soil air (Steinmann *et al.* 2004) and after 4 growing seasons, newly developed leaves consisted of 100%, and new tree-rings of 91% recent C (Keel, Siegwolf & Körner 2006). In fine roots (< 1 mm) formed in ingrowth cores during years five and six of the study, only 51% of the C carried the isotopic signature of the CO_2 released in the canopy, implying long fine root C turnover rates of *c.* 12 years or utilization of old non-structural carbon reserves for root formation (Bader, Hiltbrunner & Körner 2009).

Recent photosynthates were rapidly channelled to below-ground C sinks, as evidenced by strong isotopic signals in soil air and symbiotic fungi (Steinmann *et al.* 2004; Keel, Siegwolf & Körner 2006). Three months after the beginning of CO_2 enrichment, sporocarps of mycorrhizal fungi associated with CO_2-enriched trees already consisted of 62% new C, while saprophytic fungi were devoid of ^{13}C-depleted C after 4 years of treatment suggesting decomposition of C compounds that were formed prior to the start of the experiment (Keel, Siegwolf & Körner 2006).

Given the absence of increases in above- and below-ground growth and litter production, we assumed that the extra C assimilated under elevated CO_2 might be largely respired back to the atmosphere via soil metabolism (Körner *et al.* 2005). Surprisingly, the stronger CO_2 build-up in soil under CO_2-enriched trees during growing seasons did not translate into a sustained increase in soil respiration, most likely due to reduced soil diffusivity resulting from soil water savings (Bader & Körner 2010). This contrasts with other forest FACE studies where soil CO_2 efflux in closed-canopy stands increased by 12–23% (King *et al.* 2004; Jackson *et al.* 2009). Leaching of DIC and DOC was not a major loss pathway for the 'missing C' either. The total fluxes of dissolved C were small (*c.* 15 g C m^{-2} a^{-1}) and remained unaffected by the CO_2 enrichment (Fig. 6). In the topsoil, CO_2 enrichment increased DIC fluxes by 50%, most likely reflecting the higher partial CO_2 pressure in soil pores. This increase was outbalanced by an unexpected decline in DOC leaching, which we primarily attribute to reduced solubilization of soil organic matter (SOM) in the mineral soil through acidification associated with increased soil CO_2 concentrations. Topsoil acidification was further enhanced by higher NO_3^--N leaching as two protons are released per mole of nitrate leached, thereby suppressing DOC leaching (Evans *et al.* 2008). At 15 cm depth, both DOC and DIC leaching remained unaffected by CO_2 enrichment, thus ruling out this 'leak' pathway for the additionally assimilated C, perhaps because this forest grows on shallow soil over calcareous rock and rock debris. At the Aspen and Duke FACE sites, DIC leaching increased considerably under elevated CO_2 (Karberg *et al.* 2005; Jackson *et al.* 2009).

There is a growing awareness that CO_2 fertilization effects will be smaller than anticipated (Leuzinger *et al.* 2011) and will certainly not be uniform across landscapes but rather follow availability patterns of colimiting growth resources such as water and nutrients (Oren *et al.* 2001; Körner 2006; McCarthy *et al.* 2010). Moreover, potential CO_2 fertilization effects on tree growth might be offset over time by progressive N limitation in soils resulting from N sequestration in long-lived biomass or SOM pools or by reduced longevity (Luo *et al.* 2004; Norby *et al.* 2010; Bugmann & Bigler 2011). For instance, after 9 years of CO_2 enrichment at the Oak Ridge site, progressive N limitation caused a decline of the strong NPP response that was previously dominated by fine root production (Norby *et al.* 2010). At our site however, the lacking growth responses to CO_2 enrichment cannot be attributed to N deficiency since this region receives 20–25 kg N ha^{-1} a^{-1} wet nitrogen deposition, which is close to the upper threshold of critical loads for this type of ecosystem (Thimonier *et al.* 2010). Moreover, CO_2-induced increases in soil moisture and nitrate availability resulted in enhanced nitrate leaching at this already N-rich site. Also, the increase in the natural abundance of ^{15}N in nitrate captured by resin bags suggests accelerated net nitrification under elevated CO_2 (Schleppi *et al.* 2012). The temporal $\delta^{15}N$ dynamics derived from archived leaf samples of our tall study trees remained remarkably stable under

elevated CO_2 suggesting unaltered and ample N supply even during years when lower $\delta^{15}N$ values of control foliage indicated declining N availability under ambient conditions (Fig. 8). This was most obvious in 2004, probably representing a carry-over effect from the preceding centennial drought year 2003 that most likely had a greater impact on soil N cycling processes under control trees compared to CO_2-enriched trees simply because of the soil water savings observed under elevated CO_2 (Leuzinger *et al.* 2005). Reductions in fine root biomass may be associated with diminished nitrate consumption under elevated CO_2, however, given the largely unaltered leaf N concentrations, accelerated SOM decomposition offers a more likely explanation for the accumulation of nitrate in the soil solution under high CO_2. This assumption is supported by a 14% increase in soil microbial biomass under CO_2-enriched trees (Bader & Körner 2010). Based on our and other FACE data, we postulate two concurrent causes underlying the observed stimulation of soil microbes and soil nitrate accretion. CO_2-induced soil water savings may have stimulated soil microbial biomass and nitrification rates, which have been shown to increase with increasing soil moisture (Stark & Firestone 1995). In addition to this water-driven effect, CO_2-enriched trees can fuel microbial activity in their rhizosphere through enhanced root exudation as has been demonstrated for tropical woody species in large model communities (Körner & Arnone 1992) and more recently for *Pinus taeda* at the Duke FACE site (Phillips, Finzi & Bernhardt 2011). There, this priming effect caused faster rates of SOM mineralization and nitrification, which prevented (at least for the period considered) progressive nitrogen limitation in the inherently N-poor forest soil (Drake *et al.* 2011). Increases in soil solution nitrate at our site may have been driven by such a priming effect stimulating microbial activity and thus N mineralization. The decrease in DON that paralleled the increase in soil nitrate supports this hypothesis.

In conclusion, our data imply that tree growth in this late-successional temperate forest is not limited by current atmospheric CO_2 concentrations, suggesting that these types of stands are unlikely to grow faster in a future high-CO_2 world. Whether or not tree growth will be stimulated, such experiments are principally unable to infer long-term changes in ecosystem C pools (C sequestration, Körner 2006), since C storage in biomass is in essence a demographic phenomenon (Bugmann & Bigler 2011), and soil C pools are responding too slowly to allow separation of transient from steady-state responses. It remains unresolved which resources become rate-limiting under humid, C and N saturated conditions, but phosphate and key cations are likely candidates. Our findings point towards enhanced DIC and nitrate leaching from such forest soils with rising atmospheric CO_2 levels. As a result, soil acidity and mineral weathering may intensify in a CO_2-rich future impacting on soil biota and nutrient cycling, while at the same time, nitrate contamination of groundwater is likely to become an increasing problem, especially in already predisposed regions.

Overall, we conclude that indirect CO_2 effects inducing changes in the forest N-cycle and soil water regime are far more important than first-order CO_2 effects on growth in this system. With regard to future climate predictions, our findings and those from other FACE studies challenge the assumption of a sustained and universal CO_2 fertilization effect on tree growth that most climate–carbon cycle models critically rely on.

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References

Asshoff, R., Zotz, G. & Körner, C. (2006) Growth and phenology of mature temperate forest trees in elevated CO_2. *Global Change Biology*, **12**, 848–861.

Bader, M., Hiltbrunner, E. & Körner, C. (2009) Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE). *Functional Ecology*, **23**, 913–921.

Bader, M.K.F. & Körner, C. (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. *Global Change Biology*, **16**, 2830–2843.

Bader, M.K.F., Siegwolf, R. & Körner, C. (2010) Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. *Planta*, **232**, 1115–1125.

Brown, A.L.P., Day, F.P., Hungate, B.A., Drake, B.G. & Hinkle, C.R. (2007) Root biomass and nutrient dynamics in a scrub-oak ecosystem under the influence of elevated atmospheric CO_2. *Plant and Soil*, **292**, 219–232.

Bugmann, H. & Bigler, C. (2011) Will the CO_2 fertilization effect in forests be offset by reduced tree longevity? *Oecologia*, **165**, 533–544.

Bunn, A.G. (2008) A dendrochronology program library in R (dplR). *Dendrochronologia*, **26**, 115–124.

Darbah, J.N.T., Kubiske, M.E., Nelson, N., Kets, K., Riikonen, J., Sober, A., Rouse, L. & Karnosky, D.F. (2010) Will photosynthetic capacity of aspen trees acclimate after long-term exposure to elevated CO_2 and O_3? *Environmental Pollution*, **158**, 983–991.

Dawes, M.A., Hättenschwiler, S., Bebi, P., Hagedorn, F., Handa, I.T., Körner, C. & Rixen, C. (2011) Species-specific tree growth responses to 9 years of CO_2 enrichment at the alpine treeline. *Journal of Ecology*, **99**, 383–394.

Dawes, M.A., Hagedorn, F., Handa, I.T., Streit, K., Ekblad, A., Rixen, C., Körner, C. & Hättenschwiler, S. (2013) An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study. *Oecologia*, **171**, 623–637.

Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhardt, E.S., Billings, S.A., Jackson, R.B. *et al.* (2011) Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters*, **14**, 349–357.

Ellsworth, D.S., Thomas, R., Crous, K.Y., Palmroth, S., Ward, E., Maier, C., DeLucia, E.H. & Oren, R. (2012) Elevated CO_2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. *Global Change Biology*, **18**, 223–242.

Evans, C.D., Goodale, C.L., Caporn, S.J.M., Dise, N.B., Fernandez, I.J., Field, C.D., Findlay, S.E.G., Lovett, G.M., Meesenburg, H., Moldan, F. & Sheppard, L.J. (2008) Does elevated nitrogen deposition or ecosystem recovery from acidification drive increased dissolved organic carbon loss from upland soil? A review of evidence from field nitrogen addition experiments. *Biogeochemistry*, **91**, 13–35.

Friedlingstein, P., Cox, P., Betts, R., Bopp, L., Von Bloh, W., Brovkin, V. *et al.* (2006) Climate-carbon cycle feedback analysis: results from the (CMIP)-M-4 model intercomparison. *Journal of Climate*, **19**, 3337–3353.

Handa, I.T., Hagedorn, F. & Hättenschwiler, S. (2008) No stimulation in root production in response to 4 years of in situ CO_2 enrichment at the Swiss treeline. *Functional Ecology*, **22**, 348–358.

Hättenschwiler, S., Miglietta, F., Raschi, A. & Körner, C. (1997) Thirty years of in situ tree growth under elevated CO_2: a model for future forest responses? *Global Change Biology*, **3**, 463–471.

Holtum, J.A.M. & Winter, K. (2010) Elevated CO_2 and forest vegetation: more a water issue than a carbon issue? *Functional Plant Biology*, **37**, 694–702.

Jackson, R.B., Cook, C.W., Pippen, J.S. & Palmer, S.M. (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, **90**, 3352–3366.

Jansson, P.-E. & Karlberg, L. (2004) *Coupled Heat and Mass Transfer Model For Soil-Plant-Atmosphere Systems*. Royal Institute of Technology, Department of Civil and Environmental Engineering, Stockholm.

Jobbágy, E.G. & Jackson, R.B. (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, **10**, 423–436.

Karberg, N.J., Pregitzer, K.S., King, J.S., Friend, A.L. & Wood, J.R. (2005) Soil carbon dioxide partial pressure and dissolved inorganic carbonate chemistry under elevated carbon dioxide and ozone. *Oecologia*, **142**, 296–306.

Keel, S.G., Siegwolf, R.T.W. & Körner, C. (2006) Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. *New Phytologist*, **172**, 319–329.

Keel, S.G., Pepin, S., Leuzinger, S. & Körner, C. (2007) Stomatal conductance in mature deciduous forest trees exposed to elevated CO_2. *Trees-Structure and Function*, **21**, 151–159.

King, J.S., Hanson, P.J., Bernhardt, E., DeAngelis, P., Norby, R.J. & Pregitzer, K.S. (2004) A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments. *Global Change Biology*, **10**, 1027–1042.

Körner, C. (2006) Plant CO_2 responses: an issue of definition, time and resource supply. *New Phytologist*, **172**, 393–411.

Körner, C. (2009) Responses of humid tropical trees to rising CO_2. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 61–79.

Körner, C. & Arnone, J.A. (1992) Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science*, **257**, 1672–1675.

Körner, C., Asshoff, R., Bignucolo, O., Hättenschwiler, S., Keel, S.G., Pelaez-Riedl, S., Pepin, S., Siegwolf, R.T.W. & Zotz, G. (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. *Science*, **309**, 1360–1362.

Kubiske, M.E., Quinn, V.S., Heilman, W.E., McDonald, E.P., Marquardt, P.E., Teclaw, R.M., Friend, A.L. & Karnosky, D.F. (2006) Interannual climatic variation mediates elevated CO_2 and O_3 effects on forest growth. *Global Change Biology*, **12**, 1054–1068.

LeQuéré, C., Raupach, M.R., Canadell, J.G., Marland, G., Bopp, L., Ciais, P. *et al.* (2009) Trends in the sources and sinks of carbon dioxide. *Nature Geoscience*, **2**, 831–836.

Leuzinger, S. & Körner, C. (2007) Water savings in mature deciduous forest trees under elevated CO_2. *Global Change Biology*, **13**, 2498–2508.

Leuzinger, S. & Körner, C. (2010) Rainfall distribution is the main driver of runoff under future CO_2 concentration in a temperate deciduous forest. *Global Change Biology*, **16**, 246–254.

Leuzinger, S., Luo, Y.Q., Beier, C., Dieleman, W., Vicca, S. & Körner, C. (2011) Do global change experiments overestimate impacts on terrestrial ecosystems? *Trends in Ecology & Evolution*, **26**, 236–241.

Liberloo, M., Calfapietra, C., Lukac, M., Godbold, D., Luos, Z.B., Polle, A. *et al.* (2006) Woody biomass production during the second rotation of a bioenergy *Populus* plantation increases in a future high CO_2 world. *Global Change Biology*, **12**, 1094–1106.

Liberloo, M., Tulva, I., Raim, O., Kull, O. & Ceulemans, R. (2007) Photosynthetic stimulation under long-term CO_2 enrichment and fertilization is sustained across a closed Populus canopy profile (EUROFACE). *New Phytologist*, **173**, 537–549.

Luo, Y., Su, B., Currie, W.S., Dukes, J.S., Finzi, A.C., Hartwig, U. *et al.* (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience*, **54**, 731–739.

Matthews, H.D. (2007) Implications of CO_2 fertilization for future climate change in a coupled climate-carbon model. *Global Change Biology*, **13**, 1068–1078.

McCarthy, H.R., Oren, R., Johnsen, K.H., Gallet-Budynek, A., Pritchard, S.G., Cook, C.W., LaDeau, S.L., Jackson, R.B. & Finzi, A.C. (2010) Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric [CO_2] with nitrogen and water availability over stand development. *New Phytologist*, **185**, 514–528.

Medlyn, B.E., Barton, C.V.M., Broadmeadow, M.S.J., Ceulemans, R., De Angelis, P., Forstreuter, M. *et al.* (2001) Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. *New Phytologist*, **149**, 247–264.

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T. & Gregory, J.M. (2007) Global climate projections. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor & H.L. Miller), pp. 747–846. Cambridge University Press, Cambridge, NY, USA.

Moore, D.J.P., Aref, S., Ho, R.M., Pippen, J.S., Hamilton, J.G. & De Lucia, E.H. (2006) Annual basal area increment and growth duration of *Pinus taeda* in response to eight years of free-air carbon dioxide enrichment. *Global Change Biology*, **12**, 1367–1377.

Norby, R.J. & Zak, D.R. (2011) Ecological lessons from free air CO_2 enrichment (FACE) experiments. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 181–203.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E. & McMurtrie, R.E. (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 19368–19373.

Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schafer, K.V.R., McCarthy, H., Hendrey, G., McNulty, S.G. & Katul, G.G. (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. *Nature*, **411**, 469–472.

Pepin, S. & Körner, C. (2002) Web-FACE: a new canopy free-air CO_2 enrichment system for tall trees in mature forests. *Oecologia*, **133**, 1–9.

Phillips, R.P., Finzi, A.C. & Bernhardt, E.S. (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecology Letters*, **14**, 187–194.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Roy, J., Saugier, B. & Mooney, H.A. (2001) *Terrestrial Global Productivity*. Academic Press, San Diego.

Schleppi, P., Bucher-Wallin, I., Hagedorn, F. & Körner, C. (2012) Increased nitrate availability in the soil of a mixed mature temperate forest subjected to elevated CO_2 concentration (canopy FACE). *Global Change Biology*, **18**, 757–768.

Sholtis, J.D., Gunderson, C.A., Norby, R.J. & Tissue, D.T. (2004) Persistent stimulation of photosynthesis by elevated CO_2 in a sweetgum (*Liquidambar styraciflua*) forest stand. *New Phytologist*, **162**, 343–354.

Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F. & Godbold, D.L. (2013a) Elevated CO_2 enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytologist*, **198**, 156–168.

Smith, A.R., Lukac, M., Bambrick, M., Miglietta, F. & Godbold, D.L. (2013b) Tree species diversity interacts with elevated CO_2 to induce a greater root system response. *Global Change Biology*, **19**, 217–228.

Stark, J.M. & Firestone, M.K. (1995) Mechanisms for soil moisture effects on activity of nitrifiying bacteria. *Applied and Environmental Microbiology*, **61**, 218–221.

Steinmann, K.T.W., Siegwolf, R., Saurer, M. & Körner, C. (2004) Carbon fluxes to the soil in a mature temperate forest assessed by ^{13}C isotope tracing. *Oecologia*, **141**, 489–501.

Thimonier, A., Graf-Pannatier, E., Schmitt, M., Waldner, P., Walthert, L., Schleppi, P., Dobbertin, M. & Kräuchi, N. (2010) Does exceeding the critical loads for nitrogen alter nitrate leaching, the nutrient status of trees and their crown condition at Swiss Long-term Forest Ecosystem Research (LWF) sites? *European Journal of Forest Research*, **129**, 443–461.

Tripati, A.K., Roberts, C.D. & Eagle, R.A. (2009) Coupling of CO_2 and Ice Sheet Stability Over Major Climate Transitions of the Last 20 Million Years. *Science*, **326**, 1394–1397.

Warren, J.M., Potzelsberger, E., Wullschleger, S.D., Thornton, P.E., Hasenauer, H. & Norby, R.J. (2011) Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO_2. *Ecohydrology*, **4**, 196–210.

Zotz, G., Pepin, S. & Körner, C. (2005) No down-regulation of leaf photosynthesis in mature forest trees after three years of exposure to elevated CO_2. *Plant Biology*, **7**, 369–374.

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