Photosynthetic enhancement and diurnal stem and soil carbon fluxes in a mature Norway spruce stand under elevated CO₂

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Abstract

Understanding the effects of elevated atmospheric CO₂ on carbon (C) relations of mature forest trees is central to understanding ecosystem C fluxes and pools in a future high-CO₂ world. Here, we investigated the CO₂-induced photosynthetic enhancement and the diurnal variation in shoot carbon assimilation, stem CO₂ efflux and soil respiration associated with *ca.* 110-year-old and 37 m tall Norway spruce trees (*Picea abies* (L.) H.Karst.) growing under free air CO₂ enrichment (FACE; *e*CO₂) in a mixed, near-natural forest in Northern Switzerland. Diurnal measurements of these major C fluxes were conducted simultaneously on three occasions: one week before and after the start of CO₂ enrichment, and one year later. Under controlled leaf chamber conditions, an increase in the atmospheric CO₂ concentration of *ca.* 150 ppm above ambient stimulated light-saturated rates of photosynthesis in previous- and current-year upper-canopy shoots equally by 73 ± 2 %. In the course of the day such large differences in C assimilation between *e*CO₂ and *a*CO₂ trees only occurred around midday under non-limiting light conditions. The CO₂ efflux rates from spruce stems (*CE*_{stem}) and surrounding soil (*R*_{soil}) shared a similar range during night- and

daytime ($3\text{--}5\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) but were not stimulated by $e\text{CO}_2$. Both CE_{stem} and R_{soil} were still rising when photosynthesis approached evening light compensation potentially reflecting the time lag in assimilate allocation to stem tissue and fine roots. Our findings suggest a strong photosynthetic enhancement during the initial CO_2 enrichment phase but provide no evidence for an overall or daytime-dependent stimulation of respiratory CO_2 fluxes indicating that the extra C was not quickly returned to the atmosphere through respiratory processes in spruce stems or surrounding soil.

Introduction

Besides unwanted effects of elevated atmospheric CO_2 on climatic forcing (climate warming), a CO_2 enriched atmosphere is often considered a potential 'fertilizer' of plant growth, given the rate-limiting role of ambient CO_2 concentrations for leaf photosynthesis. However, whether such a CO_2 -driven stimulation of tree growth will occur is highly questionable, because of the many other constraints of growth, mineral nutrients in particular (Körner 2006). A stimulation of growth by elevated CO_2 requires a net carbon (C) gain, that is a positive difference between carbon uptake and loss.

Photosynthetic C uptake represents the largest flux in the global C cycle followed by the respiratory C release from ecosystems to the atmosphere (Roy et al. 2001). Soil respiration (R_{soil}) comprises the total CO_2 released from auto- and heterotrophic processes in soils, and may account for up to 80 % of the CO_2 emissions in temperate conifer and hardwood forests (Wofsy et al. 1993, Malhi et al. 1999, Janssens et al. 2001). A further important pathway for assimilated CO_2 is stem efflux (CE_{stem}), a combination of CO_2 respired by local woody tissue and respiratory CO_2 originating from elsewhere in the plant-soil system, part of which dissolves in xylem sap and is transported in the transpiration stream (Teskey & McGuire 2005, 2007, Etzold et al. 2013). In temperate forests, CO_2 release from stems may contribute up to 21 % to total ecosystem respiration (Wang et al. 2010). The remaining possible pathways for C out of an ecosystem (leaching of dissolved organic and inorganic C, emissions of volatile organic and inorganic C) are of much less importance and very difficult to measure in mature ecosystems (Schleppi et al. 2012). Any attempt at quantifying a potential ecosystem net carbon gain under elevated CO_2 , requires data for the main C fluxes such as leaf photosynthesis, R_{soil} and CE_{stem} .

Free air carbon dioxide enrichment (FACE) experiments are currently the only available tool to experimentally investigate future changes in C pools and fluxes in mature forests. Multi-year FACE experiments in forests have shown that photosynthetic C uptake at the leaf-level is about 40 – 60 % enhanced under elevated CO_2 depending on the magnitude of atmospheric CO_2 -enrichment and plant species (Nowak et al. 2004; Bader et al. 2010; Ellsworth et al. 2012; Norby & Zak 2011). This stimulation appears to be persistent in many species, although cases of partial or complete photosynthetic downregulation over time have also been reported (Ellsworth et al. 2012, Norby & Zak 2011). However, no relative growth stimulation of a magnitude equivalent to the photosynthetic stimulation has ever been observed in any of the studies and in systems that had reached steady state canopy density and root turnover, no sustained stimulation had been found unless mineral fertilizer was applied (e.g. Norby & Zak 2011; Bader et al. 2013; Sigurdsson et al. 2013), leaving the question of the fate of the extra C assimilated through photosynthesis. Given the time

required for C sink limitation to come into play, one might expect stronger CO₂ 'fertilization' effects early after an experimental step increase in the atmospheric CO₂ concentration compared to a long-term effect (Leuzinger et al. 2011). Here we explore the initial carbon relations of mature spruce trees to an abrupt 150 ppm rise in canopy level CO₂ concentration.

One plausible pathway for the extra C taken up by forests exposed to elevated atmospheric CO₂ concentrations is increased soil respiration. However, the stimulation of belowground C transfer under eCO₂ in a *Pinus taeda* stand has only resulted in a modest increase in soil respiration (Jackson et al. 2009). In general, young, expanding stands showed greater enhancement of soil CO₂ efflux (23 – 54 %) than more mature stands (12 – 16 %) under eCO₂ (King et al. 2004). Long-term trends under CO₂ enrichment have been inconclusive because rates of CO₂ efflux from forest soils have been shown to decline over time at the Oak Ridge National Laboratory FACE site (ORNL-FACE), increase over time at the Duke University FACE site (Duke-FACE), remained largely unaffected at the Swiss Canopy Crane FACE site (SCC-FACE) site under deciduous trees, and tended to decline under spruce (King et al. 2004, Jackson et al. 2009, Bader & Körner 2010, Mildner et al. 2015). R_{soil} may be indirectly influenced by elevated CO₂ through changes in temperature and soil moisture sensitivity, which may even result in reduced rates of R_{soil} under CO₂ enrichment (Dieleman et al. 2012).

Temporal changes in soil respiration are ultimately driven by net ecosystem productivity unless there is long-term C accumulation such as in peatland (Raich and Nadelhoffer 1989, Caprez et al. 2012). On a seasonal basis, R_{soil} is largely controlled by climatic drivers (temperature and soil moisture; Tang et al. 2005; Mildner et al. 2015), while C supply from canopy photosynthesis plays a secondary role on this temporal scale (Högberg et al. 2001; Kuzyakov & Cheng 2001). Diurnal variation in R_{soil} is typically low (Buchmann 2000; Bader & Körner 2010), and dominated by photosynthesis, with R_{soil} lagging behind by several hours to days (Tang et al. 2005; Wertin & Teskey 2008; Kuzyakov & Gavrichkova 2010; Epron et al. 2011). In closed-canopy forests, diurnal soil temperature and moisture show little variation on rainless days and thus have only minor effects on daily R_{soil} (Tang et al. 2005; Bader & Körner 2010).

Although woody tissue respiration can consume 11 – 33 % of the total C assimilated by coniferous trees (Ryan et al. 1995), this respiratory component of the forest C budget is rarely quantified. Generally, CO₂ efflux from stems (CE_{stem}) can be divided into a growth and a maintenance component, and a third component related to ion uptake (Amthor 1984). The effects of elevated CO₂ on CE_{stem} are unclear, as both increases but also reductions have been observed (Carey et al. 1996; Edwards et al. 2002; Gielen et al. 2003; Zha et al. 2005). CE_{stem} and sap flow were found to be either positively (Levy et al. 1999), or negatively correlated (Bowman et al. 2005; Teskey & McGuire 2007), reflecting the transport of dissolved CO₂ in or out of the stem compartment via the xylem stream (Levy et al. 1999; Teskey & McGuire 2002; Moore et al. 2008; Bloemen et al. 2013, Etzold et al. 2013). Therefore, any changes in sap flow under eCO₂ relative to ambient conditions might also alter the rate of CE_{stem} . A strong correlation between CE_{stem} and wood growth has been observed (Ryan et al. 1994). Seasonal variations in CE_{stem} are primarily driven by

temperature, but tree phenology, stem-internal CO₂ concentration, and the contribution of xylem-transported CO₂ also produce sizable effects (Teskey & McGuire 2002; 2007; Cernusak et al. 2006; Etzold et al. 2013). However on a diurnal time scale, temperature seems to have a stronger impact on CE_{stem} than any other factor (Damesin et al. 2002).

In the present study, mature (ca. 110-year-old and 37 m tall) *Picea abies* trees growing in a near-natural forest in northwest Switzerland were subjected to elevated CO₂ concentrations by means of free air CO₂ enrichment (FACE) technology. We aimed to characterise the main CO₂ fluxes (photosynthetic C uptake, R_{soil} and CE_{stem}) under elevated CO₂ compared to current ambient CO₂ levels on an intra-daily timescale. This was achieved by simultaneously measuring net photosynthetic rates in branchlets using a crane gondola, and respiratory CO₂ release from stems and the soil in the understorey of *P. abies* trees before and after the onset of free air CO₂ enrichment. From current knowledge, we expected a stimulation of shoot net photosynthesis, but also enhanced C recycling to offset likely sink limitation of growth in such a natural forest setting.

Material and methods

Study site and CO₂ enrichment system

This free air CO₂ enrichment (FACE) study was conducted in a ca. 110-120 year-old, near-natural mixed forest, located 12 km southwest of Basel, Switzerland (47°33' N, 7°36' E, 500 m a.s.l.). The species-rich stand is dominated by hardwood and conifer trees (*Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl., *Carpinus betulus* L., *Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.). In forest patch selected for the CO₂ enrichment experiment *Picea abies* is the predominant tree species. The mild temperate climate in this region is characterised by a mean growing season temperature of 14.7 °C (May – September) and around 1000 mm annual precipitation (Bader & Körner 2010). In 2009, five ca. 110-year-old *Picea abies* trees of around 37 m height were fitted with a modified web-FACE system (Pepin & Körner 2002, Mildner et al. 2014) using a construction crane. In brief, the computer-controlled release of pure CO₂ occurred through a fine web of perforated lightweight tubing fixed around tree branches. The target concentration of CO₂ was 550 ppm and an air sampling system comprising multiple sampling points per tree was used to monitor canopy CO₂ concentration using infrared gas analysis. CO₂-enrichment was confined to the tree crowns between 15 and 37 m above ground and suspended when temperature dropped below 4 °C, light intensity above the canopy was < 100 μmol m⁻² s⁻¹ or when wind speed exceeded 10 m s⁻¹. The CO₂ control and monitoring system scanned across four sectors within each tree crown to ensure adequate CO₂ distribution and to maintain the target CO₂ level as closely as possible even under changing wind conditions. The uppermost two meters of a crown formed the top sector followed by three 120° sectors equally subdividing the remainder of the crown. From 2009 to 2011 the canopy CO₂ concentration averaged 541 ppm, 532 ppm, and 541 ppm, respectively; and the median CO₂ concentrations ranged from 500 to 560 ppm (Mildner et al. 2014). Five spruce trees of similar age and height growing at sufficient distance from the CO₂-treated trees served as controls (for tree map see: http://static-content.springer.com/esm/art%3A10.1007%2Fs10533-015-0084-5/MediaObjects/10533_2015_84_MOESM4_ESM.pdf).

Stability of gas-exchange measurements after branchlet removal

Since four out of the five control trees were located beyond the reach of the canopy crane, we relied on tree climbers for sample collection from the crowns of these trees. To evaluate the suitability of this approach, we monitored the stability of photosynthetic gas-exchange measurements over time following branchlet removal from a set of spruce trees that were accessible with the crane gondola: a terminal south-facing branchlet from the outer spruce crown was enclosed in the leaf chamber of the photosynthesis system and gas-exchange measurements were started in logging mode using a 1-min measurement interval. Then, the enclosed branchlet was severed and the photosynthesis system including the detached sample was transferred to the field laboratory on site where logging continued over a period of 230 minutes under saturating light conditions of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by a cold light source (Schott, KL 1500, Mainz, Germany), 25°C leaf temperature and an air-to-leaf vapour pressure deficit of around 1.1 kPa. This procedure was replicated five times on sunny days using sun-exposed branchlets from five different spruce trees.

Photosynthetic enhancement ratio, A/C_i curves, diurnal measurements of photosynthesis, stem and soil CO_2 efflux

We performed three diurnal measurement campaigns on sunny, cloudless days before, during the first week, and one year after the start of canopy CO_2 enrichment on 30 July 2009 (measurement dates: 29 July 2009, 06 August 2009, 14 July 2010). We simultaneously measured net rates of photosynthesis, stem CO_2 efflux (CE_{stem}) and soil respiration (R_{soil}) at five times throughout the day from 3 am to 5 pm (except for CE_{stem} on 14 July 2010 when only four measurements were conducted). For each of the daily measurement times, gas-exchange was recorded on previous- and current-year needles in the north- and south-facing parts of the crown (except for 14 July 2010 where only south-facing shoots were measured); two CE_{stem} measurements were taken on the north- and south-facing side of stems and three R_{soil} records per tree were taken and averaged. Stomatal conductance data and water relations of these diurnal campaigns have already been published elsewhere (Leuzinger & Bader 2012).

Instantaneous rates of shoot gas-exchange were recorded using a portable photosynthesis system equipped with a conifer chamber (LI-6400 XT, chamber 6400-05, Licor, Lincoln, NE, USA). During the pre-treatment campaign, all samples were measured at 385 ppm leaf chamber CO_2 concentration. After the start of CO_2 enrichment, shoots of control trees were measured at 385 ppm and those of trees exposed to elevated CO_2 at 550 ppm leaf chamber CO_2 concentration. The photosynthetic enhancement ratio was assessed as the needle-intrinsic ratio of light-saturated photosynthesis under 550 ppm over 385 ppm chamber CO_2 concentration (A_{550}/A_{385}), i.e. all samples were measured at both 385 and 550 ppm chamber CO_2 concentration. A/C_i curves were performed on detached shoots at ambient relative humidity and the chamber temperature set to 25°C which closely matched the ambient air temperature at that time. Saturating light levels of $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ were provided by a halogen light source (Transtector F-800, AIP WILD AG Zürich, Switzerland), mounted above the conifer chamber. The A/C_i curve procedure started at 385 ppm chamber CO_2

concentration, before this was stepwise reduced to 300, 200, 100, and 50 ppm. Thereafter, chamber CO₂ was reset to 385 ppm to check whether (partial) Rubisco deactivation had occurred. If the original photosynthetic rate could not be restored the recording was terminated and started over with a new sample. From 385 ppm the chamber CO₂ concentration was increased to 600, 800, 1000, 1500 and 2000 ppm. The analyser cells of the gas-exchange system were matched after each change in chamber CO₂ concentration. Individual response curves were typically completed within 35 min. We used a Farquhar-type photosynthesis model to derive the maximum rate of carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) from the A/C_i recordings (Long & Bernacchi 2003).

We marked the section of the sample branchlets that was enclosed by the conifer chamber and collected the needles after the completion of the recordings. The projected needle area was determined using WinSEEDLE analysis software (Regent Instruments Inc., Quebec, Canada).

Instantaneous rates of CE_{stem} were measured using a portable soil respiration system (LI-6400 XT, soil CO₂ flux chamber 6400-09, Licor, Lincoln, NE, USA). The soil respiration chamber was horizontally oriented and strapped on PVC rings (10 cm diameter) glued onto the bark (loose bits removed) at 1.3 m height using silicone sealant. Since all trees had DBH values greater than 45 cm ($a\text{CO}_2$: 46 – 57 cm, $e\text{CO}_2$: 48 – 64 cm) the surface area enclosed by the rings was virtually flat making curvature corrections redundant. Every recording comprised three measurement cycles each consisting of a passive CO₂ increase in the chamber headspace followed by a CO₂ scrubbing back to ambient CO₂ concentrations of 385 ppm.

Instantaneous rates of soil respiration were recorded using two identical custom-made static chambers fitted with diffusion-aspirated nondispersive infrared gas analysers (IRGAs) and relative humidity/temperature sensors (GMP343 carbon dioxide probe, HMP75 rH/T probe, Vaisala, Vantaa, Finland). The chamber design and technical details are described in Bader & Körner (2010). During measurements, the chambers were placed on polypropylene rings (5 cm in height and 20 cm in diameter) inserted 2 cm into the forest soil. Individual recordings of soil R_{soil} lasted 5 min with a 5 s reading interval. Rates of R_{soil} were calculated from the slope of a linear regression applied to the initial CO₂ increase within the chamber. The first minute of the recordings was omitted from the regression analysis to avoid small flux disturbances involved with chamber placement. Under identical experimental conditions, Heinemeyer & McNamara (2011) showed that using short sampling times with closed static chambers yields flux estimates that are comparable to the fluxes measured with a LICOR 8100 closed dynamic chamber reference system (LICOR, Lincoln, NE, USA).

Statistical analysis

All statistical computations were performed using the statistical analysis and graphics software R version 3.1.0 (R development Core Team 2014). The stability of photosynthetic gas-exchange measurements of detached branchlets was modelled using an ordinary least squares model. A/C_i curves recorded under pre-treatment and FACE conditions were analysed using Farquhar-type equations (Long & Bernacchi, 2003) fitted by generalised nonlinear least squares (*gnls*). Model diagnostic plots suggested heteroscedasticity, which was modelled by incorporating exponential or power variance functions using C_i as variance covariate. In order to compare $a\text{CO}_2$ vs. $e\text{CO}_2$ we formulated parameter models allowing

separate parameter estimates for each CO₂ treatment. These parameter models were then compared to a base model (assuming common parameter estimates for both CO₂ treatments) using likelihood ratio tests.

The photosynthetic enhancement ratios (A_{550}/A_{385}) prior and during FACE were analysed using generalised least squares models (*gls*, R package *nlme*, Pinheiro et al. 2014). These models contained cardinal direction, needle age, CO₂ treatment and their interaction as fixed term. Model diagnostic plots suggested heteroscedasticity in the pre-treatment and the treatment data, which was modelled by incorporating exponential or power variance functions using the fitted values as variance covariate.

Generalised additive mixed models (GAMMs) were applied to the non-aggregated data to analyse the diurnal courses of needle gas-exchange, stem and soil respiration (Wood, 2011, R package *mgcv*). The GAMMs for photosynthetic gas-exchange contained 'CO₂ treatment', 'needle age' and 'date' (date of diurnal recording) as factors and a smoothing term for 'time of day' (thin plate regression spline). The GAMMs for the stem and soil respiration data contained 'CO₂ treatment' and 'date' as factors and smoothers for 'time of day' and 'temperature' (bark or soil temperature).

'Tree individual' nested in 'date' were incorporated as random effects in all models to account for the repeated measures. Serial autocorrelation was modelled using an autocorrelation structure of order 1 (corAR1, Pinheiro et al. 2014, R package *nlme*). To test whether the diurnal course followed a common pattern across CO₂ treatments (CO₂ × time of day interaction), a model with one common smoother was compared to a model allowing separate smoothers for $a\text{CO}_2$ and $e\text{CO}_2$. The same approach was used to test the date × time of day interaction. Model comparisons were based on the Akaike Information Criterion (AIC), which measures goodness of fit and model complexity and is more robust than likelihood ratio tests when comparing GAMMs (Zuur et al. 2009). The lower the AIC the better the model fit. The difference in AIC between two candidate models is called ΔAIC and models are considered different if the ΔAIC is greater than 10 (Burnham & Anderson 2002). Graphical model validation tools (residual plots for variance homogeneity and quantile-quantile plots for normality) were used to assess the underlying model assumptions. The residual plots indicated heteroscedasticity, which was modeled using a power variance structure (varPower, R package *nlme*).

Results

Stability of photosynthetic rates after branchlet removal

In situ measurements of net photosynthesis of one-year old branchlets showed an average net photosynthetic rate of $7 \pm 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ (mean $\pm$ SE, $n = 5$, Fig. 1). After branch detachment, photosynthesis declined at a rate of less than 2 % per hour. Nearly 4 hours after branchlet detachment the photosynthetic rate had only dropped by $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. Stomatal conductance showed only minor changes in the first 45 min after branchlet removal but had increased by 22 % at the end of the recording (230 min after detachment).

Photosynthetic enhancement, V_{cmax} and J_{max}

The photosynthetic enhancement ratio was remarkably similar across designated $a\text{CO}_2$ and $e\text{CO}_2$ trees, needle age and cardinal directions averaging 1.69 ± 0.02 (mean $\pm$ SE) prior to the onset of CO_2 enrichment and 1.76 ± 0.04 shortly afterwards (Fig. 2, Table 1).

Similarly, V_{cmax} and J_{max} showed no statistically significant differences between needle age and designated $a\text{CO}_2$ and $e\text{CO}_2$ trees, apart from the significantly higher V_{cmax} seen in current-year needles of control trees before the start of CO_2 enrichment (Fig. 3). Average V_{cmax} values were in the range of $23\text{--}36 \mu\text{mol m}^{-2} \text{s}^{-1}$ and J_{max} varied between 95 and $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3).

Diurnal C fluxes

Photosynthetic gas-exchange showed a typical daily pattern with a rapid rise in the early morning hours to maximum light-saturated values before noon ($a\text{CO}_2$: $6\text{--}9 \mu\text{mol m}^{-2} \text{s}^{-1}$, $e\text{CO}_2$: $9\text{--}12 \mu\text{mol m}^{-2} \text{s}^{-1}$), followed by a nearly linear decline over the course of the afternoon (Fig. 4 a – b). Rates of nighttime shoot respiration ranged from 1 to $3 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The diurnal courses of gas-exchange showed similar patterns across recording dates, ($\Delta\text{AIC} = 32$ in support of the model with one common smoothing term for all dates) but photosynthetic rates were somewhat higher during the two 2009 recordings compared to 2010 (Table 2). Previous-year and current-year needles shared a similar diurnal pattern ($\Delta\text{AIC} = 18.4$, supporting the model with one common smoothing term for needle age), which was overall slightly lower in current-year needles (Table 2). Prior to the start of the CO_2 experiment, daily peak net photosynthetic rates measured at $385 \text{ ppm } \text{CO}_2$ were on average already between 1.11 (current-year needles) and 1.44 (previous-year needles) times higher in trees selected for CO_2 enrichment compared to control trees (Fig. 4 a – b left panels). According to the photosynthetic responses of branchlets, the start of CO_2 enrichment must have resulted in a strong instantaneous enhancement in daily net photosynthetic rates (integrated over the day) of trees exposed to $e\text{CO}_2$ compared to trees growing under $a\text{CO}_2$. Taking the pre-treatment differences into account, this CO_2 -driven photosynthetic stimulation almost doubled C assimilation in previous-year and current-year needles. High CO_2 also resulted in a steeper increase to peak photosynthesis values warranting separate fits for $a\text{CO}_2$ and $e\text{CO}_2$ ($\Delta\text{AIC} = 30.8$ in favour of the model allowing separate smoothing terms for $a\text{CO}_2$ and $e\text{CO}_2$). However, one year after the start of CO_2 enrichment (summer 2010), the photosynthetic enhancement had declined in previous year needles (1.35 -fold higher under $e\text{CO}_2$ compared to $a\text{CO}_2$) and had virtually disappeared in current-year needles, which showed even 6% less photosynthetic stimulation relative to the pre-treatment difference (Fig. 4).

Generally, CE_{stem} and R_{soil} were lowest at night or in the early morning hours and increased by up to 35% throughout the day (Fig. 4 c and d). Night values of CE_{stem} varied on average between 2.8 and $3.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ and nighttime R_{soil} ranged from 2.4 to $3.7 \mu\text{mol m}^{-2} \text{s}^{-1}$. Interestingly, the daily rise in CE_{stem} was paralleled by an increase in bark temperature, while the increase in R_{soil} occurred without a corresponding rise in soil temperature. Consequently,

T_{bark} had a significant effect on stem C release ($L = 8.54$, $df = 2$, $P = 0.01$) but daily T_{soil} did not significantly affect soil respiration ($L = 2.16$, $df = 2$, $P = 0.34$).

The highest stem CO_2 release of $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ was measured in the late afternoon during the diurnal recording in 2010, when bark temperatures reached 29°C . However, overall the magnitude and the diurnal pattern of CE_{stem} were similar between recording dates ($\Delta\text{AIC} = 2$ in favour of the model with a common smoother for recording date, Table 3). Regardless of recording date, rates of CE_{stem} were always slightly lower under ambient compared to elevated CO_2 , but these differences did not warrant separate fits and the magnitude of the differences was not significant ($\Delta\text{AIC} = 3$ supporting the model with a common smoother for CO_2 treatment, Table 3).

The daily rise in R_{soil} showed similar shapes across recording dates ($\Delta\text{AIC} = 6$ supporting the model with a common smoother for recording date) but occurred at a significantly lower level in 2010 (Fig. 4, Table 4). The diurnal increase in R_{soil} also followed a similar pattern across CO_2 treatments ($\Delta\text{AIC} = 3.3$ in support of the model with a common smoother). However, similar to the diurnal gas-exchange data, there was a significant pre-treatment difference with 20 % higher rates of soil respiration under trees selected for CO_2 enrichment compared to soil under control trees (i.e. the same daily pattern but an upward shift of the curve under CO_2 -enriched trees). This pre-treatment difference remained constant after the start of the CO_2 enrichment in summer 2009 but had nearly disappeared one year later (on a day when the soil at 10 cm depth was 4°C warmer compared to the previous year; Fig. 4) suggesting a reduction in R_{soil} under $e\text{CO}_2$.

Discussion

Stability of photosynthetic rates after shoot removal

Logistic constraints frequently impede *in situ* measurements, especially when dealing with tall trees. Shooting branches or employing tree climbers to collect samples present alternative approaches but the question lingers whether gas-exchange measurements conducted on detached plant material are truly representative. The use of a construction crane allowed us to carefully investigate this issue by continuously logging shoot photosynthesis starting as *in situ* measurements in the tree crown initiated from the crane gondola and continuing these recordings for nearly 4 hours following the detachment of the branchlet. This removal experiment demonstrated that photosynthetic rates of Norway spruce branchlets remain remarkably constant for at least an hour after detachment (Fig. 1). By comparison, g_s values only remained reasonably constant for about 45 min and showed a 22 % increase over time (Fig. 1). Consequently, our findings suggest a 45 min window of opportunity for representative gas-exchange measurements on samples collected by tree climbers, which lies well within the timeframe required to perform an A/C_i curve recording.

Photosynthetic stimulation under elevated CO_2

Averaged across cardinal directions, needle ages and CO_2 treatment, the photosynthetic enhancement in mature *Picea abies* trees reached 73 % which is in good agreement with

the values reported for current-year needles of *Pinus taeda* (+67 %) growing under elevated CO₂ at the Duke FACE site (Ellsworth et al. 2012). However, in contrast to *Picea abies*, there were marked differences between needle ages in *Pinus taeda* with only 40 % photosynthetic enhancement seen in previous-year needles. For *Pinus radiata* cuttings that had been growing in open-top chambers for five years, Greenep et al. (2003) reported photosynthetic stimulation between 34 and 43 % in young needles and between 26 and 49 % in older needles, depending on the time of year. However, these figures were not given as a photosynthetic enhancement ratio (i.e. a_{CO_2} and e_{CO_2} plants measured at both the low and the high CO₂ concentration) but were derived from comparisons between maximal photosynthetic rates at the respective growth CO₂ concentration.

V_{cmax} and J_{max} measured in our study trees were in the range of values observed in *Pinus taeda* exposed to + 200 ppm atmospheric CO₂ concentration (Ellsworth et al. 2012). Greenep et al. (2003) reported similar V_{cmax} values for *Pinus radiata* cuttings after five years of growth under elevated CO₂ in open-top chambers, however, J_{max} was substantially lower reaching values between 50 and 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Diurnal C fluxes

Estimates of C fixation and respiration rates are key to productivity and C budget models and findings from other forest FACE studies and chamber-based CO₂ experiments commonly suggest an increase in both assimilatory C uptake and respiratory C release (e.g. Ellsworth et al. 2012, Jackson et al. 2009). Here, we aimed at simultaneously quantifying the major diurnal C fluxes in c. 110-year-old and 37 m tall *Picea abies* trees and surrounding soil under e_{CO_2} . Peak daily C assimilation rates in both previous-year and current-year needles were strongly enhanced in trees receiving e_{CO_2} relative to control trees, but one year after the start of CO₂ enrichment, the photosynthetic stimulation seemed smaller particularly in current-year needles (Fig. 3). However, shoot gas-exchange measurements conducted under controlled conditions (A_{550}/A_{400}) in 2014 did not provide evidence for photosynthetic downregulation (Klein et al. 2015, in review). The only other FACE study using a conifer species was conducted at the Duke forest in North Carolina, USA, where *Pinus taeda* had been subjected to FACE over a period of 10 years and on average the exposure to e_{CO_2} enhanced net photosynthesis by 67 % in current year needles and 37 % in previous year needles (Ellsworth et al. 2012) under ambient atmospheric CO₂ concentration + 200 ppm. We did not observe CO₂-related differences in dark respiration in our *Picea* trees but Griffin et al. (2004) reported an 1.7 – 2.7-fold increase in the number of mitochondria in needles of *Pinus radiata* trees grown under e_{CO_2} in open-top chambers. The largest increase in mitochondria was observed at the tip where it led to significantly increased area-based respiration rates. However, the mid-section and base of the needles showed reduced respiration, resulting in an overall 12 % decline in needle respiration under e_{CO_2} (Griffin et al. 2004).

At our FACE site, CE_{stem} remained unaffected by e_{CO_2} , which is consistent with the findings of Gielen et al. (2003) for three *Populus* species growing in a short-rotation biomass plantation at the POP-FACE facility in Italy. However, at the ORNL and Duke FACE sites, CO₂ enrichment resulted in 33 % and 6 % higher rates of CE_{stem} in *Liquidambar styraciflua* and *Pinus taeda*, respectively, during the early stages of these experiments (Edwards et al. 2002,

Hamilton et al. 2002). Diurnal recordings on 14-year-old *Picea abies* trees subjected to CO₂ enrichment in open-top chambers also showed moderate increases in CE_{stem} of up to 16 % over control trees (Acosta et al. 2010). CO₂-related changes in sap flow may confound comparative CE_{stem} readings due to the respiratory CO₂ dissolved and transported in the transpiration stream and the amount of this CO₂ that remains stored internally. CE_{stem} has been shown to correlate negatively with sap flux density implying that a CO₂-induced reduction in whole-tree transpiration would inevitably result in greater CO₂ efflux rates from stems (Bowman et al. 2005). However, such confounding can be ruled out because sap flow in *Picea abies* remained unaffected by $e\text{CO}_2$ at the SCC FACE site (Leuzinger & Bader 2012). The absence of a CO₂ effect on CE_{stem} in the longer term is corroborated by the lack of a CO₂-induced stimulation of stem radial growth, which was assessed with girth tapes at breast height (1.3 m), crown base (20-25 m) and in the upper canopy (30 m; Klein et al. 2015, in review). Atwell et al. (2003) reported initial stimulation of the basal area increment of young, clonal *Pinus radiata* trees under $e\text{CO}_2$ and high-N conditions in open-top chambers, but not under $e\text{CO}_2$ alone. However, this initial response ceased after 1.25 years, resulting in similar relative growth rates across all $e\text{CO}_2$ and N treatment combinations.

Given the initial increase in photosynthetic C uptake under $e\text{CO}_2$ and the close coupling between photosynthesis and R_{soil} (Tang et al. 2005) we expected a rapid stimulation of soil CO₂ efflux. Stable C isotope tracing revealed a small yet clear signal of the ¹³C signature of the fossil CO₂ used for canopy enrichment in soil air approximately 12 days after the start of this FACE study (Mildner et al. 2014). However, during the first week of CO₂ enrichment R_{soil} remained unaffected and in the 2010 assessment the pre-treatment difference had nearly vanished, suggesting a slight suppression rather than a stimulation of R_{soil} , which was supported by bi-weekly seasonal measurements revealing a small but significant reduction in R_{soil} under CO₂-enriched trees compared to the controls (Mildner et al. 2015). The lack of a CO₂ driven stimulation of R_{soil} is in contrast to other forest FACE experiments where R_{soil} commonly increased in response to $e\text{CO}_2$ under various soil and canopy conditions (King et al. 2004) but it is in line with findings for soil under deciduous trees at the same site (Bader & Körner 2010) and with the seasonal figures associated with our spruce trees (Mildner et al. 2015; Klein et al. 2015, in review). Though none of the stands in the study by King et al. (2005) had achieved canopy-closure when FACE commenced, their findings suggest that the magnitude of the CO₂-driven stimulation of R_{soil} strongly depends on stand age as greater responses were reported for very young compared to more advanced stands. This may be largely related to the contribution of root respiration to the overall R_{soil} signal. The SCC site is a mature, closed-canopy stand with trees aged well over 100 years suggesting fully expanded root systems ('root closure'), that one would expect to respond less vigorously to CO₂-enrichment (Mildner et al. 2015) than expanding root systems in expanding stands as was initially the case at the Duke- and ORNL-FACE sites. Moreover, soil nutrient availability is likely to play a role. At the Duke Forest FACE site, fertiliser addition resulted in reductions in R_{soil} under both $a\text{CO}_2$ and $e\text{CO}_2$, suggesting that forests on high-fertility sites, such as ours (20 – 25 kg N ha⁻¹ a⁻¹ wet nitrogen deposition, Thimonier et al. 2010), respire less CO₂ back to atmosphere (Butnor et al. 2003).

A noticeable feature of the diurnal courses was the continuing rise in CE_{stem} stem and R_{soil} towards the evening, when shoot photosynthesis was already approaching evening light compensation. We speculate that this may reflect a time lag in assimilate allocation to stem tissue and fine roots, however, published findings about the effect of carbohydrates on

respiratory processes are mixed and thus there is no unanimous conclusion on this topic (Covey-Crump et al. 2002; Atkin et al. 2000).

For logistic reasons, step-changes in CO₂ concentrations are common in FACE experiments, however, in reality atmospheric CO₂ increases gradually over decades to centuries. A recent modelling study has looked at the difference in ecosystem response those two scenarios may entail, by simulating the Duke and ORNL FACE experiments over a 300 year time period, with no consistent pattern detected (Walker et al. 2015). In the long run (>10 years), initial responses seen in step-change CO₂ experiments are most likely mitigated due to compensatory effects (Leuzinger et al. 2011, Norby & Zak 2011), although in the case of photosynthesis, this could not be confirmed in the FACE experiment on deciduous trees at the same site as the present study took place (Bader et al. 2010).

Our data indicate a strong CO₂-driven stimulation of shoot photosynthesis under controlled leaf chamber conditions across cardinal directions in these tallest and oldest trees ever exposed to eCO₂. There appeared no overall or daytime-dependent stimulation of CO₂ release from stems or soil under FACE conditions suggesting that the extra C assimilated during the initial study phase was not rapidly returned to the atmosphere via respiratory processes. This confirms earlier results from experiments at this and other sites that the carbon balance cannot easily be closed in mature forests that are exposed to elevated CO₂ (Fatichi & Leuzinger 2013). The most likely fate for the additional C are multiple sinks along its pathway that may be too small to track individually, given the limited statistical power of such large-scale experiments (Mildner et al. 2015).

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Figure captions

Figure 1 (a) Net photosynthetic rate and **(b)** stomatal conductance (lower panel) of one-year old (2008) south-facing branchlets of *Picea abies* as a function of time after detachment. The dashed lines indicate the mean values before branchlet removal. The solid lines show the mean of five branchlets derived from different adult spruce trees ± 1 standard error (grey area). Measurements were conducted in logging mode over a period of 230 minutes using a 1-minute interval under saturating light intensity of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, 25°C leaf temperature, and an ALVPD (air-to-leaf vapour pressure deficit) of ca. 1.1 kPa. For the sake of clarity the regression line for the linear decline in the photosynthetic rate was omitted and only the linear regression equation is shown.

Figure 2 Instantaneous enhancement of light-saturated photosynthesis (PER) measured across cardinal directions in upper-crown branchlets of mature *Picea abies* trees. Measurements were conducted on previous-year and current-year shoot sections one week before the onset of canopy CO_2 -enrichment (upper panel) and one week afterwards (lower panel) at the SCC FACE site. Means $\pm$ SE, $n = 5$.

Figure 3 Maximum rate of Rubisco carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) in south-facing upper-crown branchlets of mature *Picea abies* trees. Measurements were conducted on previous-year and current-year shoot sections one week before the onset of canopy CO_2 -enrichment (upper panel) and one week afterwards (lower panel) at the SCC FACE site. Means $\pm$ SE, $n = 5$. *** $P < 0.001$ (likelihood ratio test, see material and methods).

Figure 4 (a – b) Diurnal courses of net photosynthesis (A_{net}) in previous- and current-year needles, **(c)** stem CO_2 efflux (CE_{stem}) and bark temperature (T_{bark}), and **(d)** soil respiration (R_{soil}) along with soil temperature at 10 cm depth (T_{soil}). The measurement campaigns were performed at the Swiss Canopy Crane FACE site on or under CO_2 -enriched (closed symbols, solid lines) and control trees (open symbols, dashed lines). Means $\pm$ SE, $n = 5$. Black and white bars on top indicate night- and daytime hours. Lines represent fits from generalised additive mixed models applied to the non-aggregated data.

Figures

Fig. 1

Fig. 2

Fig. 3

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Table headers

Table 1 Results from a generalised least squares model for diurnal gas-exchange of *Picea abies* needles applying individual smoothers for $a\text{CO}_2$ and $e\text{CO}_2$. CO_2 treatment = $a\text{CO}_2$ vs. $e\text{CO}_2$, needle age = previous-year vs. current-year needles, FACE 2009 = 6 Aug 2009 one week after the start of CO_2 enrichment, FACE 2010 = 14 Jul 2010, one year after the start of CO_2 enrichment (the pre-treatment recording is included as baseline in the intercept term). *edf* = estimated degrees of freedom, *s* = smoother term.

Table 2 Results from a generalised additive mixed model for diurnal gas-exchange of *Picea abies* needles applying individual smoothers for $a\text{CO}_2$ and $e\text{CO}_2$. CO_2 treatment = $a\text{CO}_2$ vs. $e\text{CO}_2$, needle age = previous-year vs. current-year needles, FACE 2009 = 6 Aug 2009 one week after the start of CO_2 enrichment, FACE 2010 = 14 Jul 2010, one year after the start of CO_2 enrichment (the pre-treatment recording is included as baseline in the intercept term). *edf* = estimated degrees of freedom, *s* = smoother term.

Table 3 Results from a generalised additive mixed model for diurnal C release from *Picea abies* stems. CO_2 treatment = $a\text{CO}_2$ vs. $e\text{CO}_2$, Diurnal FACE 2009 = recording of 6 Aug 2009 one week after the start of CO_2 enrichment, Diurnal FACE 2010 = recording of 14 Jul 2010, one year after the start of CO_2 enrichment (the pre-treatment recording is included as baseline in the intercept term). *edf* = estimated degrees of freedom, *s* = smoother term.

Table 4 Results from a generalised additive mixed model for diurnal soil respiration under *Picea abies* trees. CO_2 treatment = $a\text{CO}_2$ vs. $e\text{CO}_2$, Diurnal FACE 2009 = recording of 6 Aug 2009 one week after the start of CO_2 enrichment, Diurnal FACE 2010 = recording of 14 Jul 2010, one year after the start of CO_2 enrichment (the pre-treatment recording is included as baseline in the intercept term). *edf* = estimated degrees of freedom, *s* = smoother term.

Table 1

<i>Parameter</i>	<i>DF_{num}</i>	<i>DF_{den}</i>	<i>F</i>	<i>P</i>
<i>Pre-treatment</i>				
Intercept	1	64	11429.77	<0.001 ***
Cardinal direction	3	64	0.82	0.486
Needle age	1	64	0.05	0.825
CO ₂	1	64	0.47	0.494
Cardinal direction × needle age	3	64	0.31	0.821
Cardinal direction × CO ₂	3	64	0.421	0.739
Needle age × CO ₂	1	64	1.71	0.196
Cardinal direction × needle age × CO ₂	3	64	0.80	0.499
<i>FACE</i>				
Intercept	1	61	8886.20	<0.001 ***
Cardinal direction	3	61	2.47	0.070
Needle age	1	61	0.18	0.673
CO ₂	1	61	0.49	0.487
Cardinal direction × needle age	3	61	0.81	0.494
Cardinal direction × CO ₂	3	61	1.69	0.178
Needle age × CO ₂	1	61	0.01	0.943
Cardinal direction × needle age × CO ₂	3	61	0.85	0.473

Table 2

<i>Parametric coefficients</i>	Estimate (SE)	<i>t</i>	<i>P</i>
Intercept	3.25 (0.23)	13.87	<0.001 ***
CO ₂ treatment	2.07 (0.33)	6.35	<0.001 ***
Needle age	-0.36 (0.10)	-3.59	<0.001 ***
Diurnal FACE 2009	-0.02 (0.20)	-0.12	0.91
Diurnal FACE 2010	-0.63 (0.20)	-3.18	<0.01 **

Approx. significance of smooth terms

	<i>edf</i>	<i>F</i>	<i>P</i>
s(time of day) : CO ₂	2.93	153.9	<0.001 ***

Table 3

<i>Parametric coefficients</i>	Estimate (SE)	<i>t</i>	<i>P</i>
Intercept	3.10 (0.35)	8.91	<0.001 ***
CO ₂ treatment	0.45 (0.35)	1.29	0.20
Diurnal FACE 2009	0.55 (0.44)	1.29	0.20
Diurnal FACE 2010	0.55 (0.43)	1.29	0.20

Approx. significance of smooth terms

	<i>edf</i>	<i>F</i>	<i>P</i>
s(time of day)	1.00	32.65	<0.001 ***
s(bark temperature)	2.73	6.58	<0.001 ***

Table 4

<i>Parametric coefficients</i>	Estimate (SE)	<i>t</i>	<i>P</i>
Intercept	3.58 (0.14)	26.00	<0.001 ***
CO ₂ treatment	0.58 (0.14)	4.21	<0.001 ***
Diurnal FACE 2009	-0.14 (0.17)	-0.83	0.41
Diurnal FACE 2010	-0.79 (0.17)	-4.71	<0.001 ***

<i>Approx. significance of smooth terms</i>			
	<i>edf</i>	<i>F</i>	<i>P</i>
s(time of day)	1.00	41.85	<0.001 ***