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Growth and carbon relations of mature *Picea abies* trees under 5 years of free-air CO_2 enrichment

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Summary

1. Are mature forests carbon limited? To explore this question, we exposed ca. 110-year-old, 40-m tall *Picea abies* trees to a 550-ppm CO_2 concentration in a mixed lowland forest in NW Switzerland. The site receives substantial soluble nitrogen (N) via atmospheric deposition, and thus, trees are unlikely N-limited. We used a construction crane to operate the free-air CO_2 release system and for canopy access. Here, we summarize the major results for growth and carbon (C) fluxes.

2. Tissue ^{13}C signals confirmed the effectiveness of the CO_2 enrichment system and permitted tracing the continuous flow of new C in trees. Tree responses were individually standardized by pretreatment signals. Over the five experimental years, needles retained their photosynthetic capacity and absorbed up to 37% more CO_2 under elevated (E) compared to ambient (A) conditions. However, we did not detect an effect on stem radial growth, branch apical growth and needle litter production. Neither stem nor soil CO_2 efflux was stimulated under elevated CO_2. The rate at which fine roots filled soil ingrowth cores did not significantly differ between A- and E-trees.

3. Since trees showed no stomatal responses to elevated CO_2, sap flow remained unresponsive, both in the long run as well as during short-term CO_2 on–off experiments. As a consequence, soil moisture remained unaffected. We trapped significantly more nitrate in the root sphere of E-trees suggesting a CO_2-stimulated breakdown of soil organic matter, presumably induced by extra carbohydrate exudation ('priming').

4. *Synthesis*. The lack of a single enhanced C sink to match the increased C uptake meant a missing C sink. Increased C transport to below-ground sinks was indicated by C transfer to ectomycorrhiza and on to neighbouring trees and by increased C export to soil. We conclude that these tall *Picea abies* trees are not C limited at current CO_2 concentrations and further atmospheric CO_2 enrichment will have at most subtle effects on growth, despite enhanced N availability.

Key-words: carbon isotopes, conifers, elevated CO_2, FACE, forest, height profile, wood anatomy

Introduction

Whether carbon (C) is a growth-limiting resource for forests in a 400-ppm world is currently debated (Körner 2003, 2006, 2015; Würth *et al.* 2005; Norby & Zak 2011; Smith *et al.* 2015). Since C can only be invested into new biomass to the extent other essential chemical elements are available, the question of CO_2 fertilization becomes a nutrient cycle issue. Unlike agricultural crops, most forests depend on the natural nutrient cycle, the provision of finite soil nutrients such as phosphorus, potassium, magnesium and manganese. A CO_2-driven growth stimulation as is often seen in young, expanding tree plantations or fertilized trees, is unlikely to be observed in mature forests and over long time spans (Körner 2006; Leuzinger *et al.* 2011).

Under such conditions, elevated atmospheric CO_2 can stimulate tree growth and forest net primary production only if the natural nutrient supply is enhanced as well. There is some

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evidence that extra photoassimilates released as root exudates can prime the breakdown of soil organic matter and cause a release of nutrients that otherwise might have stayed recalcitrant in the soil organic matter pool (Finzi *et al.* 2007; Phillips, Finzi & Bernhardt 2011; Schleppi *et al.* 2012). Since the soil C reservoir is finite, such priming effects cannot persist and thus will be transitory, if they occur. In regions with high soluble nitrogen (N) deposition, the extra N might not permit faster growth, unless other nutrient elements become more readily available as well. The crucial role of soil nutrients for a CO_2-driven growth response of trees has been demonstrated for tropical (Winter *et al.* 2000), temperate (Oren *et al.* 2001) and boreal forest trees (Sigurdsson *et al.* 2013). No lasting growth response in closed-canopy forests without nutrient addition was observed as a result of a step increase in CO_2 concentration by 150–200 ppm, neither in deciduous stands (Norby *et al.* 2010; Norby & Zak 2011; Bader *et al.* 2013) nor in a boreal forest setting (Sigurdsson *et al.* 2013). This cautions against projections of faster tree growth based on the photosynthetic stimulation by high CO_2 concentration alone (Körner 2006, 2009).

Thus, it is essential to test the CO_2 fertilization hypothesis under settings that match natural forest growth conditions as closely as possible. With a ca. 85 % contribution to global terrestrial biomass, forests are clearly the single most important ecosystem for terrestrial biomass C storage, and the majority of these forests is still largely unmanaged (most tropical and boreal forests, and an increasing area of secondary temperate forest). Exposing such forests to future CO_2 concentrations exerts a major experimental challenge and has repeatedly been urged as one of the most pending tasks in global change research (Calfapietra *et al.* 2009; Cernusak *et al.* 2013). Mature trees growing under competition for light and soil resources in closed stands do not fit in any growth or open top chambers. Even the classical free-air CO_2 enrichment (FACE), where CO_2 is released into the unconstrained canopy by towers protruding it, can only be employed in such tall natural forests at an extreme cost and the risk of tree damage. Such towers would have to be 40–50 m tall even in moderate size mature temperate forests and anchored with massive foundations and cables, potentially damaging soils, roots and branches.

Therefore, we designed a CO_2 release technique that does not require such invasive infrastructure (Pepin & Körner 2002). We successfully released pure CO_2 directly into 35-m tall broad-leaved tree canopies by thin, perforated tubes woven into the forest roof by means of a 45-m tall canopy crane (Körner *et al.* 2005; Bader *et al.* 2013). The same technique has been used in *Picea abies* trees for the current project. With all its limitations, this seems like the most feasible way to expose tall trees to future CO_2 concentrations without disturbing the forest or damaging it during thunderstorms. The limitations are still many. The sheer size of the trees and thus the need of a construction crane plus a large quantity of food quality CO_2 (ca. 2 t day^{-1} in our case, at peak season) sets limits to the numbers of exposed trees, parcel size and spatial replication, under the current funding policies. This is the reason why natural, mature forests are so heavily underrepresented in CO_2-impact research on plants. To some extent, these limitations can be mitigated by careful site and tree selection, standardizing tree responses by their growth history using individual tree's tree ring data, and by collecting other pre-treatment signals. The effectiveness of the CO_2 administration can be verified using the stable C isotope signal of the enrichment gas (given that its $^{13}CO_2$ content differs significantly from that of ambient air) and trace its fate throughout the forest from tree tops to root tips, for the study of metabolic processes (Mildner *et al.* 2014). Yet, while offering the most realistic experimental conditions in terms of forest ecology, such big experiments inevitably run at the limit of statistical power. With a single construction crane and given the size of the trees, any analysis remains constrained to 'trees' as the replicated unit, rather than a group of trees or the forest as a whole. Despite these issues, we gleamed insight into tree responses by capitalizing on pre-treatment data.

Growth is certainly not the only process that can potentially respond to elevated CO_2. Past research has shown other important effects, including (i) enhanced photosynthesis; (ii) downregulation of stomatal conductance and consequently decreased tree water use (Medlyn *et al.* 2001; Holtum & Winter 2010); (iii) increased intrinsic water-use efficiency, related to (i) and (ii) above (Battipaglia *et al.* 2013; Keenan *et al.* 2013); and (iv) increased non-structural C storage, for example in form of starch (Handa, Körner & Hättenschwiler 2005; Bader *et al.* 2013). Notably, any of these and other processes can has considerable consequences to future tree and forest function and hence deserves careful measurement in any CO_2 enrichment experiment.

In the former 8-year experiment at this site, using the same CO_2 enrichment technique on 10 tall deciduous trees, we found no consistent growth enhancement under elevated CO_2 (Körner *et al.* 2005; Bader *et al.* 2013), which is in line with the latest findings in the only other deciduous forest studied at such a scale in Tennessee, USA (Norby & Zak 2011). The strength of our previous experiment was the experimental inclusion of different tree species, thus offering more robust responses of such forests in general compared with experiments on a single deciduous species. Here, we employed the same infrastructure to explore CO_2 responses in the single most important European timber tree species, *Picea abies*. Using the canopy crane, we exposed five, 110-year-old, almost 40-m tall individuals to a 550-ppm future CO_2 concentration in a mixed forest in NW Switzerland between August 2009 and October 2014. We summarize the major results for C fluxes and growth at 3 heights along a vertical profile, including elaborated branch morphology and wood anatomy analyses.

We hypothesized that these trees grow at the site's soil carrying capacity and with regard to long-term growth, they will not benefit from CO_2 enrichment. Should there be a growth response, we expected to see it early in the experiment, after a step increase of CO_2 concentration, with the response fading as other growth constraints become effective (Leuzinger *et al.*

2011). Since we expected that the rate of photosynthesis will not be downregulated under elevated CO_2, resulting with increased C gain, we hypothesized that there will be counterbalancing C fluxes through enhanced respiratory metabolism, root turnover and soil CO_2 release.

Materials and methods

STUDY SITE AND EXPERIMENTAL SET-UP

A free-air CO_2 enrichment experiment was established in a diverse mixed forest 12 km southwest of Basel, Switzerland (47°33′N, 7°36′E, 550 m a.s.l). The site is dominated by ca. 100- to 120-year-old coniferous trees (mostly *Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L. and *Abies alba* Mill.) and deciduous trees (mostly *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl. and *Carpinus betulus* L.). Trees are forming a closed canopy with heights of 30–40 m and a leaf area index of ca. 5 (Leuzinger & Körner 2007). The soil is acidic, shallow silty-loamy rendzina on calcareous bedrock with maximum depth of 25 cm. Concentrations of soil nitrate, ammonium and dissolved organic N are not limiting (Schleppi *et al.* 2012), also evidenced by high [N] in leaves of key tree species (Bader, Siegwolf & Körner 2010). The climate is mild temperate, with mean January and July temperatures of 2.1 and 19.1 °C and mean temperature during the growing season (May–September) of 14.7 °C. Mean annual precipitation is ca. 900 mm.

Between 30 July 2009 and 30 October 2014, five 37–40 m tall, ca. 110-year-old Norway spruce (*Picea abies*) individuals were equipped with a web-FACE system using a 45-m tall canopy crane (Pepin & Körner 2002). CO_2 was released into the tree canopies through laser-punched tubes woven around the tree branches and allowing for computer-controlled adjustment of flows according to weather conditions (see Figs S1–S6 in the Supporting Information). The FACE treatment was discontinued when temperatures were < 4 °C, photosynthetic photon flux density, PPFD, was < 100 μmol m^{-2} s^{-1}, or wind speed was > 10 m s^{-1}. Therefore, FACE was largely off during night and storms and during the coldest period from early November until early March (4 months). Throughout the rest of the year (March–October), CO_2 concentrations measured directly with 60 IRGA gas sampling points (LI-820, Li-Cor, Lincoln, NE, USA) along the 5.5 years were 554 ± 14 ppm in the crowns of elevated trees, thus reflecting an efficient FACE treatment. The annual median CO_2 concentrations among the years were 384–466 ppm and 524–605 ppm in ambient (A-) and elevated (E)-trees, respectively. CO_2 release was constrained to the height of tree crowns, that is 20–40 m above the ground, without downward flow and without CO_2 transfer to the soil surface (Klein, Siegwolf & Körner 2016). Five similarly tall trees (36–40 m) away from the E-trees served as controls under ambient CO_2 (A-trees). All but one of these A-trees were outside the reach of the canopy cranes' jib. Major tree size parameters and a detailed site map are provided in Table S1 and Fig. S1, respectively. Crowns of A- and E-trees were higher than their surrounding canopy by 6.0 ± 1.3 m and 4.1 ± 1.5 m, and their lowest branches were at 16.2 ± 1.7 m and 16.8 ± 1.6 m, respectively (Table S1). The CO_2 gas employed for elevating CO_2 levels, carried a constant ^{13}C isotope signal ($\delta^{13}C = -30‰$; compared to −8.2‰ of ambient CO_2), permitting the tracing of C flux in trees and soils and also testing the efficiency of the FACE system (Mildner *et al.* 2014). CO_2 enrichment efficiency was also verified using 50 plant isometers distributed throughout the canopy. These isometers consisted of small pots planted with a C_4 photosynthesis pathway grass (*Echinochloa crus-galli*) without apparent enzymatic ^{13}C fractionation. The $\delta^{13}C$ of isometers grown in the tree canopy under elevated CO_2 was on average 4.8‰ lower than that of isometer plant tissue grown under ambient CO_2, representing a CO_2 mixing ratio of 536 ± 10 ppm, compared to 404 ± 8 ppm around A-trees (following the derivation described in Pepin & Körner 2002).

CONTINUOUS MEASUREMENTS

We continuously measured microclimate, soil moisture, sap flow and stem diameter variations, which will be reported in detail separately (T. Klein, R. T. W. Siegwolf & C. Körner, unpublished data). Soil moisture was monitored continuously 2–5 m from stems of all study trees at 10 cm depth, close to the middle of the shallow rhizosphere (see site description above). Here, we report the effects of CO_2 enrichment on the whole-tree transpiration flux, as derived from sap flow measurements using 30-mm heat dissipation probes (Dynamax, Houston, TX, USA) installed directly onto stems of all ten trees, two each, facing S and N. Sensors were installed on 6 May 2014 and insulated with styrofoam and reflecting foil. These sensors substituted similar sensors installed in May 2009, just prior to FACE application (Leuzinger & Bader 2012). Voltage signals (30 min averages) were recorded by a DL2e logger (Delta-T Devices Ltd., Cambridge, UK) and processed as described in Leuzinger & Bader (2012). The recording frequency was increased to 10 min during a short-term FACE shutdown experiment. For that experiment, CO_2 enrichment of the five elevated *Picea* trees was interrupted between 9:00 and 10:30 on 1 and 5 August 2014, and sap flow rates were compared to those measured immediately before and after switch-off and during similar times on 2 and 6 August. All 4 days were clear, sunny days with similar temperature and humidity dynamics.

NEEDLE GAS EXCHANGE

Needle stomatal conductance (g_s), net assimilation (A_{net}) and transpiration (T) were measured on 1-year-old needles in ambient and elevated trees at both ambient and elevated CO_2 levels (400 and 550 ppm) using a portable gas exchange system (Li-6400XT, Li-Cor, Lincoln, NE, USA) equipped with a CO_2 mixer to control the CO_2 level in the chamber. In three field campaigns in summer 2013 (18 June, 2 July and 19 September 2013), the gas exchange system was operated from the crane gondola, with measurements between 11:30 and 14:00 in the upper canopy at 35 m above-ground under ambient light (150–1800 μmol m^{-2} s^{-1}) using a conifer chamber (Li-6400-05, 7.5 × 5 cm). Chamber temperature and vapour pressure deficit in the June, July and September campaigns were 30, 22 and 17 °C, and 3.1, 2.1 and 1.2 kPa, respectively. These measurements were limited by the crane's access to tree crowns and hence included north and south sides of elevated trees and only one ambient tree, with all trees measured in 2014. A month before the end of the FACE experiment, on 23 and 26 September 2014, needle gas exchange was measured on needles from all ten trees under saturating light (1500 μmol m^{-2} s^{-1}) using a conifer chamber with a powerful RGB light source (Li-6400-22L, 7.5 × 7 cm). With the help of tree climbers, we also sampled the four A-trees outside the crane's reach. We sampled cut branchlets (20–30 cm) from sunlit parts of tree crowns (30–35 m above-ground at major directions N, E, S and W) between 10:00 and 14:30, alternating between ambient and elevated trees. Each measurement was performed within 1–8 min following branchlet collection. Three specimens were remeasured at 10 and 20 min following cutting, and gas exchange rates did not decline before 20 min, as also shown in Bader

et al. (2016). Data were logged as soon as the photosynthetic rate remained constant, typically within 2–3 min. Total needle area was measured with an area metre (Li-3100, Li-Cor, Lincoln, NE, USA) taking the average of three sequential measurements. The ratio between gas exchange at 550 and 400 ppm was determined as the enhancement ratio induced by elevated CO_2.

NEEDLE LITTERFALL

Leaf litter was collected by 30 litter traps (0.2 m^2) placed at compass directions of 120°, 240° and 360° around each of the 10 trees. Traps were emptied once a month in autumn 2009 and 2–5 times per year in 2010–2014. Litter fall in the year 2009 was considered to still represent foliage produced under pre-treatment conditions. The litter was sorted into *P. abies* and non-*Picea* litter, oven-dried at 80 °C for at least 48 h and weighed.

BRANCH AND NEEDLE GROWTH

At the end of the FACE experiment, on 23 and 26 September 2014, ten 1–2 m long branches were cut from each of the ten trees, bagged and brought to the laboratory. Branches were cut in the sunlit part of tree crowns, that is 22–35 m above-ground at major cardinal directions N, E, S and W, using the crane gondola for six of the trees and by tree climbers for the remaining four A-trees. Branches were stored up to 3 weeks at 4 °C before processing. In *Picea abies* growing at our forest site, needles are retained for 6–8 years. Each branch was cut into annual length increment segments of the last 7 years, starting from the 2014 branches at the tip and back until the 2008 increment. These annual segments (700 in total) were oven-dried and then separated into needles and stems and weighed. We also took a random sample of 20 needles per branch and measured the diameter and length of the annual main branch segments and the number of side branchlets. The bases of the main branches of 2008 were further cut into small discs using a fresh razor blade for tree-ring analysis at a precision of 0.01 mm using the LINTAB tree-ring station connected to TSAP-Win software (Rinntech, Heidelberg, Germany) equipped with a binocular microscope (Leica, Heerbrugg, Switzerland). Annual basal area increments (BAI) of these branches were determined using the mean of ring width measurements from four directions. We used the 2009 branch BAI to standardize for the pre-treatment growth of each of the 100 branches.

RADIAL STEM GROWTH

In parallel with the branch sampling campaign (23 and 26 September 2014; see above) six trunk cores were collected from each tree, two at breast height (1.3 m), two at crown base (20–25 m, depending on the individual tree) and two in the upper canopy (30 m above-ground) at 90° and 270° compass direction per height. Cores were taken from the crane gondola or by tree climbers using a 200-mm increment borer (core diameter 5.15 mm; Haglof, Sweden). Stem cores were dried at 80 °C for 72 h and scraped using a scalpel for better reading of the tree-ring structure. Ring widths of the past 15–25 years were measured at a precision of 0.01 mm using the LINTAB tree-ring station. The ring widths measured from each core were verified by visual cross matching, comparing intra- and intertree cores. BAI was calculated from mean ring width for each tree, height and year. Stem growth chronologies were then standardized by the 2005–2009 mean, providing the pre-treatment growth pattern. We limited the standardization period to 5 years to minimize the effects of past canopy structure dynamics on tree vigour and also avoiding the irregular 2004 growth following the 2003 heat-wave over Europe (Leuzinger *et al.* 2005).

Bulk wood density was determined in earlywood and latewood of annual growth rings from cores taken at breast height. We measured the volume (slice disc area × thickness; measured with a caliper at 0.01 mm precision) and dry mass of thin slices of earlywood and latewood of annual growth rings. The bulk wood density (g cm^{-3}) was calculated by dividing slice mass by its volume.

Transverse sections were prepared from trunk cores sampled at crown base of all ten trees taken by a hand microtome (GSL1, WSL, Switzerland). To facilitate the production of long, continuous sections spanning across the 2004–2014 tree rings, the tissue was stabilized using a starch-based non-Newtonian fluid (Schneider & Gärtner 2013). The xylem structure was observed under a microscope (Olympus BH-2, Tokyo, Japan) and captured by an interfaced camera (Olympus E-330). Each transverse core section was captured by 20–40 sequential photomicrographs with overlapping fields to allow stitching, using an image-processing software (Adobe Photoshop, Adobe Systems, San Jose, CA, USA). We measured the diameter (d_r) and the number of cell rows (n_r) of the earlywood and latewood of all growth rings formed between 2004 and 2014. In each growth ring, we identified the largest cell and measured its diameter (d_c) and lumen diameter (d_l). We calculated mean cell diameter from d_r/n_r and maximum cell wall diameter from $\frac{1}{2} \times (d_c - d_l)$.

NON-STRUCTURAL CARBOHYDRATES IN STEMS

Trunk cores taken from the east-facing sides of all trees in September 2014, at breast height (1.3 m), crown base (20–25 m, depending on the individual tree) and upper canopy (30 m above-ground) were used to determine non-structural carbohydrate content (NSC). Samples were prepared from tree rings of 2010–2014, with tree rings of 2004–2008 serving as a pre-treatment reference. The difficulty of this approach is the typical decline in NSC content with increasing sapwood depth, quantified at 33% reduction between 10 and 30 mm below the cambium in our trees (Hoch, Richter & Körner 2003). Assuming a similar radial gradient, and considering the average depths of tree rings of 2010–2014 and 2004–2008, that is 0–11 and 15–23 mm, we expected a sapwood age effect of −21% in the pre-treatment NSC compared to the FACE period NSC. Hence, all pre-treatment NSC values were increased by 21% to make them comparable with the 2010–2014 NSC values. All samples were ground using a ball mill (Retsch, Hann, Germany) at a frequency of 25 s^{-1} until tissues had turned into fine powder (~ 5 min). NSC analyses followed the method by Wong (1990), modified as described in Hoch, Richter & Körner (2003). Dried wood powder (8–12 mg) was extracted with 2 mL deionized water at 100 °C for 30 min. An aliquot of each sample extract was taken for the determination of low molecular weight carbohydrates using invertase (from baker's yeast, Sigma-Aldrich, Buchs, Switzerland) to break sucrose into glucose and fructose. Glucose and fructose were converted into gluconate-6-phosphate using glucose hexokinase (Sigma Diagnostics, St. Louis, MO, USA) and phosphogluconate isomerase (from baker's yeast, Sigma-Aldrich). The total amount of gluconate-6-phosphate was determined as the increase in $NADH + H^+$ using a photometer (HR 700; Hamilton, Reno, NV, USA). For NSC determination, the remaining extract was incubated at 40 °C for 15 h with amyloglucosidase (from *Aspergillus niger*, Sigma-Aldrich) to break starch into glucose. NSC was determined as the total amount of glucose as described above. Starch content was calculated as total NSC minus free sugars. All concentrations were calculated on a % dry matter basis.

FINE ROOT GROWTH

On 12 April 2013, we took 90 soil cores (12 cm in depth × 3.6 cm diameter): nine cores per tree in the main rooting sphere (2 m around the tree trunks) in triplets placed at directions of 120°, 240° and 360° around each trunk. The three cores per triplet were 10 cm apart and were averaged after biomass assessment to balance microscale heterogeneity. We immediately installed similarly sized in-growth cores in these coring holes: cylinders were made of a 2-mm stiff polyethylene mesh (Sefar AG, Heiden, Switzerland), filled with sieved, root-free soil collected on-site and gently compacted. On 23 September 2014 (17 months later), the in-growth cores were gently recovered using a knife. Soil and in-growth cores were kept frozen at −20 °C and were defrosted prior to analysis in cold water (4 °C) for 48 h. Fine roots were extracted using a sieve (1-mm mesh) and tweezers, and then classified into the following categories: *P. abies*, non-*Picea* and dead fine roots. Live *P. abies* fine roots were further classified into three diameter classes (< 0.5, 0.5–1, and > 1–2 mm). *Picea abies* fine roots were selected by comparison to pure *P. abies* and pure *Fagus sylvatica* (the major neighbouring species) reference root collections from nearby sites. The distinct morphology of *P. abies* roots warranted the separation of *P. abies* roots from roots of *F. sylvatica* and other species. All fine root classes were dried at 80 °C for 48 h and weighed for biomass determination.

CARBON ISOTOPE COMPOSITION

Since the CO_2 gas employed for elevating CO_2 levels carried a constant ^{13}C isotope signal ($\delta^{13}C = -30‰$), $\delta^{13}C$ measurements were used to trace the C flows in trees and soils and to test the efficiency of the FACE system. These measurements were performed on tree-ring wood, needles, branches, fine roots and plant isometers. Dried samples were milled, weighed into tin capsules in aliquots of 0.3 to 0.8 mg and analysed for C isotopes. The isotope analysis was performed at the Paul Scherrer Institute, Villigen, Switzerland. Samples were analysed using a mass spectrometer operating in continuous flow mode (Delta S, Thermo Finnigan MAT, Bremen, Germany) following combustion in an elemental analyzer (EA-1110 CHN, Carlo Erba Thermoquest, Milan, Italy) and having passed a variable open-split interface (Conflo II, Thermo Finnigan MAT). The precision of $\delta^{13}C$ analyses was < 0.1‰. The δ-notation expressed the isotopic deviation from the international reference standard (Vienna Pee Dee Belemnite: V-PDB): $\delta^{13}C = (R_{sample}/R_{standard} - 1)$ (‰) where R is the molar ratio of ^{13}C to ^{12}C for the sample and the standard, respectively.

RESPIRATORY FLUXES

Rates of CO_2 release from soil and stem were measured in 2008–2014 and in 2009–2011, respectively. We measured CO_2 release from soil (soil respiration, R_{soil}; μmol CO_2 m^{-2} s^{-1}) with two identical custom-made, closed, non-steady-state, non-through-flow chambers, equipped with open path, non-dispersive infrared gas analyzers (IRGA) and relative humidity/T sensors (GMP343 carbon dioxide probe, HMP75 rH/T probe; Vaisala, Vantaa, Finland; detailed description of the system in Bader & Körner 2010). Polypropylene collars (Ø 20 cm, 5–7 cm height) inserted ca. 2–3 cm into the soil served as a socket and seal for the chambers. We installed three collars per tree in 2 m distance to the stem base at a 120° angle around each tree, serving as subsamples for each tree. These collars were left in place throughout the course of the experiment. Photosynthetic active tissue inside the collars (very minor understorey herbs) was removed prior to R_{soil} measurements, but litter was left in place to ensure natural conditions. Monthly measurements started in July 2008 (a year before FACE onset) were intensified in 2009 and lasted through the growing seasons of 2010, 2011, 2013 and 2014. These measurements were performed at maximum daytime R_{soil} rates (13:00–18:00) and alternating between E- and A-trees to reduce any temporal bias. R_{soil} rates were calculated using a linear regression for the increase of the CO_2 concentration inside the chamber headspace per time unit (60 recordings per 5 min, with the records of the first min discarded to account for potential chamber placement effects; Davidson *et al.* 2002). Stem CO_2 release (stem respiration; μmol CO_2 m^{-2} s^{-1}) was measured using the LI-COR 6400-09 Soil CO_2 Flux Chamber connected to the LI-6400XT Portable Photosynthesis System (LI-Cor, Lincoln, NE, USA) and calculated as described in Mildner *et al.* (2015).

SOIL SOLUTION ANALYSES

Soil solution sampling was enabled by a system of ceramic suction cups installed in 2 groups of 2–3 cups at 15 cm depth around each of the ten trees and connected via a sampling bottle to vacuum systems as described in Schleppi *et al.* (2012). Samples were collected once a month throughout the entire experimental period, except when temperatures were below 0 °C in winter. All samples were immediately refrigerated, filtered at 0.45 μm and analysed for nitrate, dissolved reduced nitrogen (DRN), organic carbon (DOC) and inorganic carbon (DIC). We calculated DRN and not dissolved organic nitrogen (DON) because ammonium concentration was often below the detection limit, that is not subtractable. A detailed account of these data will be published separately.

STATISTICAL ANALYSIS

The replicated unit in our study was the single tree, with five trees under ambient and five trees under elevated CO_2, and hence, all measurements per tree were averaged. Among the multiple factors for which effects were tested by analysis of variance (ANOVA), CO_2 level (400 ppm, ambient; and 550 ppm, elevated) was the single factor repeated across all responses. Other factors were PPFD, sample height, year and interactions among these factors and with CO_2 level. Responses included needle photosynthesis rates, needle litterfall, branch and needle parameters, stem basal area increments, stem wood and xylem anatomy properties, fine root production, stem and soil CO_2 efflux rates and all tissue-specific ^{13}C isotope signals. For each response, we also calculated the 95% confidence intervals for the difference between ambient and elevated trees. Soil solution analyses were tested with repeated-measures ANOVA. All statistical analyses were performed in JMP Pro 11 (Cary, NC, USA), with $\alpha = 0.05$ across all tests.

Results

EFFECTIVENESS OF CO_2 ENRICHMENT

We used the ^{13}C isotope signals, which were introduced to the canopy from the pure tank CO_2, to trace the fate of new C. Data are shown in diagrams together with the biomass responses (Figs 2, 3 and 5). Needle and branch wood $\delta^{13}C$ of trees in elevated CO_2 (E) was on average −32.3 and −31.4‰, that is ca. 5–6‰ lower than that in trees growing at ambient CO_2 (A), confirming effective CO_2 application. Stem wood $\delta^{13}C$ significantly decreased in E-trees in 2009 rings

and in the following years ($P < 0.001$). The mean stem $\delta^{13}C$ of A-trees across all three sampling heights was $-26.16 \pm 0.07‰$, with minor interannual variation. In contrast, the stem $\delta^{13}C$ of E-trees under FACE was $-30.00 \pm 0.23‰$ (a difference of ca. 4 ‰), with interannual variations, partly due to old C feeding new tissue growth (in 2009–2010). At 30 m above-ground, stem $\delta^{13}C$ of E-trees was lower, with a mean of $-31.14 \pm 0.34‰$ (signal similar to branches, i.e. 5 ‰) probably due to the proximity of fully illuminated branches resulting in a higher photosynthetic rate, incorporating more ^{13}C-depleted CO_2. Latewood $\delta^{13}C$ of E-trees under FACE was usually less negative than earlywood $\delta^{13}C$ but not in A-trees (Fig. 2). Fine root $\delta^{13}C$ under E-trees was $-30.24 \pm 0.81‰$, that is 3‰ lower than in A-trees ($-27.35 \pm 0.62‰$; $P = 0.023$).

NEEDLE GAS EXCHANGE AND WHOLE-TREE TRANSPIRATION

Stomatal conductance (g_s) in needles of A- and E-trees was not affected by elevated CO_2. Under constant, saturating PPFD and 20 °C, g_s was 0.080 mol H_2O m^{-2} s^{-1} in July and between 0.005 and 0.021 in late September, on average 0.010 ± 0.003 mol H_2O m^{-2} s^{-1} in both A- and E-trees (a stomatal enhancement ratio of 1.0; Fig. 1a). To further test the effect of elevated CO_2 on whole-tree transpiration, we followed sap flow dynamics during CO_2 on/off experiments between 9:00 and 10:30 on two clear, sunny days in the 2014 growing season (1 and 5 August 2014). We were unable to find any effect of a sharp change in canopy CO_2 concentration on concurrent sap flow. Sap flow rates were unresponsive to ambient and elevated CO_2 (FACE off vs. on) and were also identical at the same time of day during the days following the on/off experiments (2 and 6 August 2014; Fig. 1b). In general, sap flow dynamics were similar across A- and E-trees (Fig. 1b). At chamber CO_2 concentration of 400 ppm, photosynthetic net assimilation (A_{net}) was 11.4 ± 1.2 and 11.7 ± 0.8 in A- and E-trees, respectively (Table S2). Photosynthesis increased from 11.6 ± 0.7 to 15.01 ± 0.7 μmol CO_2 m^{-2} s^{-1} between 400 and 550 ppm CO_2 (photosynthetic enhancement ratio of 1.35 ± 0.3 in both A- and E-trees; Fig. 1c,d). The 95% confidence interval for the difference in photosynthesis between A- and E-trees was (−2.70, +0.86 μmol CO_2 m^{-2} s^{-1}). Prior to the FACE experiment,

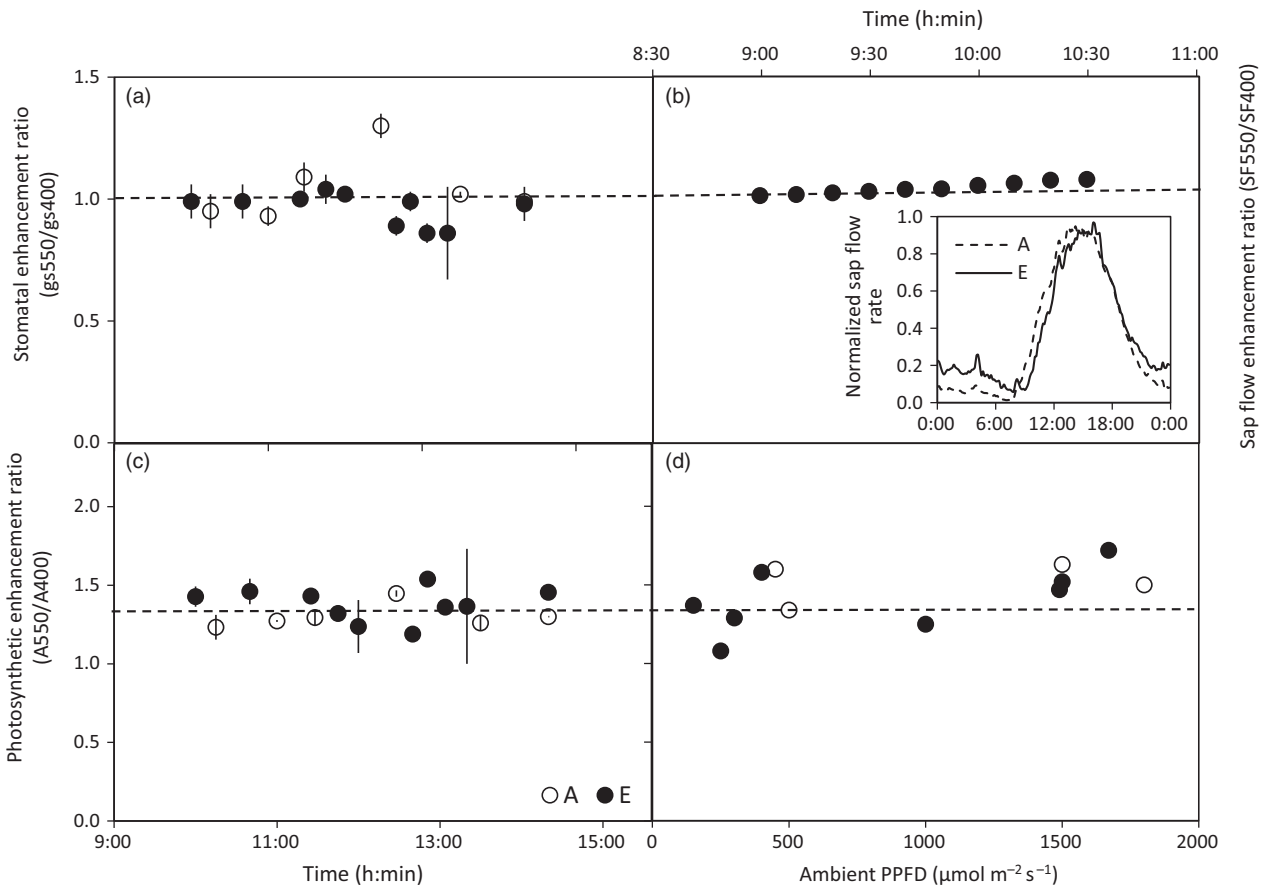

Fig. 1. The ratio between needle stomatal conductance (a), tree sap flow (b) and photosynthesis (c, d) at 550 and 400 ppm CO_2 in needles and stems of tall *Picea abies* trees growing under ambient (A) and elevated CO_2 (E). Sap flow enhancement ratio (b) was calculated for E-trees only by a FACE shutdown experiment on 1 August 2014, compared to 2 August 2014. The inset shows diurnal sap flow curves for A- and E-trees on 2 August 2014. Each data point is a mean ± SE of two measurements at specific time of the day (a, c) or a single observation at specific ambient light intensity (d). Error bars do not appear when too small. The dash horizontal line in (a, b) denotes no enhancement (ratio = 1.0); the dashed horizontal line in (c, d) denotes an arithmetic enhancement (ratio = 550/400, i.e. 1.375).

A_{net} was slightly, but not significantly, higher in E- vs. A-trees (Bader *et al.* 2016). Ambient light level (150–1800 μmol m^{-2} s^{-1}) and duration of CO_2 exposure had no significant effect A_{net} (Table 1); hence, there was no downward adjustment of A_{net}.

RADIAL STEM GROWTH

We found no significant effect of elevated CO_2 on stem growth at any height ($P = 0.774$) while radial increment changed significantly between years ($P = 0.001$) and sampling heights ($P = 0.015$) irrespective of treatment. The 95% confidence interval for the difference in radial stem growth between A- and E-trees was (−0.25, +0.09). Interannual growth variations generally decreased with tree height (Fig. 2). During 2010–2014, there was a mild increase in wood density compared to 2004–2008, in both early- and latewood of both A- and E-trees (Table 2). This increase was slightly more pronounced in latewood of E-trees, but overall the differences between A- and E-trees were not significant ($P = 0.747$). Together with the observation of similar growth patterns, we did not detect any measurable FACE treatment effect on stem biomass increment. The number of cell rows in both early- and latewood was slightly higher in E- vs. A-trees suggesting smaller cells (Table 2), but this difference was already observed before 2009 and thus relates to *a priori* differences between individual trees and not to the CO_2 enrichment. Mean cell diameters in the earlywood and latewood of all trees across both periods were 37–40 μm and 20–23 μm, respectively.

Table 1. ANOVA results for tall *Picea abies* growth, photosynthesis, needle litterfall and $\delta^{13}C$ under ambient and elevated CO_2

Factor	d.f.	Df_{DEN}	*F* value	*P*
Response: *Picea abies* needle photosynthesis (2013–2014)				
CO_2	1	96	0.02	0.882
PPFD	1	96	0.97	0.328
CO_2 × PPFD	1	96	0.03	0.859
Response: *Picea abies* stem radial growth (2010–2014)				
Year	1	129	3.25	0.074
Height	2	129	6.15	0.003**
CO_2	1	129	0.44	0.508
CO_2 × height	2	129	0.72	0.486
Year × height	2	129	0.31	0.736
Year × CO_2	1	129	0.37	0.544
Year × CO_2 × height	2	129	0.28	0.752
CO_2 effects on *Picea abies* stem radial growth at three heights				
Upper canopy	1	39	2.74	0.106
Crown base	1	39	0.09	0.762
Breast height	1	39	0.02	0.901
Response: *Picea abies* stem wood $\delta^{13}C$ (2011–2014)				
Year	3	205	4.88	0.003**
Height	2	205	5.17	0.007**
CO_2	1	205	123.61	< 0.001***
Early/latewood	1	205	0.18	0.668
CO_2 × height	2	205	17.28	< 0.001***
Year × height	6	205	1.05	0.393
Year × CO_2	3	205	4.60	0.004**
Year × CO_2 × height	6	205	1.07	0.383
CO_2 effects on *Picea abies* branch parameters (2010–2014)				
Main branch wood biomass	1	39	0.02	0.902
Main branch diameter	1	39	0.05	0.824
Main branch length	1	39	0.13	0.722
Side branchlet biomass	1	39	1.97	0.168
Single branchlet biomass	1	39	0.10	0.752
Side branches per branch	1	39	2.01	0.164
Response: *Picea abies* needle litterfall (2010–2014)				
CO_2	1	38	0.26	0.614
Year	1	38	0.03	0.870
CO_2 × year	1	38	2.90	0.097
CO_2 effects on *Picea abies* fine root biomass (2013–2014)				
Soil cores	1	20	0.65	0.429
In-growth cores	1	20	1.71	0.206
CO_2 effects on *Picea abies* respiratory fluxes				
Stem respiration (2010–2011)	1	11	2.30	0.062
Soil respiration (2001–2014)	1	43	3.54	0.025*

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

STEM CARBON STORAGE

Stem non-structural carbohydrate (NSC) concentrations were expectedly low, on average 0.6–1.3 % d.m. (Table 2). Sampled in October 2014, values from the 2004–2008 tree rings were corrected for an age-related decline with increasing sapwood depth to simulate pre-treatment concentrations (see Materials and methods) but, in general, were still lower than those measured in the 2010–2014 tree rings of both A- and E-trees. The 2004–2008 tree rings of A-trees had significantly more soluble sugars at the crown base than E-trees. Under FACE, E-trees had significantly more starch at 30 m than A-trees (Table 2; $P = 0.027$), so the presumed high CO_2 effect faded with distance from the upper crown.

BRANCH AND NEEDLE GROWTH

Annual growth includes the formation of new terminal branch segments and side branchlets, while branch diameter increases with age due to secondary growth (Fig. 3a,b). Main branch diameter was similar across A- and E-trees, including the 2011 segments. At final harvest, main branch segment length varied between 82 and 127 mm, with no difference between E- and A-trees (Fig. 3c). The 95% confidence interval for the difference in main branch segment length between A- and E-trees was (−9.64, +17.00 mm). Side branchlets biomass per branch generally increased from 1 year to the next, although not in 2014 across all trees (Fig. 3d). So, the annual biomass of new side branchlets was quite similar in E- and A-trees, except for 2014 (Fig. 3d). This increase resulted from a larger number of side branchlets in E- than in A-trees in that year at otherwise similar branchlet biomass (Fig. 3e,f). This difference in branchlet number under FACE was not significant and had a 95% confidence interval of (−2.90, 0.50). Also, this difference had no significant effect on the total branch wood biomass, due to the larger fraction of the main branch segment of total branch biomass (Fig. 3a; $P = 0.902$). Accordingly, total needle biomass was higher in E- vs. A-trees in 2013 and 2014 (data not shown). The small difference in mean needle mass between E- and A-trees was not

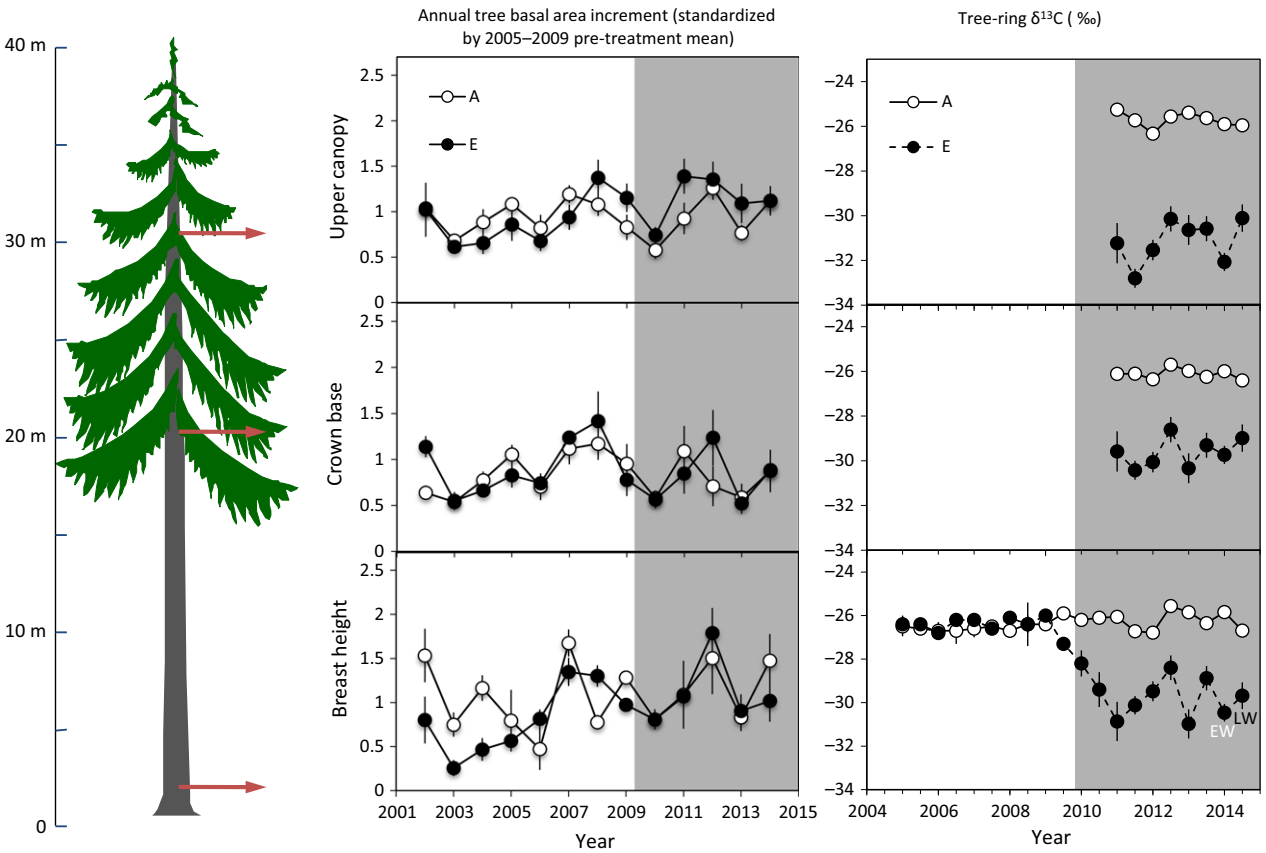

Fig. 2. Stem annual radial growth (left panels; standardized by the 2005–2009 pre-treatment mean) and tree-ring $\delta^{13}C$ (right panels) of tall *Picea abies* trees growing under ambient (A) and elevated CO_2 (E) along their height profile. FACE period is denoted by grey background. Each data point is a mean ± SE of five trees, each with two cores. *P*-values are for the difference between A- and E-trees during 2011–2014 using ANOVA. EW, earlywood; LW, latewood.

Table 2. Stem wood density, xylem anatomy and non-structural carbohydrates (NSC) in tall *Picea abies* under ambient and elevated CO_2 before and during FACE. Sampling heights (Ht) are breast height (BH), crown base (CB) and 30 m above-ground (upper canopy, UC). EW is earlywood and LW, latewood. Numbers are means (standard errors). Asterisks denote significantly higher value among ambient and elevated trees by ANOVA ($\alpha < 0.05$)

		Ambient *Picea abies*		Elevated *Picea abies*	
Parameter	Ht	2004–2008 Pre-treatment	2010–2014 FACE	2004–2008 Pre-treatment	2010–2014 FACE
Wood density EW (g cm^{-3})	BH	0.40 (0.01)	0.44 (0.02)	0.43 (0.02)	0.48 (0.02)
Wood density LW (g cm^{-3})	BH	0.77 (0.04)	0.80 (0.01)	0.70 (0.04)	0.76 (0.04)
Number of cell rows EW	CB	28.4 (4.4)	27.8 (6.4)	36.5 (6.7)	40.6 (7.5)
Number of cell rows LW	CB	9.7 (0.8)	9.7 (1.3)	13.0 (2.5)	12.5 (2.0)
Mean cell diameter EW (μm)	CB	40.3 (2.6)	40.2 (1.7)	37.2 (1.7)	39.2 (1.3)
Mean cell diameter LW (μm)	CB	23.3 (4.2)	21.4 (2.5)	22.0 (3.2)	20.1 (1.1)
Max. cell diameter (μm)	CB	58.4 (2.3)	60.2 (2.7)	58.3 (2.8)	60.2 (2.7)
Max. cell wall diameter (μm)	CB	8.7 (1.1)	8.7 (0.4)	9.3 (0.7)	9.1 (0.6)
NSC (% d.m.)	BH	0.76 (0.26)	0.96 (0.06)	0.59 (0.06)	1.03 (0.10)
	CB	0.76 (0.13)	0.88 (0.12)	0.72 (0.15)	0.96 (0.19)
	UC	0.94 (0.14)	0.99 (0.07)	0.80 (0.11)	1.26 (0.27)
Starch (% d.m.)	BH	0.37 (0.12)	0.37 (0.08)	0.21 (0.11)	0.38 (0.07)
	CB	0.22 (0.14)	0.27 (0.09)	0.41 (0.12)	0.33 (0.11)
	UC	0.39 (0.22)	0.19 (0.08)	0.40 (0.13)	0.72 (0.16)*
Soluble sugars (% d.m.)	BH	0.40 (0.15)	0.59 (0.14)	0.47 (0.15)	0.66 (0.08)
	CB	0.74 (0.11)*	0.61 (0.08)	0.31 (0.06)	0.63 (0.09)
	UC	0.59 (0.13)	0.80 (0.06)	0.39 (0.04)	0.54 (0.12)

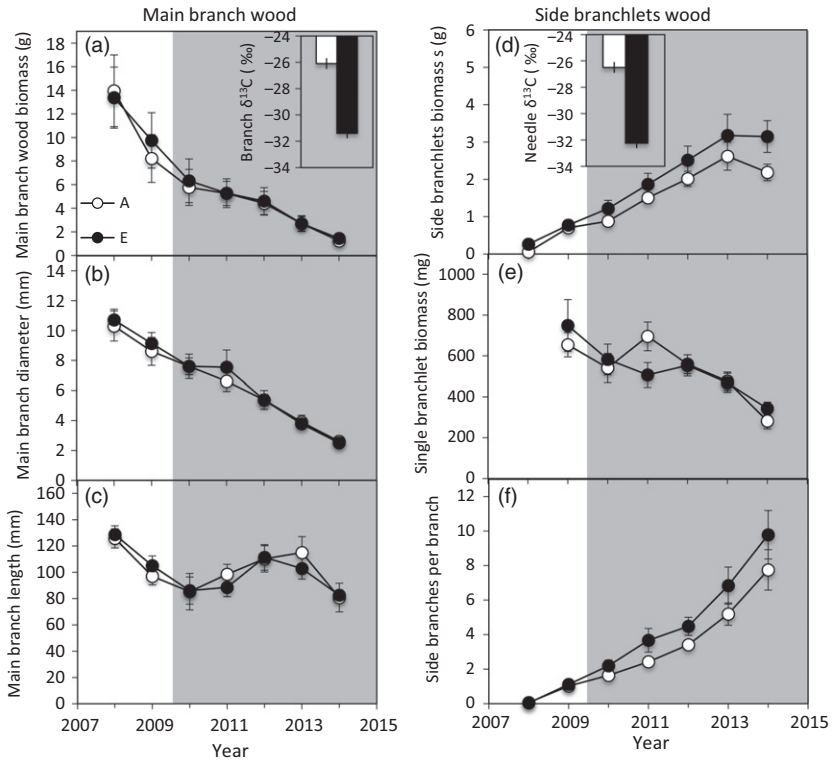

Fig. 3. Development of terminal branches (a–c) and side branchlets (d–f) of tall *Picea abies* trees (standardized by the 2009 main branch basal area increment) growing under ambient (A) and elevated CO_2 (E). FACE period is denoted by grey background. Each data point is a mean ± SE of five trees, each with ten observation branches. Insets in (a) and (g) show the mean ± SE $\delta^{13}C$ in new branches (2010–2011) and needles (2010–2013), respectively. *P*-values are for the difference between A- and E-trees using ANOVA.

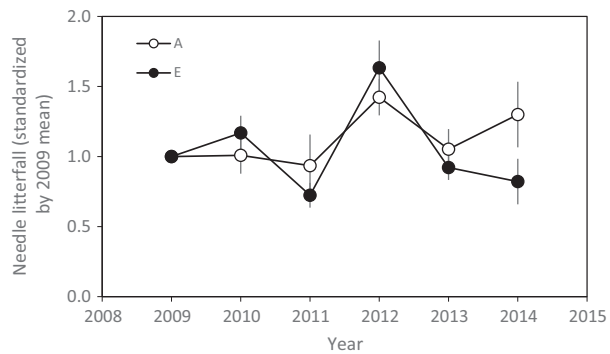

Fig. 4. Annual needle litterfall of tall *Picea abies* trees (standardized by the 2009 mean) growing under ambient (A) and elevated CO_2 (E). Each data point is a mean ± SE of five trees, each with three litter traps.

significant, and all needles became heavier with age, suggesting higher retention of heavy vs. lightweight needles with time across A- and E-trees. Dividing the annual needle biomass by the single needle biomass of 20 random needles and standardizing for pre-treatment branch diameter increment, the calculated number of needles per branch was generally similar between E- and A-trees ($P = 0.077$). Comparing annual means separately, needle number per branch was higher in E- vs. A-trees in 2013 ($P = 0.003$), but not in any other year. Hence, these patterns were not consistent.

NEEDLE LITTERFALL

Needle litter collected in 30 traps placed under A- and E-trees over 18 sampling occasions, commonly sampled at 1- to 3-month intervals, showed a large variation (2.0–145.1 g m^{-2}) due to the heterogeneity of the forest canopy, interval duration and wind. Yet the annual needle litterfall sum was similar (86.7 ± 15.0 g m^{-2} $year^{-1}$) across A- and E-trees and over the 5 years of the experiment. The 95% confidence interval for the difference in needle litterfall between A- and E-trees was −0.15, +0.33. Overall, we were unable to detect any significant changes in litter dynamics between treatments (Fig. 4).

FINE ROOT GROWTH AND SOIL WATER CHEMISTRY

Soil cores were sampled twice during the course of the experiment. A first sample taken at the beginning of the treatment in March 2010 showed no significant E vs. A difference in fine root mass (Mildner *et al.* 2015). Soil cores sampled in early 2013 also revealed similar fine root content around A- and E-trees (Fig. 5a; Table 1). In-growth cores were installed immediately after each soil core sampling and recovered after 17–20 months. In-growth cores recovered from the same boreholes in December 2011 by Mildner *et al.* (2015) revealed again similar fine root content around A- and E-trees. But in-growth cores recovered in September 2014 (17 months after the 2013 installation) showed non-significant difference for fine root mass around E-trees vs. A-trees, namely 109 ± 32 and 64 ± 12 g m^{-2}, respectively (Fig. 5b), but there was a weak, though, consistent trend towards higher fine root mass under E-trees. This difference was consistent across all fine root size classes, but was not significant for any of these classes (Fig. 5b). The 95% confidence interval for the difference in

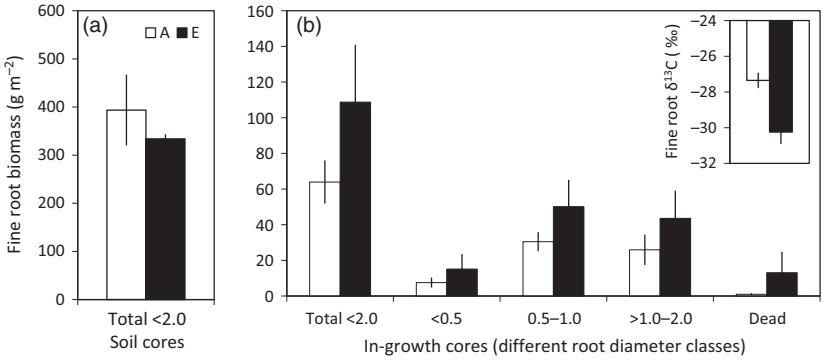

Fig. 5. Fine root biomass in soil (a) and in-growth cores (b) of tall *Picea abies* trees growing under ambient (A) and elevated CO_2 (E). Each bar is a mean ± SE of five trees, each with nine cores. The inset in (b) show the mean ± SE $\delta^{13}C$ in fine roots formed in 2013–2014. *P*-values are for the difference between A- and E-trees using ANOVA.

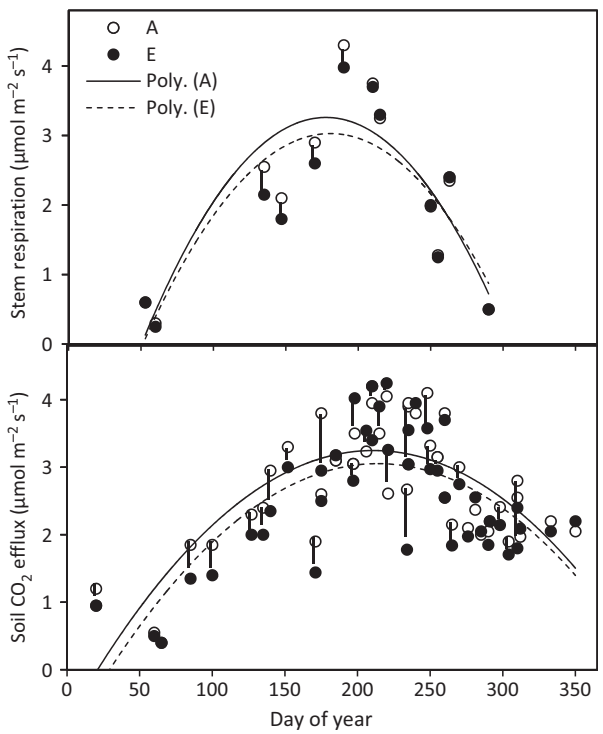

Fig. 6. Seasonal curves of CO_2 release from stem (top; 2009–2011) and soil (bottom; 2009–2014) respiration of tall *Picea abies* trees growing under ambient (A) and elevated CO_2 (E). Each data point is a mean of five trees, each with three measurement rings. Vertical lines connect means of A- and E-trees from each measurement day (when not overlapping). The polynomial fit curves for ambient and elevated trees are $y = -2*10^{-4}x^2 + 0.072x - 3.10$ ($r^2 = 0.78$) and $y = -2*10^{-4}x^2 + 0.065x - 2.88$ ($r^2 = 0.72$) for stem respiration and $y = -9*10^{-5}x^2 + 0.038x - 0.76$ ($r^2 = 0.57$) and $y = -9*10^{-5}x^2 + 0.038x - 1.04$ ($r^2 = 0.54$) for soil respiration.

root biomass between A- and E-trees was (−114, +24 g m^{-2}).

The concentration of dissolved organic and inorganic C at 15 cm soil depth was similar around A- and E-trees: DOC 7.7 ± 3.0 and 8.3 ± 5.0 mg L^{-1}, respectively; and DIC 12.2 ± 4.7 and 13.5 ± 4.3 mg L^{-1}, respectively. In contrast, free nitrate nitrogen was higher around E- compared to A-trees (repeated-measures ANOVA $P = 0.052$): 6.65 ± 3.05 vs. 1.35 ± 1.29 mg L$^{-1,}$ respectively, whereas dissolved reduced nitrogen was similar in A- and E-trees: 0.38 ± 0.12 and 0.21 ± 0.13 mg L^{-1} ($P = 0.09$).

RESPIRATORY FLUXES

Stem and soil CO_2 efflux showed the expected parabolic seasonal curves peaking at 3–4 μmol m^{-2} s^{-1} in July (around day of year 200; Fig. 6). Stem respiration was more confined to the growing season than soil respiration and dropped below 1.0 μmol m^{-2} s^{-1} in October through to February. A- and E-trees had similar seasonal curves, with A-trees showing mildly higher rates of stem and soil respiration. Mean stem respiration rates were similar in A- and E-trees (1.9 ± 0.4 and 1.8 ± 0.4 μmol m^{-2} s^{-1}, respectively), and the 95% confidence interval for the difference was (−0.96, +1.18 μmol m^{-2} s^{-1}). Mean soil respiration rates were 2.7 ± 0.1 and 2.5 ± 0.1 μmol m^{-2} s^{-1} in A- and E-trees, respectively, and the 95% confidence interval for the difference was (−0.21, +0.58 μmol m^{-2} s^{-1}). This small, but consistent, difference was significant ($P = 0.025$) indicating a subtle negative CO_2 effect on soil respiration.

Discussion

Stable isotope signals prior to and during the 5.5 years of the experiment confirm that FACE imposed a significant CO_2 enrichment signal across all tissues. This signal is integrated by isometers as well as IRGA-derived signals, which agree on a ca. 150 ppm increase in atmospheric CO_2 around these tall tree crowns (see Materials and methods). Under this CO_2 level, C supply to the mesophyll increased, as there was no stomatal response or down-regulation of photosynthesis (Fig. 1) from the very beginning of the experiment (Leuzinger & Bader 2012; Bader *et al.* 2016). Consequently, stem water flux did not change, and soil moisture was similar around A- and E-trees in the first year (Leuzinger & Bader 2012) and throughout the entire experiment (data not shown). As hypothesized, we did not find any effect on stem growth, not even a trend or initial temporary response (Fig. 2). We did not find any effect on branch length growth and no difference in needle litter fall between A- and E-trees (Figs 3,4). A trend towards increased fine root production was not significant, although consistent across all fine root size categories (Fig. 5) and with a 95% confidence interval reflecting the lower biomass in A- vs. E-trees (−114, +24 g m^{-2}). Contrary to expectations, soil respiration slightly decreased in E-trees by 7% and we did not detect an effect on stem respiration at the

base (Fig. 6) as observed early on (Mildner *et al.* 2015), also indicated by a 95% confidence interval of (−0.96, +1.18 μmol m^{-2} s^{-1}).

Total needle mass and the number of side branchlets increased in E- vs. A-trees in 2013 and 2014, while individual needle mass and branch radial increment were the same in E- and A-trees (Fig. 3). In contrast, needle litter fall in 2013–2014 was slightly lower under E- vs. A-trees, suggesting that higher needle biomass at the single branch level might have been compensated for by less branches at the whole-tree level. The tall *Picea* trees studied here are soaring above the mixed forest canopy by 5.0 ± 1.0 m (see Materials and methods) and hence receive more light than neighbouring trees of other species. This could have made branchlet density more responsive to CO_2. But other than this limited branchlet effect that did not scale to main branch diameter and branch length responses, we found no stimulation of biomass accumulation. Earlier CO_2 enrichment experiments in *Picea*, using branch bags, also found no CO_2 effect on branch and needle development (Barton & Jarvis 1999; Roberntz 1999). The marginally significant increase in branching and associated total needle biomass per branch could be a transient response because (i) gaps within the crown should close; (ii) allometric relations should limit any canopy adjustment not matched by respective changes in stem and roots; and (iii) in our FACE with deciduous trees, an increase in branching index in *Quercus* and *Fagus* (but not in *Carpinus*) was observed in the first or second year under elevated CO_2 but not later (Asshoff, Zotz & Körner 2006). Since neither branch length nor branch basal area responded to elevated CO_2, the needle mass to conduit area ratio was slightly less at branch level in E-trees.

Many of the CO_2 enrichment signals and physiological responses were already observed 2.5 years after the onset of FACE (Mildner *et al.* 2014, 2015; Bader *et al.* 2016). Yet, the dynamics of wood $\delta^{13}C$ at breast height (Fig. 2) and the increasing trend in fine root production (Fig. 5) were unexpected based on earlier observations. Some of the temporal variability in wood $\delta^{13}C$ of E-trees after 2011 (Fig. 2) can be explained by environmental variability, since it was also observed, to a lesser extent, in wood $\delta^{13}C$ of A-trees. But the higher fluctuations in E- vs. A-trees are not fully understood and could partly relate to the variability of CO_2 concentration around branches of E-trees (Streit *et al.* 2014) and also between years. Alternatively, it likely reflects the higher reliance of wood formation on ^{13}C-depleted starch, which was formed in the previous year (Hoch, Richter & Körner 2003), leading to more negative $\delta^{13}C$ in earlywood vs. latewood as in our E-trees. The increasing trend in fine root biomass, although not significant, is in line with reports from multiple sites (Iversen 2010) and might be a delayed response to higher C supply at otherwise unchanged soil moisture. In our former FACE with deciduous tree species, we observed a 30% reduction in fine root biomass together with reduced tree water consumption and resultant soil water savings in E-trees (Bader, Hiltbrunner & Körner 2009). In planted young *Picea abies* under 570 ppm, fine root density significantly increased during the expansive stage of the stand (Spinnler, Egli & Körner 2002) and similarly, Pokorný, Tomášková & Marek (2013) reported for young *Picea* under 750 ppm, a 37% increase in below-ground biomass with 43% increase in roots < 1 mm. Such changes relate to faster initial space occupancy and must not be confused with steady-state signals in mature stands (Anderson-Teixeira *et al.* 2013; Miller *et al.* 2015). A transitory increase in root production was also observed at the ORNL FACE (Norby *et al.* 2010). Further, the Czech FACE revealed enhanced needle photosynthesis, without any change in needle anatomy and chemistry (Lhotáková *et al.* 2012; Urban *et al.* 2012), which is in agreement with our observations of unchanged single needle biomass and length (data not shown).

The sustained enhancement of photosynthesis in our earlier FACE with deciduous trees was also without any effect on carboxylation efficiency and maximal electron transport, and, based on litterfall data, there was also no change in LAI (Bader, Siegwolf & Körner 2010; Bader *et al.* 2013). Yet, unlike *Picea*, there was a mean 10% downregulation of stomatal conductance under elevated CO_2, which mostly came from *Carpinus*, which was less pronounced in *Fagus*, and absent in *Quercus* (Keel *et al.* 2007). Across all deciduous species, sap flow decreased by 10–15% (Leuzinger & Körner 2007; Bader, Hiltbrunner & Körner 2009). In the Duke *Pinus taeda* FACE, photosynthesis was also enhanced at unchanged g_s (Ellsworth *et al.* 2012) as reported earlier for *Pinus radiata*, (Tissue *et al.* 2001). In contrast, young *Populus* spp. (POP-EUROFACE in Italy) and *Liquidamber styraciflua* (ORNL FACE in Tennessee, USA) showed a reduction in g_s of 14–44% (Gunderson *et al.* 2002; Tricker *et al.* 2005; respectively). In turn, the ratio A_{net}/g_s, known as intrinsic water-use efficiency (WUE_i), increased in our experiment by 38% at the needle level, simply as a result of higher A_{net} at constant g_s, in line with some previous observations (De Kauwe *et al.* 2013; Streit *et al.* 2014) but less than ca. 77%, reported for other FACE experiments on planted or younger trees (Battipaglia *et al.* 2013). Our signal scales to the whole-tree level, because LAI was not affected, but the ecologically more relevant WUE, that is the ratio between biomass gain and water loss, did not change.

A 33% enhancement of photosynthesis under 670–700 ppm CO_2 was observed in the *Picea abies* FACE in Flakaliden, Sweden, in whole-tree chambers (Hall *et al.* 2013; Wallin *et al.* 2013). At that boreal site, with frost events common throughout spring, the growing season of elevated CO_2 trees was elongated by 4 weeks inside the tree chambers, but nevertheless, it did not affect total tree growth (Sigurdsson *et al.* 2013). In our freestanding temperate zone *Picea*, there was no CO_2 effect on tree phenology (T. Klein, R. T. W. Siegwolf & C. Körner, unpublished data). Radial growth of the deciduous species in our former FACE also remained unaffected by elevated CO_2 over 8 years (Körner *et al.* 2005; Bader *et al.* 2013).

The small differences in wood structure and chemistry we observed in E- vs. A-trees (Table 2) are consistent with those reported in earlier studies with *Picea*. Wood density in saplings increased from 0.48 at 420 ppm CO_2 to 0.50 g cm^{-3} at

560 ppm CO_2 in earlywood and was 0.81 g cm^{-3} in latewood across E- and A-trees (Hättenschwiler, Schweingruber & Körner 1996), in agreement with our measurements of 0.44 and 0.48 g cm^{-3} in earlywood and 0.80 and 0.76 g cm^{-3} in latewood of adult A- and E-trees under 400 and 550 ppm CO_2, respectively. Since the very small CO_2 effect observed by Hättenschwiler, Schweingruber & Körner (1996) was significant, it meant stronger wood in those young trees, a signal that seems to diminish at our larger scale. In that experiment with saplings, stem wood starch content increased from 1.07 to 1.44 % d.m., which is (in relative terms) in agreement with the wood starch increase from 0.19 to 0.72% d.m observed here in the stem in the upper canopy. Minor CO_2 effects on wood were also found in the *Picea* FACE in Flakaliden, with reduced tracheid lumen diameter and wood soluble sugars (Kostiainen *et al.* 2004). The decrease in soluble sugars was from 1.32 to 0.84 % d.m., similar in relative terms to our observation in stem cores, from 0.74 to 0.31% d.m., and in both studies this effect was significant in the lower canopy but not at breast height (Table 2).

However, the minute increase in wood starch concentration in the canopy of trees under 550 ppm CO_2 cannot explain the large surplus of C, based on branchlet photosynthesis. In general, C uptake amounts must be balanced by the sum of five fluxes, that is respiration, growth, export, litter and the net exchange between C storage and consumption (Klein & Hoch 2015). Here, we showed that under elevated CO_2 (i) respiration of stems was either reduced or unchanged; (ii) growth of stems and branches was unchanged, with only non-significant increases in root growth, side branchlet biomass and earlywood density; (iii) changes in C export to soil remained largely unknown but with an indication for priming (increased nitrate in the soil solution); (iv) litter production was unchanged; and (v) there was a net increase in starch (and reduction in soluble sugars) in the upper part of the stem. Therefore, in our search for the 'missing C sink' (Fatichi & Leuzinger 2013), respiration and above-ground growth can be ruled out (contrary and in line with our hypothesis, respectively). It is possible that C transport to below-ground sinks has increased, as indicated by an increasing trend (although not significant) in fine root production, the transfer of labelled C to ectomycorrhiza (Mildner *et al.* 2014) and to trees of other species sharing the same mycorrhizal networks (Klein, Siegwolf & Körner 2016). C export to soil might have increased too, although soil respiration at elevated CO_2 was not enhanced, and DIC and DOC were unaffected at the shallow soil depth explored here. The observed increase in nitrate concentration in the rhizosphere, at similar moisture levels and without any sign of reduced tree nitrogen (N) uptake (no downregulation of photosynthesis), suggests higher microbial N mineralization, presumably due to enhanced root exudation (priming effect; Körner & Arnone 1992; Iversen *et al.* 2011; Norby & Zak 2011; Phillips, Finzi & Bernhardt 2011; Phillips *et al.* 2012; Schleppi *et al.* 2012). A decoupling between increased fine root production and unaffected soil respiration has already been observed in spruce under elevated CO_2 (Spinnler, Egli & Körner 2002). In that study, increased fine root density was accompanied by enhanced soil respiration on calcareous soil, but not on acidic soil. The low soil pH at our site (5.8; Schleppi *et al.* 2012) is in agreement with that observation. Lastly, the extra C could have been released through a multitude of 'vents', each too small to track individually, along the entire assimilate transport chain from canopy to soil microbes (Mildner *et al.* 2014; Savage *et al.* 2015).

Summarizing the results of our 5.5 years of spruce FACE, and reviewing results from our former, 8 years of FACE with deciduous trees, and many other CO_2 enrichment experiments across the globe, an apparent paradox is emerging: CO_2 uptake is the basis for autotrophic life, but at current CO_2 availability, it does not control most plant processes, including growth (Leuzinger & Hättenschwiler 2013; Smith *et al.* 2015). Instead, photosynthesis, growth, respiration and C storage all appear regulated by other limiting resources, most likely soil nutrients other than N (Körner 2003, 2015). At the Flakaliden FACE, all effects of elevated CO_2 on photosynthesis were predisposed by light level in the canopy (Hall *et al.* 2013) but did not scale to a tree growth signal. The predominance of nutrient availability over CO_2 elevation as a growth driver has been repeatedly reported: Boreal *Picea abies* at 670–700 ppm CO_2 showed 25% growth increase only at improved (optimal) nutrient availability (Ryan 2013; Sigurdsson *et al.* 2013). When soil N availability was reduced below a critical level, all CO_2 tree responses in the ORNL and Duke FACE diminished (Franklin *et al.* 2009). Radial stem increment of *Picea* saplings increased with increasing rates of N deposition but not with elevated CO_2 (Hättenschwiler, Schweingruber & Körner 1996). Similarly young tropical trees showed no growth response to elevated CO_2 when they were not fertilized (Winter *et al.* 2000). Changes of wood density will depend on the balance between N and C availabilities (Hättenschwiler & Körner 1998): under abundant nutrient availability, the wood of *Picea* softens; when nutrients are scarce and C is oversupplied, stems get stiff (Meyer, Paulsen & Körner 2008).

In summary, we found no evidence that CO_2 concentrations beyond current levels will enhance above-ground growth and productivity of Norway spruce. Yet, considering the universal, continuous CO_2 increase, important species- and tissue-specific responses can still lead to competitive advantages or disadvantages among species. Indirect CO_2 effects such as those on soil nitrate concentration in both our FACE experiments at this site, a steady 22% increase in leaf NSC in *Quercus* and increased stomatal sensitivity in *Carpinus* (but not in the other 4 species; Körner *et al.* 2005; Bader *et al.* 2013) are good examples. Except for the absence of a CO_2-driven growth stimulation, species-specific and subtle responses of other traits can exert long-term changes in ecosystem properties, calling for experimentation with different species, over long periods, and in experimental settings as natural as possible with regard to the nutrient cycle.

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Data accessibility

The complete data sets used in this publication are archived in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.29mb7 (Klein *et al.* 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Major size elements of the ten studied *Picea abies* trees.

Table S2. Needle gas exchange rates measured on 1-year-old needles in ambient (A) and elevated (E) trees at both ambient and elevated CO2 levels (400 and 550 ppm) during five summer field campaigns (18 June, 2 July, and 19 September 2013; 23 and 26 September 2014).

Fig. S1. Canopy overview map of the Swiss canopy crane mixed forest research site.

Fig. S2. View of the study site at the Swiss canopy crane research station in a mixed forest 12 km southwest of Basel, Switzerland (47°33′N, 7°36′E, 550 m a.s.l).

Fig. S3. View of the crane and the mixed forest canopy from the forest floor.

Fig. S4. The crane gondola at work, bringing down branch samples from the crown of a one of the ten *Picea abies* study trees.

Fig. S5. Schematic of the web free air CO_2 enrichment system for the experiment on five *Picea abies* trees at the Swiss canopy crane mixed forest site conducted between 2009 and 2014.

Fig. S6. A typical canopy branch of a tall *Picea abies* carrying the porous tubes emitting the labelled CO_2.