Response of mature Norway spruce (*Picea abies*) to elevated atmospheric CO_2

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

PHILOSOPHISCH-NATURWISSENSCHAFTLICHEN FAKULTÄT

der Universität Basel

von

MANUEL MILDNER
aus Freiburg i. Br., Deutschland

Freiburg im Breisgau, 2016

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

Prof. Dr. Christian Körner
Prof. Dr. Ansgar Kahmen

Basel, den 24. Juni 2014

Prof. Dr. Jörg Schibler
Dekan

Acknowledgements

First and foremost I would like to thank Christian Körner for offering this exceptional opportunity to do a PhD in his department, and for his patience during this long journey of my PhD thesis. He has never resigned to support and to guide my work. As a newcomer in the field of Ecophysiology I benefitted from his outstanding skills, especially how to execute 'real' science.

I am particularly indebted to Rolf Siegwolf who co-supervised my PhD and familiarized me with the field of stable isotopes. It always was a relief having had such an authority in isotope research by my side. His talent for explaining abstract contexts is superior; I would never have understood the power of stable isotopes in ecophysiological research. He always had an open ear for any sort of problems and I always enjoyed my trips to his lab. In this context, I also need thank the group at the Laboratory of Atmospheric Chemistry, notably Matthias Saurer for his valuable comments on all isotope aspects and Katharina Lötscher for working through the countless samples I send for analysis. Without their help this thesis would never have existed.

Many thanks go to Ansgar Kahmen who agreed to co-examine my thesis despite being incredibly busy.

I owe following two persons all my experience to survive Hofstetten, statistics, conferences (ESA), and kids: Martin Bader and Sebastian Leuzinger. Thank you for your endurence in teaching a tenderfoot everything important.

I am very much obliged to following people:
Erwin Amstutz for demonstrating that tough-guys do have a heart of gold; and for allowing me to be part of his 'Hofstetten' microcosm;
Georges Grun for always controlling the web-FACE in the background;
Martin Trischler for not just teaming up in Hofstetten, but also for being a 'Jack of all trades';
Christina Baumann and Sonja Jakob, with whom I experienced the 'Hofstetten unpredictability' for the first time;
Tamir Klein for absorbing the losses of my absence in Hofstetten;
Urs Weber for also being addicted to Scotland and for his CK translations;
Erika Hiltbrunner not just for having been the very first person I spoke to at the Institute, but mainly for her expertise in every single domain of science;
Günter Hoch for his whit;
Elena Puri, Juliana Neumann, Remo Wild for fine root cleaning and separation;
Rigobert Keller, Markus Wilhelm and the Mycological Association Basel for identification of frozen fungal sporocarps.

There were so many people at our Institute not directly involved in this project but who kept me smiling most of the time: Chris Kollas, Riccarda Caprez, Nicole Inauen, Thijs van den Bergh, Armando Lenz, Yann Vitasse, Christophe Randin, Franziska Grob, and Susanna Riedl. Thanks to you all.

However, you all come in second. There is nothing more rewarding than having a caring family in the background. Even though not the best point in time, but becoming a father during my PhD changed my live and feelings dramatically. For this, all my love goes to you Charlotte and to my daughter Liese. Finally, I want to thank my parents and my brothers, they all know of the arduousness I was faced during this journey.

Funding was provided by the Swiss National Science Foundation (Grants 31003AB-126028, 31003A_140753, 31-67775.02, 3100-059769.99, 3100-067775.02, 3100AO-111914/1). The crane was sponsored by the Swiss Federal Office of the Environment (FOEN).

Table of Contents

Chapter 1

General introduction

General introduction

1.1. Global change with emphasis on carbon dioxide

Global change includes a multitude of anthropogenic alterations of the earth's atmosphere, biosphere, hydrosphere, and pedosphere, all potentially threatening life on earth (Körner 2003). The sheer quantity of human beings on earth, and their activities in an industrialized world affect our planet by e.g. land use change (agriculture, deforestation, urban development, resource exploitation), and alterations of the atmosphere's chemical composition through combustion of fossil fuels, gas release by industries (namely cement production), and vegetation burning (Le Quéré et al. 2013; IPCC 2013). The effects of human activities on the earth's climate system relate to the emission of greenhouse gases that lead to global warming, ozone depletion, and the advent of weather peculiarities. The unprecedentedly rapid rise of the greenhouse gas carbon dioxide (CO_2) in the atmosphere since the industrial revolution 150 years ago is clearly shifting the diet of the planet, given its crucial role for plant photosynthesis. Since the beginning of the Industrial Era (1750), the earth is faced with an ongoing increase of the atmospheric CO_2 concentrations from ca. 280 ppm to 396 ppm in March 2013 (Dlugokencky & Tans 2014), a rise that has not been witnessed for the last ca. 800.000 years (Petit et al. 1999; Siegenthaler et al. 2005). Climate model predictions forecast CO_2 concentrations of at most 936 ppm by the end of this century (RCP8.5; IPCC, 2013). Since these high CO_2 levels will exert direct influences on ecosystems, intense efforts have been undertaken to find solutions for this global threat. The photosynthetic assimilation of carbon (C) by autotrophic organisms, taken from CO_2 in the atmosphere, might potentially make a contribution to reduce atmospheric CO_2 levels (Houghton et al. 1999), should this lead to greater terrestrial C stores (for which we have no proof yet).

1.2. CO_2 effects on plants

In the context of global change, anthropogenic CO_2 emissions play a dominant role for plants, and trees particularly because of their long live span. Global warming may affect trees indirectly (via the greenhouse effect), but also directly because of the importance of C as tree food. Trees take up C by photosynthesis (PS), with subsequent conversion into organic matter. Importantly, PS of C_3 plants is not saturated up to CO_2 levels of ca. 1000 ppm (Körner 2006), as confirmed by most CO_2 enrichment experiments to date (Ainsworth & Long 2005; Ainsworth and Rogers 2007; Leakey et al. 2009; Bader et al. 2010). Thus, this strong C capacity of the photosynthetic machinery underlines the potential importance of trees to partly mitigate the drastic rise in atmospheric CO_2 (Schimel 1995; Sitch et al. 2013), provided such assimilates are fed into recalcitrant C pools. The mitigation effect is however, controversially discussed (Falkowski et al. 2000) because increased C uptake by PS stimulation does not necessarily translate into long-term C fixation in tree biomass or soil organic matter (Hagedorn et al. 2003; Norby & Zak 2011; Bader et al. 2013) since other factors co-determine tree growth at elevated CO_2 concentrations (Körner 2000, 2003, 2006). Of these, the most important factors are space and nutrient (e.g. nitrogen and phosphorous) limitations, and the tree's developmental stage dictating the likely effects of high CO_2 levels on plant performance (Leuzinger & Hättenschwiler 2013). Only recently, long-term free air CO_2 enrichment (FACE) experiments and model predictions evidenced resource-limitation constraints (mainly nitrogen), developing over time that limit the stimulatory effect of CO_2 on biomass production (Luo et al. 2004; McCarthy et al. 2010; Norby et al. 2010; Zaehle et al. 2014), or even zero it (Körner et al. 2005; Bader et al. 2013; Sigurdsson et al. 2013). Additionally, three important factors (i.e. age, space constraints, and species identity) were found to affect the magnitude of a CO_2 response (Körner 2006). Whereas young trees prospered vigorously at elevated CO_2, during their exponential phase of growth with ample (soil) space available, mature trees in a steady state of growth (i.e. constant fine root biomass and leaf area index; Körner 2006) are assumed to be marginally affected by elevated CO_2 (Leuzinger et al. 2011; Bader et al. 2013).

C allocation might shift from aboveground to belowground pools in order to cover the enhanced nutrient demand at elevated CO_2. Such a C allocation shift could be observed at the Oak Ridge FACE study on *Liquidambar styraciflua*, showing increased fine root biomass (Norby et al. 2004), a signal that had disappeared with time in this FACE experiment (Norby et al. 2010). C allocation to soils had been observed in some (Jastrow et al. 2005, Iversen et al. 2008, 2012), but clearly not in other experiments that rather indicates a CO_2 priming effect on soil C, and a loss in soil organic matter (Kuzyakov et al. 2000; Phillips et al 2012; Hungate et al. 2013). A stimulation may persist for several years until soil nutrient availability (mainly N) declines, thus weakening belowground root growth (Norby et al. 2010). Moreover, limited soil space is likely to decrease belowground growth stimulation in mature trees growing in natural environments (Dawes et al. 2013).

Even if more C enters the plant system under elevated CO_2, resulting in stimulation of growth, it remains very uncertain whether this will effect C sequestration because of counteracting CO_2 release to the atmosphere (e.g. respiration by plants, or by heterotrophic organisms). This facet of the C balance is complex and elusive, since the major CO_2 return in terrestrial ecosystems is emitted from soils (Raich & Potter 1995), and soils contain the largest terrestrial C pool (Dixon et al. 1994). Thus, accelerated soil C cycling under elevated CO_2 might preclude increased growth, leaving the global C budget largely

unaffected (Lukac et al. 2009; Dawes et al. 2013). The stimulatory CO_2 effect on soil CO_2 release diminished with tree age (King et al. 2004), or could not be observed at all (Bader & Körner 2010). Soils are difficult to examine, and soil respiration is independently stimulated by rising global temperature (Heath et al. 1996). Therefore, CO_2 enrichment in connection with temperature increases (not examined in the scope of this study) are both likely to dampen the C sequestration potential of forest ecosystems (Leuzinger et al. 2011; Dieleman et al. 2012).

1.3. C allocation in plants

Explaining the responses of plants to higher levels of atmospheric CO_2 requires basic knowledge about their internal C translocation. In long-lived plants such as trees, this involves large intermediate C pools, long residence times, and long transfer distances. C mixing (i.e. the dilution of old C reserves inside the tree with new assimilates) in deciduous trees was found to be strongly species-, tissue-, and season-specific (Keel et al. 2006, 2007). While foliage mainly depends on new assimilates for tissue formation and metabolic activity, the fraction of new C incorporated in new tissue in stems and roots is assumed to decrease because of increasing dilution of recent assimilates into older storage pools, and it is this mixture of C reserves that becomes invested in structural growth. In contrast to deciduous trees, evergreen conifers perform year-round photosynthetic activity, and maintain their foliage several years. Seasonal patterns in C utilization of either new or old C should, thus, fluctuate less in evergreen conifers compared to deciduous trees because of almost year-round supply of assimilates. Seasonal C allocation patterns are basically controlled by the physiological activity of a certain tree tissue. One may expect that assimilates fixed during high metabolic or growth activity are directly fed into these sinks, whereas assimilates produced during periods of low growth activity are channeled to reserve pools for later structural demands.

1.4. C isotopes as tool to study C allocation

Understanding C allocation in living trees in situ requires nondestructive methods that are easy to apply. Early studies largely used seedlings and juvenile trees for reasons of practicability. Mature trees in their natural environment have been investigated only more recently (Pepin & Körner 2002). As trees grow, technical challenges also increase. Further, tree-internal transfer processes are concealed within the tree body, a black box, difficult to look into. The only applicable approach to track the fate of newly assimilated C is the use of physico-chemical markers such as stable C isotopes. Few studies attempted to resolve the difficulty of studying C allocation in mature trees by using a tracer technique (Epron et al. 2011; Kuptz et al. 2011). In this context, FACE studies are unique because they produce C isotope labeling as a by-product, the tracer application is not confined to single tree units (e.g. branches), but to the entire canopy, and it is continuous rather than pulsed. A permanent and long-term $\delta^{13}C$ label applied in FACE experiments (Andrews et al. 1999; Körner et al. 2005), as opposed to pulse labeling, allows tracking seasonal changes in C translocation. In order to apply the isotope technique for the study of C translocation in FACE studies, C allocation patterns in trees must not be altered significantly by CO_2 enrichment because there is no 'control' (i.e. trees that are not CO_2-enriched, but receive the same C isotope label). Recently, whole-canopy labeling with extended ^{13}C pulses could successfully be deployed with only slight increases in the CO_2 concentration in the canopy (Epron et al. 2011; Kuptz et al. 2011). The drawback of such studies is that a stronger label must be applied ($\delta^{13}C$ of ca. -50‰ in Kuptz et al. 2011, or pure ^{13}C in Epron et al. 2011) which is considerably more expensive. Therefore, FACE experiments in conjunction with isotope labeling provide the best possible means to assess C allocation in mature trees.

1.5. The Swiss Canopy Crane (SCC) web-FACE experiment

A FACE experiment was set up in a near-natural forest in NW Switzerland (near Basel) in 2009 to assess (1) C allocation patterns, and (2) the CO_2 responses of mature *P. abies* trees (i.e. 110-year-old and ca. 37m tall). *P. abies* is both ecologically and economically one of the most important tree species in Northern and Central Europe (Brändli 2010). It is unknown whether and how these trees will respond to a CO_2-rich atmosphere, and whether we can expect a so-called CO_2-fertilization effect (Lindner et al. 2010; Reyer et al. 2014). Here, we subjected the canopies (15-37 m above ground) of five mature *P. abies* trees to elevated CO_2 concentrations that are likely to become a reality within the next 60 years (500-560 ppm). FACE started in late July 2009, thus, after current year radial stem increment, but allowing to mature tissues, and to build stores for the following year under elevated CO_2. The so-called web-FACE facility had already been applied successfully for over 9 years to expose mature deciduous tree species to elevated CO_2 concentrations (for an extended description and synthesis of the web-FACE study on deciduous trees see Pepin & Körner 2002; Bader et al. 2013). After the conclusion of the former web-FACE experiment, the system was revised to match the tree architecture of *Picea abies*.

C allocation patterns were studied using the C isotope labeling approach that had already been successfully applied in the previous web-FACE study (Steinmann et al. 2004; Keel et al. 2006; Bader et al. 2013). The tank CO_2 employed originated from burning of fossil fuel with a carbon isotope signature that is distinctively more negative than that of the atmosphere ($\delta^{13}C$ of -30‰ versus ca. -8‰) and is, thus, isotopically labeled. Therefore, the $\delta^{13}C$ of the CO_2 in the canopies' atmosphere (and of the tree assimilates produced of the labeled CO_2) differed from that of control trees. The unavoidable labeling through FACE permitted tracing of tree-internal C allocation processes in space and time, provided that C allocation patterns were not significantly changed by high levels of CO_2. An

assessment of potential shifts in the translocation preferences under elevated CO_2 would have required equally labeled trees under ambient CO_2. The special setting of the FACE system ensured the input of extra CO_2 exclusively into the canopies of the trees and, therefore, eliminated the potential for contamination of below-canopy tissues and soils by downward draft of labeled CO_2.

The effectiveness of CO_2 enrichment was monitored by IRGA measurements, and by the use of C_4 grasses (isometers) seasonally (May-September) installed within the canopies of CO_2-treated and control trees. The C needed for tissue production of the C_4 grasses is derived entirely from the surrounding CO_2, without any utilization of stored carbohydrates. Consequently, the C isotope label imprinted on grass tissues grown in an elevated CO_2 atmosphere can be used to calculate the seasonally integrated canopy CO_2 concentrations when compared to isometers grown at ambient CO_2 concentrations. Further, the difference between isometers at ambient and elevated CO_2 reflects the 100% C isotope signal that can be reached by a tree compartment when the C utilized was drawn exclusively from atmospheric CO_2.

These features (i.e. FACE, C_4 grasses, and isotope label) provided the means to unravel the temporal appearance and contribution of new assimilates in specific tree compartments. Together with the former web-FACE study on deciduous trees at the same site (Pepin & Körner 2002), this is the first time that such tall trees, in this case *P. abies*, were exposed to elevated CO_2 in a natural Central European forest ecosystem.

1.6. This doctoral thesis

My PhD thesis is divided into four parts, a general introduction, three chapters on original science, and an overall summary.

Chapter 2 presents the C isotope labeling results of the first 2.5 years after the onset of FACE, irrespective of the CO_2 treatment (published 2014 in Oecologia). This chapter provides an extended picture of the contributions of new C to tissue production and maintenance in needles, branchlets, stem, fine roots, and fungal partners, and fluxes (stem and soil CO_2 efflux) in mature *P. abies* trees over a longer time frame (i.e. 2.5 years). Further, seasonal preference shifts (i.e. preferential utilization of new or old C) could successfully be traced in the test trees.

Chapter 3 reports respiratory (i.e. soil and stem CO_2 efflux) and fine root responses to increased levels of atmospheric CO_2 (published 2015 in Biogeochemistry). The results compile the first 2.5 years of FACE, with special emphasis on potential tree-specific differences that were already present prior to the onset of FACE. We asked whether elevated CO_2 induced C to be preferentially allocated towards belowground pools, and whether this C excess in the soil also stimulated soil CO_2 release.

Chapter 4 examines changes in diurnal variations of C fluxes in these trees caused by elevated CO_2 (co-authorship; published 2016 in Environmental and Experimental Botany). We followed the diurnal cycle of respiratory fluxes (stem and soil), and net photosynthetic rate during three late-summer days (pre-treatment 2009, FACE 2009, and FACE 2010).

Chapter 5 is the general summary that concludes the thesis.

References

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist 165:351-371

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising CO_2: Mechanisms and environmental interactions. Plant Cell and Environment 30:258-270

Andrews JA, Harrison KG, Matamala R, Schlesinger WH (1999) Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). Soil Science Society of America Journal 63:1429-1435

Bader MKF, Körner C (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Global Change Biology 16:2830-2843

Bader MKF, Siegwolf RTW, Körner C (2010) Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. Planta 232:1115-1125

Bader MKF, Leuzinger S, Keel SG, Siegwolf RTW, Hagedorn F, Schleppi P, Körner C (2013) Central European hardwood trees in a high-CO_2 future: synthesis of an 8-year forest canopy CO_2 enrichment project. Journal of Ecology. doi: 10.1111/1365-2745.12149

Brändli U-B (2010) Schweizerisches Landesforstinventar. Ergebnisse der dritten Erhebung 2004–2006. Birmensdorf, Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft WSL, Bern, Bundesamt für Umwelt, BAFU:0-312

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

Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes JS, King J, Leuzinger S, Linder S, Luo YQ, Oren R, De Angelis P, Tingey D, Hoosbeek MR, Janssens IA (2012) Simple additive effects are rare: A quantitative review of plant biomass and soil process

responses to combined manipulations of CO_2 and temperature. Global Change Biology 18:2681-2693

Dlugokencky E, Tans P. Trends in atmospheric carbon dioxide. National Oceanic & Atmospheric Administration, Earth System Research Laboratory (NOAA/ESRL). http://www.esrl.noaa.gov/gmd/ccgg/trends. last access: 15 May 2014.

Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J (1994) Carbon pools and flux of global forest ecosystems. Science 263:185-190

Epron D, Bahn M, Derrien D, Lattanzi FA, Pumpanen J, Gessler A, Högberg P, Maillard P, Dannoura M, Gerant D, Buchmann N (2012) Pulse-labelling trees to study carbon allocation dynamics: A review of methods, current knowledge and future prospects. Tree Physiology 32:776-798

Falkowski P, Scholes RJ, Boyle E, Canadell J, Canfield D, Elser J, Gruber N, Hibbard K, Högberg P, Linder S, Mackenzie FT, Moore B, Pedersen T, Rosenthal Y, Seitzinger S, Smetacek V, Steffen W (2000) The global carbon cycle: A test of our knowledge of earth as a system. Science 290:291-296

Hagedorn F, Spinnler D, Bundt M, Blaser P, Siegwolf R (2003) The input and fate of new C in two forest soils under elevated CO_2. Global Change Biology 9:862-872

Hanewinkel M, Cullmann DA, Schelhaas M-J, Nabuurs G-J, Zimmermann NE (2013) Climate change may cause severe loss in the economic value of European forest land. Nature Climate Change 3:203-207

Houghton RA, Hackler JL, Lawrence KT (1999) The U.S. carbon budget: contributions from land-use change. Science 285:574-578

IPCC (2013) Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Iversen CM, Ledford J, Norby RJ (2008) CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. New Phytologist 179:837-847

Iversen CM, Keller JK, Garten CT, Norby RJ (2012) Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of CO_2-enrichment. Global Change Biology 18:1684-1697

Jastrow JD, Miller RM, Matamala R, Norby RJ, Boutton TW, Rice CW, Owensby CE (2005) Elevated atmospheric carbon dioxide increases soil carbon. Global Change Biology 11:2057-2064

Keel SG, Siegwolf RTW, Körner C (2006) Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. New Phytologist 172:319-329

Keel SG, Siegwolf RTW, Jäggi M, Körner C (2007) Rapid mixing between old and new C pools in the canopy of mature forest trees. Plant Cell and Environment 30:963-972

King JS, Hanson PJ, Bernhardt E, De Angelis P, Norby RJ, Pregitzer KS (2004) A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments. Global Change Biology 10:1027-1042

Körner C (2000) Biosphere responses to CO_2 enrichment. Ecological Applications 10:1590-1619.

Körner C (2003) Carbon limitation in trees. Journal of Ecology 91:4-17

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

Körner C (2006) Plant CO2 responses: An issue of definition, time and resource supply. New Phytologist 172:393-411

Kuptz D, Fleischmann F, Matyssek R, Grams TEE (2011a) Seasonal patterns of carbon allocation to respiratory pools in 60-yr-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling. New Phytologist 191:160-172

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantifications of priming effects. Soil Biology & Biochemistry 32:1485-1498.

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. Journal of Experimental Botany 60:2859-2876

Le Quéré, C, Peters GP, Andres RJ, Andrew RM, Boden T, Ciais P, Friedlingstein P, Houghton RA, Marland G, Moriarty R, Sitch S, Tans P, Arneth A, Arvanitis A, Bakker DCE, Bopp L, Canadell JG, Chini LP, Doney SC, Harper A, Harris I, House JI, Jain AK, Jones SD,

Kato E, Keeling RF, Klein Goldewijk K, Körtzinger A, Koven C, Lefèvre N, Omar A, Ono T, Park G-H, Pfeil B, Poulter B, Raupach MR, Regnier P, Rödenbeck C, Saito S, Schwinger J, Segschneider J, Stocker BD, Tilbrook B, van Heuven S, Viovy N, Wanninkhof R, Wiltshire A, Zaehle S, Yue C (2013) Global Carbon Budget 2013. Earth System Science Data Discussions 6:689-760

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

Leuzinger S, Bader MKF (2012) Experimental vs. Modeled water use in mature norway spruce (*Picea abies*) exposed to elevated CO_2. Frontiers in Plant Science 3:229-229

Leuzinger S, Hättenschwiler S (2013) Beyond global change: Lessons from 25 years of CO_2 research. Oecologia 171:639-651

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolstro M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecology and Management 259:698-709

Lukac M, Lagomarsino A, Moscatelli MC, De Angelis P, Cotrufo MF, Godbold DL (2009) Forest soil carbon cycle under elevated CO_2 - a case of increased throughput? Forestry 82:75-86

Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. BioScience 54:731–739

McCarthy HR, Oren R, Johnsen KH, Gallet-Budynek A, Pritchard SG, Cook CW, LaDeau SL, Jackson RB, Finzi AC (2010) Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: Interactions of atmospheric CO_2 with nitrogen and water availability over stand development. New Phytologist 185:514-528

Mildner M, Bader MKF, Leuzinger S, Siegwolf RTW, Körner C (2014) Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*. Oecologia:DOI 10.1007/s00442-014-2935-5

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. Proceedings of the National Academy of Sciences of the United States of America 101:9689-9693

Norby RJ, Zak DR (2011) Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annual Review of Ecology, Evolution, and Systematics 42:181-203

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proceedings of the National Academy of Sciences of the United States of America 107:19368-19373.

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

Petit JR, Raynaud D, Barkov NI, Barnola JM, Basile I, Bender M, Chappellaz J, Davis M, Delaygue G, Delmotte M, Kotlyakov VM, Legrand M, Lipenkov VY, Lorius C, Pepin L, Ritz C, Saltzman E, Stievenard M (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399:429-436

Phillips RP, Meier IC, Bernhardt ES, Grandy AS, Wickings K, Finzi AC (2012) Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecology Letters 15:1042-1049

Raich JW, Potter CS (1995) Global patterns of carbon-dioxide emissions from soils. Global Biogeochemical Cycles 9:23–36

Reyer C, Lasch-Born P, Suckow F, Gutsch M, Murawski A, Pilz T (2014) Projections of regional changes in forest net primary productivity for different tree species in Europe driven by climate change and carbon dioxide. Annals of Forest Science 71:211-225

Schimel DS (1995) Terrestrial ecosystems and the carbon-cycle. Global Change Biology 1:77-91

Siegenthaler U, Stocker TF, Monnin E, Luthi D, Schwander J, Stauffer B, Raynaud D, Barnola JM, Fischer H, Masson-Delmotte V, Jouzel J (2005) Stable carbon cycle climate relationship during the late Pleistocene. Science 310:1313-1317

Sigurdsson BD, Medhurst JL, Wallin G, Eggertsson O, Linder S (2013) Growth of mature boreal Norway spruce was not affected by elevated CO_2 and/or air temperature unless nutrient availability was improved. Tree Physiol 33:1192–1205

Sitch S, Friedlingstein P, Gruber N, Jones SD, Murray-Tortarolo G, Ahlström A, Doney SC, Graven H, Heinze C, Huntingford C, Levis S, Levy PE, Lomas M, Poulter B, Viovy N, Zaehle S, Zeng N, Arneth A, Bonan G, Bopp L, Canadell JG, Chevallier F, Ciais P, Ellis R,

Gloor M, Peylin P, Piao S, Le Quéré C, Smith B, Zhu Z, Myneni R (2013) Trends and drivers of regional sources and sinks of carbon dioxide over the past two decades. Biogeosciences Discussion 10, doi:10.5194/bgd-10-20113-2013.

Steinmann KTW, Siegwolf RTW, Saurer M, Körner C (2004) Carbon fluxes to the soil in a mature temperate forest assessed by ^{13}C isotope tracing. Oecologia 141:489–501

Trumbore SE, Chadwick OA, Amundson R (1996) Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. Science 272:393–396

Zaehle S, Medlyn BE, De Kauwe MG, Prentice IC, Walker AP, Thornton P, Iversen CM, Hanson PJ, Norby RJ, Dietze MC, Hickler T, Luo Y, Mccarthy H, Wang Y-P, El-Masri B, Jain A, Wang S, Warlind D, Weng E, Parton W, Gallet-Budynek A, Finzi A, Oren R (2014) Evaluation of 11 terrestrial carbon-nitrogen cycle models against observations from two temperate Free-Air CO_2 Enrichment studies. New Phytologist 202:803-822

Chapter 2

Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*

Manuel Mildner, Martin K.-F. Bader, Sebastian Leuzinger, Rolf T.W. Siegwolf and Christian Körner

published in *Oecologia 175* (2014):747-762. DOI 10.1007/s00442-014-2935-5

Oecologia
DOI 10.1007/s00442-014-2935-5

PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*

Manuel Mildner · Martin K.-F. Bader · Sebastian Leuzinger · Rolf T. W. Siegwolf · Christian Körner

Received: 25 October 2013 / Accepted: 15 March 2014

Abstract There is evidence of continued stimulation of foliage photosynthesis in trees exposed to elevated atmospheric CO_2 concentrations; however, this is mostly without a proportional growth response. Consequently, we lack information on the fate of this extra carbon (C) acquired. By a steady application of a $^{13}CO_2$ label in a free air CO_2 enrichment (FACE) experiment, we traced the fate of C in 37-m-tall, ca. 110-year-old *Picea abies* trees in a natural forest in Switzerland. Hence, we are not reporting tree responses to elevated CO_2 (which would require equally ^{13}C labeled controls), but are providing insights into assimilate processing in such trees. Sunlit needles and branchlets grow almost exclusively from current assimilates, whereas shaded parts of the crowns also rely on stored C. Only 2.5 years after FACE initiation, tree rings contained 100 % new C. Stem-respiratory CO_2 averaged 50 % of new C over the entire FACE period. Fine roots and mycorrhizal fungi contained 49–56 and 26–43 % new C, respectively, after 2.5 years. The isotopic signals in soil CO_2 arrived 12 days after the onset of FACE, yet it contained only ca. 15 % new C thereafter. We conclude that new C first feeds into fast turnover C pools in the canopy and becomes increasingly mixed with older C sources as one moves away (downward) from the crown. We speculate that enhanced C turnover (its metabolic cost) along the phloem path, as evidenced by basipetal isotope signal depletion, explains part of the 'missing carbon' in trees that assimilated more C under elevated CO_2.

Keywords Carbon isotopes · Elevated CO_2 · FACE · Forest · Respiration

Communicated by Rowan Sage.

M. Mildner (✉) · M. K.-F. Bader · S. Leuzinger · C. Körner
Institute of Botany, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland
e-mail: manuel.mildner@unibas.ch

M. K.-F. Bader
New Zealand Forest Research Institute (SCION), Te Papa Tipu Innovation Park, 49 Sala Street, 3046 Rotorua, New Zealand

S. Leuzinger
Institute for Applied Ecology New Zealand, School of Applied Sciences, Auckland University of Technology, 31-33 Symonds Street, 1142 Auckland, New Zealand

R. T. W. Siegwolf
Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, PSI, 5323 Villigen, Switzerland

Introduction

Atmospheric CO_2 concentrations are rising and have now been shown to follow the most extreme of the trajectories predicted earlier (Le Quéré et al. 2009; Friedlingstein et al. 2010). Carbon (C) uptake and transpiration of trees exposed to future atmospheric CO_2 concentrations have been shown to strongly depend on the age of trees (Ceulemans and Mousseau 1994; Medlyn et al. 2001), tree species and tree spacing in a forest (Körner 2000), on soil conditions (Spinnler et al. 2002), and on the duration of the treatment (Idso 1999; Körner 2006; Leuzinger et al. 2011). Therefore, no generally valid response patterns are found for trees exposed to elevated CO_2 (Norby and Zak 2011). The most realistic responses can be expected when trees are experimentally exposed to elevated CO_2 in situ in closed forest stands.

To date, only three free air CO_2 enrichment (FACE) experiments have been completed (with a fourth experiment using whole-tree chambers). A *Pinus taeda*

Published online: 03 April 2014

plantation in North Carolina, USA (Duke FACE; Schlesinger et al. 2006), revealed sustained stimulation of stem growth, which, however, was strongly influenced by high nitrogen (and possibly other nutrients) availability (Oren et al. 2001; Schäfer et al. 2003; McCarthy et al. 2010). The second experiment, a *Liquidambar styraciflua* plantation in Tennessee, USA (Oak Ridge FACE), showed a strong initial but no long-term stimulation of growth and productivity (Norby and Zak 2011). Higher nitrogen demand to keep up with the greater C uptake eventually provoked soil nitrogen limitation, followed by a complete loss of the photosynthetic and NPP response to elevated CO_2 by the end of the experiment (Norby et al. 2010). A third experiment, using adult trees in a mature deciduous forest in Switzerland [Swiss Canopy Crane (SCC) web-FACE], reported no growth response to elevated CO_2 at all (Körner et al. 2005; Bader et al. 2013), although some species-specific water savings induced a soil priming effect (nitrate release; Schleppi et al. 2012). The fourth experiment, using whole-tree chambers on 40-year-old *P. abies* trees in a boreal forest in northern Sweden (Medhurst et al. 2006), showed a growth response to elevated CO_2 only when mineral fertilizer was applied, and none under natural nutrient conditions (Comstedt et al. 2006; Sigurdsson et al. 2013).

In summary, these results suggest that C is not generally a limiting resource for tree growth at current CO_2 concentrations. However, a common observation in most CO_2-enrichment experiments is that leaf photosynthesis remains stimulated by elevated CO_2 (Ainsworth and Rogers 2007; Bader et al. 2010; but see Norby et al. 2010). Even in cases where some downward adjustment had been seen, the diminished photosynthetic stimulation still did not produce a proportional growth response. Thus, the question of the fate of the additionally assimilated C remains (Fatichi and Leuzinger 2013).

Tracing the fate of C in a forest needs a marker that can be applied at canopy scale. The stable ^{13}C isotope is the only suitable marker for such large-scale and long-term in situ labeling investigations. Pulse labeling (a strong, short-term peak with highly enriched $^{13}CO_2$; usually 99 % ^{13}C) is suitable for studying short-term dynamics of the C distribution (Epron et al. 2012). The other option is to expose the foliage continuously with ^{13}C depleted CO_2 originating from fossil fuel (e.g., −30 ‰ relative to the V-PDB standard), permitting the continuous tracing of the fate of novel C over a longer period (Andrews et al. 1999; Hättenschwiler et al. 2002; Körner et al. 2005; Comstedt et al. 2006; Keel et al. 2007; von Felten et al. 2007).

Recent studies on in situ C allocation in field-grown conifers (<25 m tall) used the whole tree/canopy ^{13}C pulse (or extended pulse) method with trees, which are normally growing under ambient CO_2 concentration (Kagawa et al. 2006; Högberg et al. 2008; Endrulat et al. 2010; Dannoura et al. 2011; Kuptz et al. 2011a; Warren et al. 2012). Such short-term pulse experiments in conifers revealed a rapid appearance (a few days) of labeled C in respiratory CO_2 released from soils (Högberg et al. 2008; Ritter et al. 2011), indicating a strong linkage between assimilation and soil CO_2 release. However, transport velocity and basipetal C propagation was found to vary with tree size and season, and is controlled by weather conditions and sink strength, as was demonstrated by repeated pulse labeling of 12-year-old *Pinus pinaster* growing in a plantation (Dannoura et al. 2011; Epron et al. 2011). Moreover, the dilution of new assimilates in large, old non-structural C pools prior to utilization (Keel et al. 2007) led to only a small and seasonally fluctuating contribution of new C to respiratory CO_2 release from stems and coarse roots of a 60-year-old *P. abies* stand in Germany (Kuptz et al. 2011a). Similar C mixing and seasonal patterns for structural C were observed elsewhere (Lippu 1994; Kagawa et al. 2006), and in deciduous trees at our study site (Keel et al. 2007; Bader et al. 2013). Given that tissue formation in evergreen trees profits from a year-round supply of new assimilates (Hansen et al. 1996), we can assume a rapid manifestation of the isotopic signal contained in new photoassimilates, particularly in foliage and adjacent branchlets (branch autonomy; Gordon and Larson 1968; Hoch 2005). Signals of new C should dilute basipetally, e.g., in stem wood, and extend the time until old C is completely replaced by new C (Keel et al. 2007).

Using the SCC web-FACE facility in Switzerland (Pepin and Körner 2002), we started CO_2 enrichment of canopies of mature *P. abies*, and, thus, labeling with ^{13}C depleted CO_2 in a near-natural forest ecosystem on 30 July 2009. The work presented here is not aimed at elucidating tree responses to elevated CO_2, but should illustrate the basic patterns of C allocation in mature forest trees. Establishing the fate of novel C should help in identifying sites and processes that explain 'missing C' in experiments with elevated atmospheric CO_2. We present C distribution patterns using ^{13}C signals, spanning a pre-treatment period and two full growing seasons under elevated CO_2. By consecutive sampling with short intervals in various tree compartments from tree tops to roots, we aim at analyzing (1) seasonal shifts in assimilate allocation, (2) locations of C-investment, and (3) residence times (turnover) of mobile C pools. We expect that the ^{13}C signal would be transferred from needles to respiratory fluxes within a few days, but that it will take several years before new structural biomass such as stem wood consists fully of new C. Although we cannot assess CO_2 effects on new/old C mixing ratios due to missing similarly labeled control trees, we are able to quantify

the main pathways and the duration of C transfer following assimilation for the first time in mature coniferous trees.

Materials and methods

Description of the free air CO_2-enrichment facility

In 2009, a web-FACE system (Pepin and Körner 2002) was installed in five 37-m-tall, then ca. 110-year-old *Picea abies* individuals. With this system, the trees were exposed to increased atmospheric CO_2 concentration at ca. 550 ppm in their natural forest environment. The site is located near the village of Hofstetten (47°33′N, 7°36′E, 500 m a.s.l), 12 km south-west of Basel, Switzerland. All five *P. abies* trees that were subjected to elevated CO_2 are located within max. 5 m distance from another. Five similarly tall trees served as controls. Of these controls, one tree is situated only 6 m from the treated trees (separated from the treated trees by a forest road), whereas the other trees grew at a distance of 17–30 m from the CO_2-enriched trees. The only control tree that was closer to the treated trees was 'unpolluted' by tank CO_2 as evidenced by the ^{13}C data. While the canopies of all CO_2-treated trees and one control tree were accessible with a crane gondola, the crowns of the remaining control trees could only be reached by professional tree climbers.

The CO_2 enrichment started on 30 July 2009, when earlywood and current year foliage formation of that year had been completed. Pure CO_2 (in food industry quality, with a $\delta^{13}C$ of −30 ‰) was released exclusively into the tree canopies (15–37 m above ground) through laser-punched tubes woven around the tree branches. Control of CO_2 release was separated into four sectors per tree to ensure an optimal CO_2 distribution and concentration in response to wind directions: the tree tops, and three 120° sections around the remainder of the crowns (i.e. 4 sectors per tree, 20 sectors in total). Each sector was separately monitored for CO_2 concentrations and was individually supplied with pure CO_2 to ensure a mean CO_2 concentration in the tree canopy of 550 ppm. Each sampling line in the canopy for CO_2 monitoring ended in a triplet, so that each tree crown was sampled at 12 sampling points (60 for all 5 trees). Four extra gas-sampling lines served as a control (at the crane top, 45 m above ground, the sub-canopy, and a calibration gas bottle). CO_2 concentrations were measured with two non-dispersive infrared gas analyzer (IRGA; LI-820; Li-Cor, Lincoln, NE, USA). Using C_4 grasses grown in the canopy, the $\delta^{13}C$ of the CO_2 mixture supplied was monitored (plant isometers, see below). CO_2 release was suspended when temperature was below 6 °C, and when above-canopy photon flux density was below 100 μmol m^{-2} s^{-1}. With this FACE system, we could reach median elevated CO_2 concentrations of 500–560 ppm based on IRGA readings, with a mean 20–50 ppm higher given the skewed frequency distribution.

Table 1 CO_2 concentrations and $\delta^{13}C$ signatures in the canopies of trees subjected to ambient and elevated CO_2 calculated from isometer (C_4 grass *Echinochloa crus-galli*) $\delta^{13}C$ values

	Mean ± SE	*n*	Mean ± SE	*n*
Canopy $\delta^{13}C$ (‰)				
2009	−8.0 ± 0.1	2	−13.7 ± 0.3	110
2010	−8.7 ± 0.1	2	−13.5 ± 0.3	82
2011	−8.5 ± 0.2	2	−13.8 ± 0.3	98
Canopy CO_2 concentration (ppm)				
2009	382 ± 2	2	541 ± 13	110
2010	397 ± 3	2	532 ± 13	82
2011	396 ± 4	2	541 ± 12	98

Ambient CO_2 values were obtained from isometers installed at the crane top. Mean ± SE for the annual FACE period for *n* isometers

C_4 grass isometers

The values from IRGA measurements could be confirmed by the isotopic compositions of canopy-grown C_4 grasses ('isometers'; *Echinochloa crus-galli* in 50-ml flasks filled with sand-clay soil, mounted in elevated and ambient tree crowns, and, thus, freely exposed to the same atmosphere as the tree crowns) in order to obtain the integrated $\delta^{13}C$ values and the corresponding CO_2 concentrations (Table 1; see Pepin and Körner 2002 for calculation). Since C_4 grasses synthesize new biomass entirely from newly assimilated CO_2 without further enzymatic fractionation, the isotopic signal in C_4 grass tissue reflects the long-term $\delta^{13}C$ within the tree crowns (ambient vs. elevated $\delta^{13}C$ differences in 2009, 2010, and 2011 (mean ± SE): −5.7 ± 0.6, −4.8 ± 0.5, and −5.3 ± 0.3 ‰, respectively,). These differences indicate 100 % new C incorporated and served as a yearly reference for the new C fraction in the different tree tissues investigated. Assuming that the isometers capture the same atmospheric CO_2 mixture in the canopy that becomes effective for the treated *P. abies* trees, the difference in the isotopic signals of isometers grown in elevated and ambient CO_2 concentrations should also be observed between the tissues of the treated and control trees (the maximum possible new C integration into tissues). However, the scattered distribution of a limited number (ca. 14) of containers with C_4 grasses within the tree canopies during peak summer only (spring and autumn are too cold for C_4 grasses) may cause a difference between the isometer signal and the maximum tree tissue signal captured over an entire season. Although we are using isometer signals as a 100 % reference, they are still a proxy for the maximum new C integration into

tissues, and needles may spatially experience slightly less or more labeled tank CO_2. A signal >100 % thus indicates that isometers underestimated the signal strength at some locations. In any case, such a signal means that the new tissue was built from novel C only.

Branchlet needle and xylem sampling

During four sampling campaigns (pre-treatment: 1–16 July 2009; treatment periods: 18–27 August 2009; 29 September 2010; 29 July 2011), professional tree climbers collected three sun-exposed and three shaded branchlets from all four cardinal directions of the control trees that were not accessible via the crane gondola. The same kind of samples were collected from the remaining study trees using the crane (24 branchlets per tree, except during the pre-treatment campaign when only sun-exposed branchlets were used). From these branchlets, we investigated sun-exposed and shaded bulk needle material and sun-exposed branchlet xylem tissue. In 2009, we collected needle and xylem samples from branchlet extensions initiated in the years 2008 and 2009. In the years 2010 and 2011, we sampled 2008 and 2009 branchlet parts again, along with the same kind of material from one or two more recent years. We obtained bulk xylem tissue by peeling off bark and phloem immediately after sampling. Additional needle tissue was collected every 2–6 days in August 2009 for 3 weeks following the FACE onset, later we continued with 1- to 3-monthly sampling intervals. Three to five needles of each of three sun-exposed and shaded branchlets per cardinal direction and per tree were collected. Prior to analysis, we pooled the needle and the xylem samples of the three branchlets from all four cardinal directions per treatment since we did not find azimuthal differences within either sunlit or shaded needles. Immediately after sampling, the material was heated in an on-site microwave oven (90 s at 600 W) to stop any metabolic processes (Popp et al. 1996). Canopy CO_2 enrichment could cause a shift in photosynthetic ^{13}C discrimination in both *P. abies* trees and the C_4 grasses that could add to the CO_2-enrichment signal caused by tank CO_2. Since such a biochemical adjustment is likely to be small (<1 ‰) and differ between sun and shade needles, we refrained from assuming a change and applying a uniform data adjustment, but present the data as measured.

Wood core sampling

Wood cores were collected at breast height (1.3 m) in east direction from each tree. Using a 5-mm-diameter increment corer, we took the samples in March 2010 during the trees' winter dormancy after completion of the latewood formation in 2009 (earlywood was formed before the beginning of the CO_2 enrichment on 30 July 2009). Tree rings formed in 2005–2009 were also analyzed for $\delta^{13}C$ values. Except for latewood 2009, we expected $\delta^{13}C$ signals in these tree rings to reflect only pre-treatment conditions before the start of the experiment. There may, however, be a very small influence of storage reserves in older ray parenchyma in the trees receiving CO_2 enrichment since midsummer 2009. Wood cores were again taken in February 2012 to assess the changes of the isotopic signal in the 2008–2011 tree rings throughout the experiment. Earlywood and latewood of each year were separated under a binocular using a razor blade. The transition zones between early and latewood, and between the years were removed in order to obtain a clearly separated isotopic signals. The wood segments were then analyzed for $\delta^{13}C$.

Fine root sampling

In March 2010, 9 soil cores per tree (12 cm in depth × 3.6 cm diameter) were collected in the inner part of the root disk (2 m from the stem) of each tree, adding up to a total of 90 root samples for treated and control trees. The nine cores per tree were grouped into three 'joint' cores collected at 10 cm distance from one another, with the triplets separated at a 120° angle around each tree. The values of these triplicate samples were pooled after the isotope analysis in order to give a more robust signal. The resulting holes were used to install ingrowth cores of the same size (2-mm mesh cylinders), filled with sieved root-free soil from on-site molehills to assess CO_2 effects on fine root growth development (Bader et al. 2009). The bulk density of soil in the ingrowth cores was adjusted to the bulk density of the soil outside (mass to volume ratio). To capture the isotopic signal of newly formed fine roots entirely developed during FACE, the use of ingrowth cores was the preferential method. In December 2011, 20 months after installation, the ingrowth cores were removed using a knife. Both soil and ingrowth cores were stored at −20 °C until processing in January 2012. Prior to fine root separation, cores were placed in cold water and defrosted for 48 h at 4 °C to keep the microbial degradation of fine root biomass as slow as possible. Roots were extracted by wet-sieving (1 mm mesh), and *P. abies* roots were identified using a reference root collection. Since the five trees exposed to elevated CO_2 formed a monospecific group in the stand, the inner part of the treated area belonged almost exclusively to *Picea*, whereas at the periphery of the plot, roots of other species (e.g., *Fagus sylvatica, Carpinus betulus, Quercus petraea*) co-occur. However, *Picea* roots are very distinct and can be clearly identified. Fine roots were separated into three diameter classes (<0.5, 0.5 < 1, 1–2 mm). We could not distinguish between still intact dead and live fine roots.

Fungal sporocarp sampling

During late summer and early fall 2010 and 2011, we collected fungal fruit bodies (sporocarps) within three distance intervals to a tree stem subjected to elevated CO_2 (0–6, 6–12, 12–18 m; the last being considered as an untreated control). The sporocarps were frozen and stored at −20 °C until taxonomic and functional group classification (either saprobiotic, mycorrhizal, or parasitic) and further preparation for C isotope analysis.

Stem and soil air sampling

The CO_2 emitted by stems was captured by four aluminum cups per tree (Ø 8.2 cm, height 2.6 cm; using Terostat-IX; Teroson, Ludwigsburg, Germany) mounted in the four compass directions to each stem at breast height (1.3 m) after removing loose bark. Given the rise in CO_2 partial pressure within the cups due to continuous CO_2 release from tree stems, we assumed that small leaks would not affect the isotope signals. Cups were fitted with an open-ended tube (2–3 cm long, inner Ø 3 mm) through a bottom hole. This tube permitted gas sampling with a syringe, but at the same time reduced gas exchange between the cups' interior and surrounding air. Since the CO_2 concentration in the cups did not reach equilibrium (Ubierna et al. 2009a), we had to account for counter diffusion by atmospheric CO_2. Therefore, the Keeling plot approach was applied to calculate the $\delta^{13}C$ in stem-emitted CO_2 (see below). Note that only the tree canopy at 20–37 m height was receiving labeled tank CO_2, with the understory remaining unaffected.

We used gas wells to collect air in equilibrium with soil air. These wells consisted of PVC tubes (inner Ø 18 mm, 12 cm long) in which small holes were drilled at each ca. 70° angle in five rows between 3 and 11 cm of the gas well height to allow air to diffuse into the tubes from the respective depths (Steinmann et al. 2004). The gas wells were inserted in vertical pre-punched holes in the soil in 2009. The bottom of each well was left open, and the top was covered with an air-tight rubber septum. Ten gas wells per tree were installed in two circles of 2 and 3 m radius around each tree stem (each with five gas wells per circle at ca. 70° angle).

At each sampling time, 15 ml air was extracted through the septum using a 60-ml syringe. The air was injected in a 12-ml pre-evacuated glass vial (Exetainer gas testing vial; Labco, High Wycombe, UK) closed with an airtight rubber septum. The overpressure produced in the vials eliminates contamination with ambient air. These vials containing the gas samples were stored in a N_2 atmosphere prior to analysis, which was performed no later than 7 days after sampling.

One day before FACE launch on 30 July 2009, we collected 15 ml gas from all soil gas wells and all stem cups to receive pre-treatment values. Starting 1 day after FACE onset, two soil gas wells per circle and tree and two cups per stem were randomly sampled. During the first 2 weeks, we sampled daily, thereafter biweekly until August 2009, followed by about a bimonthly sampling interval. Starting in May 2010, the sampling intensity was reduced to one mixed 15-ml sample per soil circle from all five gas wells per circle (each 3 ml per gas well) as well as one mixed sample of all four cups per tree stem (each 3.75 ml per cup).

We employed the Keeling plot approach (Keeling 1958) to obtain as correct as possible $\delta^{13}C$ signals in the mixture of two different CO_2 sources, respiratory and atmospheric CO_2, both diffusing in the opposite direction in the soil. We followed the approach by Steinmann et al. (2004) to obtain atmospheric reference CO_2 concentration data and the corresponding $\delta^{13}C$ values from six different European/Middle Eastern weather stations (Terceira Island, Azores, Portugal; Storhofdi, Iceland; Tenerife, Spain; Mace Head, Ireland; Ocean Station M, Norway; WIS Station, Israel) for the years 1997–2004. Note that FACE was applied to the canopy 15–37 m aboveground only; thus, tank CO_2 did not reach the ground in the test area. For applying the Keeling plot approach, CO_2 and $\delta^{13}C$ values for free air in the years 2009–2011 were needed, which were not available when we analyzed our data. Therefore, we modeled the trend of the data with a seasonal component incorporated, using Holt–Winters exponential smoothing (Holt 1957).

C isotope analysis

Within 3–4 h after sampling, all tissue samples were dried at 80 °C for 48 h, followed by grinding to a fine powder using a steel ball mill (MM 2000; Retch, Haan, Germany). Aliquots of 0.6–0.8 mg were weighed into tin capsules and analyzed for C isotope ratios. The samples were combusted in an elemental analyzer (EA-1110 CHN; Carlo Erba Thermoquest, Milan, Italy). The EA was connected to an Isotope Ratio Mass Spectrometer (IRMS, Delta S; Thermo Finnigan, Bremen, Germany) operating in continuous flow mode via a variable open-slit interface (Conflo II; Thermo Finnigan). The precision for $\delta^{13}C$ was <0.1 ‰. The isotope values are expressed in the δ-notation as deviation from the international 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. The isotope analyses were performed at Stable Isotope Facility of the Paul Scherrer Institute, Villigen, Switzerland.

Statistical analysis

The unit of replication was 'tree' (five trees under ambient and five trees under elevated CO_2 concentrations). All

statistical analyses (except for fungal $\delta^{13}C$) were performed using linear mixed effects models fitted by restricted maximum likelihood. The significance of the main effects was assessed using a backwards selection procedure based on likelihood ratio tests and the Akaike information criterion. The factor 'tree' was included as random factor (apart from fungal $\delta^{13}C$). In certain cases, a second random factor nested within 'tree' was used (xylem $\delta^{13}C$: 'year of the start of branchlet segment growth'; fine root $\delta^{13}C$: 'cardinal direction of the position of the soil/ingrowth core'). Where appropriate, we corrected for homogeneity violations using adequate variance function structures (power, exponential, and constant variance structures, or a combination thereof). Furthermore, we accounted for independence violations where necessary by applying temporal autocorrelation structures. Diagnostic plots (i.e. residual and quantile–quantile plots) were used for model validation. All statistical analyzes were performed using R, v.2-15-0 (R Development Core Team 2008–2010) and the R package *nlme*.

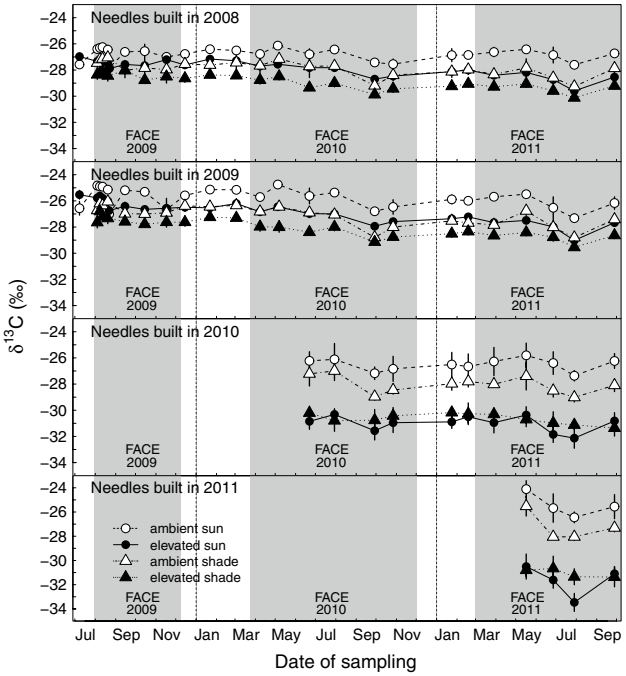

Fig. 1 Seasonal variation of carbon isotope signature ($\delta^{13}C$) in organic matter of sun-exposed and shaded needles of *Picea abies* trees exposed to ambient and elevated atmospheric CO_2 [ambient CO_2: $n = 2$ trees (except for 8 July 2009, 22 August 2009, 29 September 2010, 29 July 2011: $n = 5$ trees), elevated CO_2: $n = 5$ trees, mean ± SE]. *Diagrams* denote needles that were built in the year (from *top* to *bottom*) 2008, 2009, 2010, and 2011, respectively. The *gray-shaded* areas denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only

Results

Canopy $\delta^{13}C$ and CO_2 concentration

Assuming a $\delta^{13}C$ signature of −8.4 ‰ and a CO_2 concentration of 390 ppm in the atmosphere (2009–2011 average; NOAA), data from our regular measurements of tank CO_2 ($\delta^{13}C$ of −30 ‰), and canopy CO_2 data from the IRGA measurements, the expected $\delta^{13}C$ value of the mixed CO_2 in the canopies of treated trees was −13.8 ± 0.3 ‰ (mean ± SE of $n = 3$ years, i.e. 2009–2011). This value corresponds to an expected difference in $\delta^{13}C$ between elevated and ambient CO_2 in the canopies of −5.4 ± 0.3 ‰ (mean ± SE of $n = 3$ years, i.e. 2009–2011). The C_4 grass isometer samples revealed a similar $\delta^{13}C$ value of −13.7 ± 0.1 ‰ (mean ± SE of $n = 3$ years, i.e. 2009–2011) in the canopies of the treated trees ($\delta^{13}C$ difference between elevated and ambient CO_2: −5.3 ± 0.2 ‰), corresponding to a canopy CO_2 concentration of 538 ± 7 ppm (elevated CO_2; mean ± SE of $n = 3$ years, i.e. 2009–2011).

Pre-treatment $\delta^{13}C$ differences

Prior to the onset of FACE (pre-treatment), tissues of trees later exposed to elevated CO_2 concentrations usually showed slightly less negative $\delta^{13}C$ signals compared to control trees. Needles and branchlet xylem of treated trees formed in 2008 and 2009 showed the highest deviations from control trees (0.8 ± 0.2 ‰ in sunlit needles, and 0.9 ± 0.2 ‰ in sunlit branchlets; Figs. 1, 2; no data for shaded needles and branchlets). These canopy signal differences attenuated downstream of the plant body. Tree rings at breast height were first sampled in March 2010. Thus, earlier year rings might store some novel (fixed after 30 July 2009) non-structural carbon in ray tissue causing a slightly more negative bulk xylem signal. Yet, earlywood formed in 2005 to earlywood formed in 2009 of trees later subjected to elevated CO_2 still showed a slightly less (not significantly) depleted $\delta^{13}C$ signal compared to control trees (0.3 ± 0.1 ‰; Fig. 3), suggesting little if any influence of novel C by March 2010. Fine roots were not sampled before FACE. Respiratory CO_2 sampled before FACE showed either no $\delta^{13}C$ differences (0.1 ‰ in soil CO_2 efflux; $P = 0.7$; Fig. 7), or even more negative $\delta^{13}C$ signals (0.9 ‰ in stem CO_2 efflux; $P = 0.03$; Fig. 6) in trees later subjected to elevated CO_2 concentrations versus control trees, thus not matching the pre-treatment signals in branchlet tissues. Given these small and inconsistent trends in pre-treatment $\delta^{13}C$, we did not correct $\delta^{13}C$ signals obtained after the onset of FACE by pre-treatment signals, with the assumption that the deviations fall within the error margins. This approach is conservative, because the pre-treatment trend rather runs counter the direction of tracer signals.

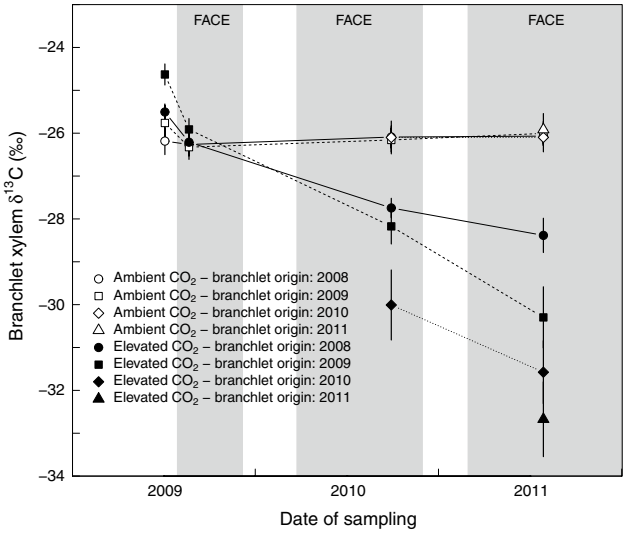

Fig. 2 Time course of the $\delta^{13}C$ of sun-exposed branchlet xylem of *P. abies* exposed to ambient and elevated atmospheric CO_2 (ambient CO_2: $n = 5$ trees, elevated CO_2: $n = 5$ trees, mean ± SE). The *gray-shaded* areas denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only. Note that the branchlet origin indicated in the diagram corresponds to the year when new branch segments were initiated. In the following years, new year rings were produced that add to branch segment thickening leading to dilution of the isotopic signal

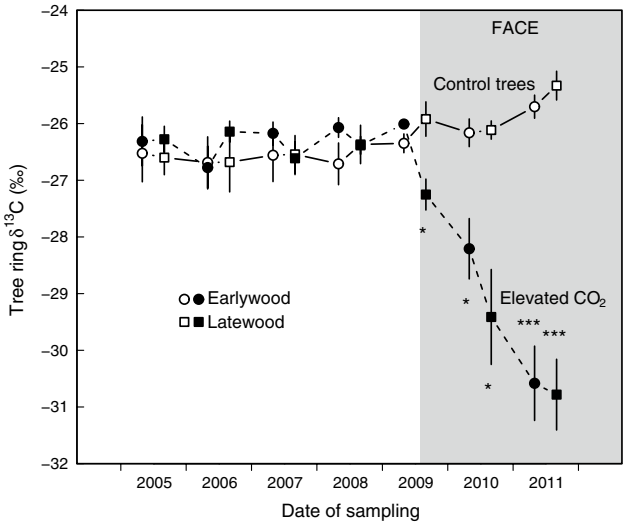

Fig. 3 Seven-year time course of $\delta^{13}C$ values in earlywood and latewood of *P. abies* subjected to ambient and elevated atmospheric CO_2 ($n = 5$ trees, mean ± SE). The *gray-shaded* area denotes the FACE period. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

$\delta^{13}C$ in needles

Generally, needles of trees subjected to elevated CO_2 always showed more negative C-isotope signals compared to trees under ambient CO_2 during the treatment period ('CO_2 treatment signal' effect: $P < 0.004$; Table 2; Fig. 1), with a significant 'exposure × CO_2 × day of year' interaction ($P < 0.001$). Furthermore, signals did not become significantly stronger with time within an age cohort and exposure class ('exposure × day of year' effect: $P = 0.063$). However, isotopic signals became more negative from one needle age class to the next (Fig. 1); the younger the needles, the more pronounced was the $\delta^{13}C$ difference between needles of trees under ambient and elevated CO_2. Sampled during CO_2 enrichment, needles of CO_2-enriched trees which were formed prior to the experiment (e.g., needles formed in 2008 and 2009; Fig. 1), showed more negative $\delta^{13}C$ values by 1.1 ± 0.1 ‰ (mean treatment difference ± SE) compared to controls, irrespective of the position in the canopy, suggesting an exchange of non-structural carbohydrates (NSC) by new ones.

Sampled during the FACE period, shaded needles exposed to elevated CO_2 showed a progressive mean depletion from 1.0 ± 0.1 ‰ (needles formed in 2008 and 2009; Fig. 1), to 2.6 ± 0.2 ‰ (needles formed in 2010; Fig. 1), and to 3.8 ± 0.6 ‰ (needles formed in 2011; Fig. 1) compared to needles in ambient CO_2. Sunlit needles formed during FACE, and sampled after the onset of FACE, were even more depleted under elevated CO_2 by 1.2 ± 0.1 ‰ (needles formed in 2008 and 2009; Fig. 1), 4.5 ± 0.1 ‰ (needles formed in 2010; Fig. 1), and 6.2 ± 0.3 ‰ (needles formed in 2011; Fig. 1) compared to sunlit needles of control trees.

In control trees, shaded needles were always more depleted compared to sunlit needles on the same trees, irrespective of the year of needle formation (1.3 ± 0.1 ‰; $P < 0.001$; Table 2). The same pattern was observed for treated needles formed prior to FACE initiation (i.e. formed in 2008 and 2009), and sampled during FACE (1.1 ± 0 ‰; $P < 0.001$; Table 2). Interestingly, this did not apply to needles grown under elevated CO_2 in 2010 and 2011. Here, shaded needles showed less negative $\delta^{13}C$ values by 0.5 ± 0.1 ‰ compared to sun-exposed needles.

Averaged over samples collected during the entire FACE period, sunlit and shaded needles formed prior to FACE (e.g., 2008 and 2009) incorporated a steady state 20 ± 0.2 % new C during the treatment years 2009–2011. Only 2 years after the FACE onset, we measured 72–117 % new C in needles formed in 2011 compared to the isometer reference signal (see "Materials and methods"). Drawing on new assimilates was intensified in sunlit needles. Overall, the amount of C incorporated did not vary at a given type of position once new needles had fully matured.

$\delta^{13}C$ in branchlet xylem

For statistical analysis, we used only xylem $\delta^{13}C$ of branchlet segments of the most recent shoot expansion, thus

Table 2 Linear mixed effects model results for *Picea abies* ^{13}C signatures of needles, branchlet xylem, year rings, fine roots (soil cores 2010 and ingrowth cores 2010-2011), fungi, and CO_2 efflux from stems and soil under ambient and elevated CO_2

Factor	*df*	*F* value	*P*	
Needle $\delta^{13}C$ (2009–2011)				
Exposition	1, 128	700.6	<0.001	***
CO_2	1, 8	16.3	0.004	**
Day of experiment	1, 128	64.6	<0.001	***
Exposition × day of experiment	1, 128	3.5	0.063	
CO_2 × day of experiment	1, 128	58.8	<0.001	***
Exposition × CO_2 × day of experiment	1, 128	34.0	<0.001	***
Branchlet xylem $\delta^{13}C$ (2009–2011)				
CO_2	1, 8	0.5	0.498	
Day of experiment	1, 9	43.8	<0.001	***
CO_2 × day of experiment	1, 10	96.5	<0.001	***
Year ring $\delta^{13}C$ (2005–2011)				
Latewood/earlywood	1, 124	0.1	0.716	
CO_2	1, 8	16.6	0.004	**
Year	1, 124	0	0.891	
Latewood/earlywood × CO_2	1, 124	8.7	0.004	**
Latewood/earlywood × year	1, 124	0.9	0.336	
CO_2 × year	1, 124	19.1	<0.001	***
Latewood/earlywood × CO_2 × year	1, 124	10.0	0.002	**
Fine root $\delta^{13}C$ (soil cores 2010)				
Diameter	2, 54	12.9	<0.001	***
Fine root $\delta^{13}C$ (ingrowth cores 2010–2011)				
CO_2	1, 8	28.0	0.001	***
Diameter	2, 40	3.8	0.032	*
Soil cores	1, 40	0.1	0.709	
CO_2 × diameter	2, 40	0.8	0.448	
CO_2 × soil cores	1, 40	8.9	0.005	**
Diameter × soil cores	2, 40	1.0	0.370	
CO_2 × diameter × soil cores	2, 40	3.8	0.030	*
Fungal $\delta^{13}C$ (2010 + 2011)				
Distance	1	7.3	0.008	**
Fungal type	1	46.2	<0.001	***
Distance × fungal type	1	8.8	0.004	**
Stem air $\delta^{13}C$ (2009–2011)				
CO_2	1, 8	2.1	0.190	
Day of experiment	1, 334	184.8	<0.001	***
CO_2 × day of experiment	1, 334	22.0	<0.001	***
Soil air $\delta^{13}C$ (2009–2011)				
Stem distance	1, 801	1.7	0.193	
CO_2	1, 8	18.0	0.003	**
Day of experiment	1, 801	0.4	0.510	
Stem distance × CO_2	1, 801	0.8	0.385	
Stem distance × day of experiment	1, 801	35.2	<0.001	***
CO_2 × day of experiment	1, 801	0	0.833	
Stem distance × CO_2 × day of experiment	1, 801	6.9	0.009	**

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

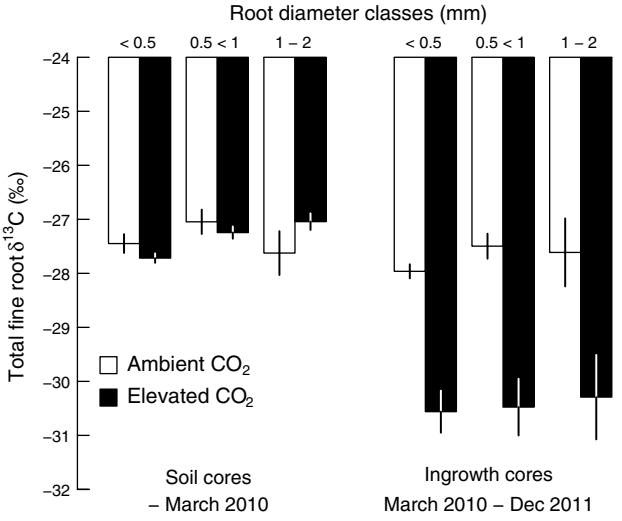

Fig. 4 $\delta^{13}C$ of fine roots under *P. abies* trees exposed to ambient and elevated ^{13}C-depleted CO_2 ($n = 5$ trees, mean ± SE). *Left panel* fine roots were excavated 8 months after the FACE onset on 30 July 2009 (with soil cores). *Right panel* $\delta^{13}C$ of fine roots that were produced exclusively during the FACE experiment from March 2010 to December 2011 (ingrowth core method)

grown exclusively under elevated CO_2 (i.e. without "contamination" of the underlying xylem and reserves from previous years). Over the entire experimental period, branchlet xylem segments collected on control trees showed stable $\delta^{13}C$ signals of -26.1 ± 0.1 ‰, irrespective of the age classes and the sampling date (Fig. 2). In contrast, isotopic signals of branchlet xylem segments formed under elevated CO_2 became consistently more negative over time compared to control tree branchlets ('CO_2 × day of year' interaction: $P < 0.001$; Table 2). This trend was more pronounced, the younger a branchlet segment: when sampled in July 2011, branchlet segments showed $\delta^{13}C$ signals of -28.4 ± 0.4, -30.3 ± 0.7, -31.6 ± 0.7, and -32.7 ± 0.9 ‰ (initiated in 2008, 2009, 2010, and 2011, respectively). These values correspond to $\delta^{13}C$ differences between elevated CO_2 and ambient CO_2 of 2.3, 4.3 5.5, and 6.8 ‰ for branchlet segments initiated in 2008, 2009, 2010, and 2011, respectively. Hence, the isotopic signals of the branchlet segments initiated in 2010 and 2011 were even stronger than would be expected compared to the 100 % new C incorporation estimated by isometer $\delta^{13}C$ signals (44, 81, 103, and 127 % new C relative to the isometer signal).

$\delta^{13}C$ in stem wood

Within just a few months after the start of CO_2 enrichment, newly assimilated C could be detected in latewood

of 2009 (isotopic depletion of 1.3 ‰ compared to control trees; Fig. 3). Signals became more negative over time, leading to a treatment-related difference in $\delta^{13}C$ of 5.5 ‰ in latewood of 2011, which corresponds to full (in fact, 103 % compared to isometers) newly incorporated C only 2.5 years after the onset of FACE. The CO_2 treatment signal was highly significant ($P = 0.004$; Table 2) with a clear 'CO_2 × year' interaction ($P < 0.001$), reflecting the increasing incorporation of new ^{13}C depleted C with time.

$\delta^{13}C$ in tree fine roots

P. abies fine roots from soil cores, collected 8 months after the onset of FACE, did not differ in $\delta^{13}C$ between treatments (not significant), but the $\delta^{13}C$ signals in the three fine root diameter classes were distinct from another ($P = 0.001$; Table 2; Fig. 4).

CO_2 enrichment had a highly significant effect on the C isotope signal in new fine roots that were formed entirely during the FACE period (ingrowth cores over a period of 20 months, installed in March 2010; $P = 0.001$; Table 2; Fig. 4). At 28 months after FACE launch, new fine roots of ingrowth cores were 2.6 ‰ (<0.5 mm), 3.0 ‰ (0.5 < 1 mm), and 2.7 ‰ (1–2 mm) more negative compared to roots sampled from control trees (Fig. 4). These values correspond to 49–56 % new C incorporated into fine roots under elevated CO_2 (relative to isometer signals; Table 2). Further, the $\delta^{13}C$ signals in new fine roots belonging to different diameter classes also differed significantly, irrespective of the treatment ($P = 0.032$).

$\delta^{13}C$ in fungal sporocarps

Altogether, 65 saprobiotic, 51 mycorrhizal, but only 2 parasitic, fungal species were collected during the years 2010 and 2011 in this mixed forest. Only four of the mycorrhizal species are known to be exclusively connected to *P. abies*. Nevertheless, the analysis included all mycorrhizal fungal species. Fungi collected at >18 m distance to the treated trees are assumed not to be influenced by our labeling. Therefore, the isotopic values of these fungi served as a control (Keel et al., in preparation). Mycorrhizal fungi collected closer to the tree base of CO_2 exposed trees showed more negative isotopic signatures compared to fungi collected at greater distance (−27.3 ± 0.5, −26.7 ± 0.3, and −26.0 ± 0.6 ‰ in 2010, and −27.7 ± 0.3, −25.6 ± 0.2, and −25.5 ± 0.3 ‰ in 2011 for the <6, <12, and <18 m distances, respectively; Fig. 5). However, the isotopic differences between the fungi at <6 m distance and the control fungi indicate only 26 % (in 2010) and 43 % (in 2011) new C incorporation. Mean $\delta^{13}C$ in all saprobiotic sporocarps was very similar regardless of the sampling distance from CO_2-enriched trees, suggesting no incorporation of

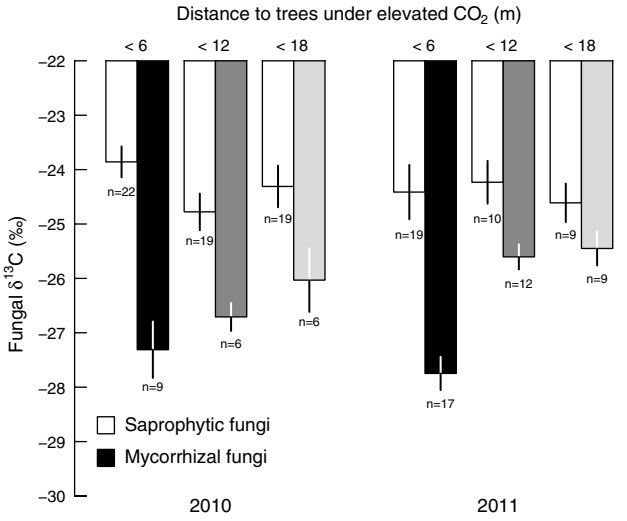

Fig. 5 $\delta^{13}C$ of saprobiotic (*white bars*) and mycorrhizal (*dark bars*) fungal sporocarps (mean ± SE; n = number of fungal species) collected in fall of the years 2010 (*left panel*), and 2011 (*right panel*). Sporocarps were sampled at various distances to stems of CO_2-enriched trees, and are grouped into three distance classes

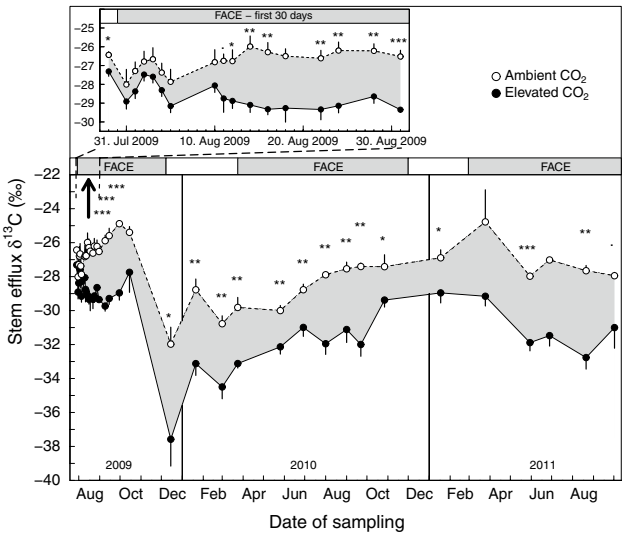

Fig. 6 Seasonal variation of $\delta^{13}C$ in stem CO_2 evolution of *P. abies* trees exposed to ambient and elevated atmospheric CO_2 ($n = 5$ trees, mean ± SE). *Inset* enlarges the first month of the FACE experiment (August 2009). The *gray bars* on *top* of the *panels* denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

new C delivered by the treated trees during that period (−24.4 ± 0.1 ‰ in each year; Table 2; Fig. 5). As a consequence, the isotopic difference between mycorrhizal and saprobiotic fungi increased with proximity to CO_2-enriched trees across all sampled individuals (3.5, 1.9, and 1.7 ‰

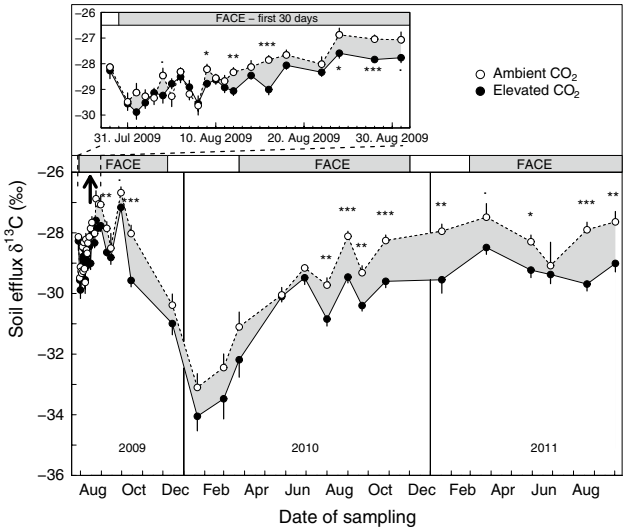

Fig. 7 Seasonal variation of $\delta^{13}C$ in soil CO_2 evolution at 3–11 cm depth under trees exposed to ambient and elevated atmospheric CO_2 ($n = 5$ trees, mean ± SE). *Inset* enlarges the first month of the FACE experiment (August 2009). The *gray bars* on *top* of the *panels* denote the main FACE periods. During the winter dormancy periods, CO_2 enrichment was performed at favorable weather conditions only. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

in 2010, and 3.3, 1.4, and 0.8 ‰ in 2011 for the <6, <12, and <18 m distances, respectively). This is supported by a significant 'distance × fungal type' interaction ($P = 0.004$; Table 2).

$\delta^{13}C$ in CO_2 released from stems and soil

Thirteen days after FACE was started, the isotopic signal in stem CO_2 efflux was 2 ‰ lower for trees exposed to elevated CO_2 than in control trees (not corrected for the 0.9 ‰ pre-treatment difference obtained during the 3 days before the onset of FACE). Subsequent measurements (mid-August 2009–October 2011) showed a significantly lower $\delta^{13}C$ value of 3.4 ± 0.2 ‰ (64 % recently fixed C) in CO_2 released from stems of treated trees, with a significant seasonal trend of the isotopic signal ($P < 0.001$; Table 2). A steep drop of the isotopic signal irrespective of the treatment occurred for no obvious reason during winter dormancy 2009/2010 (by 5.9 and 6.6 ‰ for both ambient CO_2 and elevated CO_2, respectively; Fig. 6). Thereafter, stem signals recovered during the course of the 2010 season. In the following winter, no such decline could be observed although weather conditions were similar.

There were clear C-isotope signals in CO_2 released from soils (Fig. 7). First significant deviations between ambient and elevated CO_2 were observed 12 days after the start of the FACE treatment in 2009. The time lap was similar to that in stem air $\delta^{13}C$. However, the mean difference between control and treatment was only 0.6 ‰ by that time (Fig. 7), which accounts for ca. 10 % new C emerging from soil air at this early stage of the experiment. During the entire campaign (starting with the first significant differences 12 days after the onset of FACE), CO_2 enrichment significantly reduced $\delta^{13}C$ in CO_2 released from soil under trees exposed to elevated CO_2 by 1.0 ± 0.1 ‰, corresponding to ca. 15 % new C incorporated ($P = 0.002$; Table 2). During the winter period 2009/2010, a drop in $\delta^{13}C$, very similar to that seen in stem-emitted CO_2, was observed for both ambient and elevated CO_2. This signal even persisted through the early spring season until July 2010. During this period, only minor and non-significant differences in $\delta^{13}C$ between ambient and elevated CO_2 were found. However, from June 2010 onwards, soil air $\delta^{13}C$ under elevated CO_2 was significantly depleted compared to soil air $\delta^{13}C$ under ambient CO_2 (August 2010–October 2011 mean reduction: 1.2 ± 0.1 ‰), which represents 22 % new C. The maximum reduction was 1.8 ‰ in August 2011, which corresponds to ca. 34 % of recently fixed C. Furthermore, we observed stronger ^{13}C signals in soil air collected at 2 m distance to a tree base compared to 3 m distance, further affected by the day of the experiment ($P < 0.001$; Table 2).

Discussion

Canopy-scale CO_2 enrichment has the advantage that the added CO_2 has a low $\delta^{13}C$ value (−30 ‰), which made it possible to quantify the fate of new photoassimilates in 37-m-tall *P. abies* trees in a natural mixed forest ecosystem at a high temporal and spatial resolution. This is the first whole-tree (from the needle to the soil) long-term assessment of the fate of C in tall trees in a natural forest. The intrinsic caveat of using continuous isotope labeling by canopy CO_2 enrichment is that these signals cannot be compared to controls (trees under ambient CO_2 concentrations but exposed to the same isotopic ratio as trees under elevated CO_2). Elevated CO_2 might enhance some transfer processes; hence, the dynamics of C-transfer reported here might be somewhat faster rather than slower, as compared to trees growing under a current atmospheric CO_2 concentration of 395–400 ppm. On the other hand, the current CO_2 concentration is 40 % higher than when these trees were seedlings, and the system (both treated and control trees) may be at or close to CO_2 saturation, suggesting the C-transfer rates observed here may be a reasonable approximation of current 'normal' tree behavior (Leuzinger and Bader 2012). Irrespective of the potential deviations this may incur, our data illustrate the spatial and temporal distribution pattern of freshly assimilated C in such tall trees, and how fast new C is replacing old C.

Our data indicate different rates of integration of new assimilates into the various compartments analyzed. New

foliage and branchlet xylem were built almost exclusively by new photoassimilates when grown in the first spring with FACE. Tree rings took almost 2.5 years until the new tissue consisted entirely of new assimilates. However, latewood of the first season already showed a strong signal a few weeks after the onset of FACE. In contrast, new fine roots seem to utilize more of the old, mobile C pools, and their isotope signal was only half of what we found in the needles after 28 months (ingrowth core data), indicating a slower C turnover than in aboveground tissue. The ^{13}C isotope signal rapidly appears in respiratory CO_2, revealing clear signals from stems and soil within the first 2 weeks of canopy CO_2 enrichment. However, the isotopic signal never reaches the magnitude of that in fresh assimilates (as found in needles, branchlets, and tree rings); hence, it reflects the lasting influence of older stored C sources in these CO_2-releases. In the following, we will discuss in detail the isotope signals observed in the various compartments.

Needles

Sun-exposed needles always showed less negative $\delta^{13}C$ values than shaded needles, which is related to the sun-to-shade difference in the stomatal conductance to photosynthesis ratio (Kaufmann 1982). In shaded plants or plant parts, the ratio of the leaf internal to atmospheric CO_2 concentration (c_i/c_a) is greater than in sun-exposed leaves due to a lower photosynthetic rate and/or higher stomatal conductance (Farquhar et al. 1982). A higher c_i/c_a leads to a higher discrimination of the ^{13}C isotope (i.e. becomes more negative), which is the case with decreasing irradiance and photosynthesis (Livingston et al. 1998).

The initial $\delta^{13}C$ signals in response to FACE in needles produced prior to CO_2 enrichment (i.e. in 2008 and 2009) must reflect the isotopic signature of NSC and other mobile components of needle tissue (Marshall and Linder 2013). In *P. abies*, needles contain ca. 10–27 % of NSC (Hoch et al. 2003; Schädel et al. 2010). This pool was most likely replaced by new C within a few weeks (Keel et al. 2007), corresponding to the 17–23 % of new C found in our bulk sample of old (pre-treatment) grown needles by October 2011, compared to isometer signals. The difference in $\delta^{13}C$ between trees under elevated and ambient CO_2 was clearly bigger in old sun-exposed needles relative to old shaded needles (formed prior to FACE), which is related to higher photosynthetic rates and loading with fresh assimilates (Roberntz and Stockfors 1998), and faster turnover of the non-structural C pools.

In contrast, needles formed during CO_2 enrichment (i.e. in 2010 and 2011) showed much more negative $\delta^{13}C$ values, due to the inclusion of new structural C compounds. Our results indicate that new sun-exposed needles were almost completely self-sustaining (all the new tissue is composed of new C), in line with the branch autonomy theory (Gordon and Larson 1968). In contrast, shaded needles depended more on 'old' C than sun-exposed needles.

All needle age classes exhibited slight seasonal variations with less negative $\delta^{13}C$ values in spring compared to samples analyzed later in the year. We attribute this seasonality in $\delta^{13}C$ to fluctuations in NSC concentration (Flower-Ellis 1993; Hoch et al. 2003; Schädel et al. 2009), resulting in relative shifts of tissue compounds that differ in their isotopic signals. During needle growth and maturation, the concentration in lignin, cutin, waxes, and lipid-related compounds increases. Lignin and lipids show more negative $\delta^{13}C$ signals relative to bulk needles and cellulose (Bowling et al. 2008). Thus, the $\delta^{13}C$ signal of bulk samples should decline even without CO_2 enrichment. Indeed, we found ca. 1 ‰ more negative $\delta^{13}C$ signals in mature, 1-year-old needles, compared to immature needles, which we attribute to the utilization of fresh (early spring) assimilates such as sugars and starch (less negative in $\delta^{13}C$ relative to bulk needles) for primary growth before needles reach C autonomy (Jäggi et al. 2002). A slight age-related decline in photosynthesis (Freeland 1952; Warren 2006) may contribute to that signal.

Branchlet xylem

By the time FACE was initiated on 30 July 2009, earlywood formation in branchlets and stems was already completed; therefore, we assume that only latewood and recently assimilated non-structural C compounds could be responsible for the ^{13}C drop by 0.7–1.3 ‰ in fall 2009. In the following years, $\delta^{13}C$ in branchlets incorporated a stronger signal, since secondary growth adds new xylem layers on top of older-year rings. We assume branchlets to be C autonomous, that is, largely C-supplied by the needles without utilizing stored C from other parts of the tree body (Watson and Casper 1984; Sprugel et al. 1991; Sprugel 2002). Our results suggest that, even in mature trees, primary branchlet formation is mediated almost entirely through new photosynthates produced by new and older needles, with only marginal usage of stored C during the very first stages of branchlet formation (Sprugel 2002; Hoch and Keel 2006).

Tree rings

Tree ring isotope signals indicate a significant but still small contribution (23 %) of novel C to latewood formation in the first treatment season (2009). The gradual $\delta^{13}C$ depletion in tree ring sections of the following years suggests that it takes at least 2 years for novel C to replace all C reserves that feed into xylogenesis. We had not accounted for pre-treatment differences given that we took wood core samples 8 months after the onset of FACE; thus, possible contamination of older-year rings with new assimilates through ray parenchyma cannot

be excluded, but, similar to Marshall and Linder (2013), we see no significant influence of novel C on $\delta^{13}C$ in pre-treatment tree rings. Prior to FACE (i.e. from 2005 until 30 July 2009), there was, however, very little variation in $\delta^{13}C$ signals between early- and latewood given the continuous supply of new photoassimilates due to a nearly permanent photosynthetic season of such evergreen conifers (similar to observations by Jäggi et al. 2002). Accordingly, we found $\delta^{13}C$ of early- and latewood of the same year strongly (1:1) correlated in 2005–2011 ($R^2 = 0.773$; $P < 0.001$).

Fine roots

In control trees, the $\delta^{13}C$ isotope signal of −27.5 ‰ in fine roots marks the midpoint of the range reported by others (Boström et al. 2007; Richter et al. 2009; Andersen et al. 2010), and is 1.1 ‰ less negative than in sunlit needles. This is a general pattern within plants (Hobbie et al. 2002; Badeck et al. 2005), and reflects, for instance, the known downstream post-photosynthetic fractionation processes, resulting in a cumulative enrichment during the assimilate transport from needles to roots and/or plus a change in chemical constituents with less negative $\delta^{13}C$ (e.g., dominance of starch and lignin; Hobbie and Werner 2004; Badeck et al. 2005; Cernusak et al. 2009). The considerable delay in the appearance of new ^{13}C-depleted C in the organic matter of fine roots originating from the added CO_2 suggests that photoassimilates are not directly fed into new root growth. We found no signal in March 2010, although CO_2 enrichment started on 30 July 2009. A rather weak signal compared to other tissues was found at the end of 2011 (2.6–3.0 ‰ difference between elevated and ambient CO_2). This does not contradict the significance of recent photosynthetic activity for root growth, but rather suggests that new assimilates enter a pool of mobile C reserves prior to structural investment in roots (Hansen and Beck 1994; Hobbie et al. 2002). This dilution effect of new C by old C and, therefore, the pool of older photoassimilates can be substantial, as was already concluded for deciduous tree species at the same site (Keel et al. 2007).

The strong incorporation of old C pools (from reserves) in root formation makes it impossible to infer the root age from the fraction of new versus old C in new roots, except with the ^{14}C radiocarbon method. In our case, however, we found that roots could not be older than 20 months (ingrowth cores), but their mixed tissue isotope signal would suggest several years of age (while in reality, some roots may have been only a few weeks old at harvest).

Fungal signals

Ectomycorrhizal basidiocarp formation is believed to be entirely dependent on the provision of new photoassimilates by trees (Högberg et al. 2001, 2010). In the light of this, the C isotope signals of ectomycorrhizal basidiocarps found in the main rooting sphere (<6 m radius) indicate a surprisingly small fraction of only 26–43 % new C provided by our *P. abies* trees in 2010 and 2011, respectively, but it matches the measured ^{13}C content in fine roots. This also holds for fungal species known to be host specific to *P. abies* (in these species, 43–50 % of C is new). Since these fungi may serve several hosts (Brownlee et al. 1983), a signal dilution with C from non-CO_2-enriched trees with overlapping root spheres is likely because our *Picea* trees were neighbored by *Fagus*, *Quercus*, and *Carpinus* trees. This is reflected in the steadily decreasing signal strength with increasing distance to the CO_2-enriched *Picea* trees. Moreover, we assume that the only partial labeling of fungi associated with our treated trees has to do with C-assimilate mixing (new assimilates mixed with old, stored carbohydrates) in the tree before export, possibly isotopically enriched by post-photosynthetic fractionation processes within the plant body, followed by further ^{13}C enrichment during fungal chitin synthesis (sensu Gleixner et al. 1993). As in earlier assessments on this site (Steinmann et al. 2004; Keel et al. 2006), we found a clear distinction between ectomycorrhizal and saprobiotic fungi, with the latter carrying no new C label, hence entirely relying on ‘old’ C.

Respiratory signals

^{13}C signals in CO_2 released from stems were very similar to those in soil CO_2, both in terms of signal magnitude as well as temporal variation ($R^2 = 0.46$, $P < 0.001$). The close correlation of $\delta^{13}C$ in soil CO_2 and $\delta^{13}C$ in stem efflux may indicate a simultaneous appearance of CO_2 respired from new assimilates in stems and their surrounding soils, despite the different path lengths of assimilate transport. CO_2 diffusion in stem tissues may be slower compared to soil CO_2, caused by stem–internal diffusion barriers (Etzold et al. 2013). Yet it is remarkable that the CO_2 released from metabolic processes appears more or less at the same time, which strongly indicates that the C supply for these processes has a high priority. We assume that the $\delta^{13}C$ contribution of CO_2 transported by sapflow from the rooting zone to the stem surface is small (Ubierna et al. 2009b), and that it would not affect the timing of the signal.

The seasonal trends in isotopic signals differed from year to year, unlike the tri-phase pattern reported for mature *P. abies* by Kuptz et al. (2011b). Since about half of the stem CO_2 efflux results from recent photoassimilates, we assume that the other half is from older storage pools, and that the contribution of these two pools did not significantly change over the study period. We cannot explain the observed $\delta^{13}C$ depression in soil and stem CO_2 efflux in the first but not the second winter, since we can exclude methodological

problems, and moisture or temperature regimes did not differ between the two winters. What makes this phenomenon even more difficult to understand are the results of Maunoury et al. (2007), who found $\delta^{13}C$ values of stem efflux to become more negative with rising temperatures, which is in contrast to the pattern that we observed in our study where values became more negative in winter, despite discontinuation of CO_2 enrichment during the coldest 3 months.

Soil air took 12 days after starting canopy CO_2 enrichment to exhibit a small but clear ^{13}C signal. This confirms a rapid transfer of new C to the rhizosphere. The time lag between C assimilation and soil C release exhibited by our trees is larger than the 5–6 days that were reported for late summer signals in 22-m-high *P. abies* (Ekblad et al. 2005). However, assuming a mean transfer velocity of 0.14 m h^{-1} (Jensen et al. 2012), one would expect 11- to 12-day lags for our 37-m-tall trees, which is consistent with our findings, and what was found by Steinmann et al. (2004) for broad-leaved trees. Our data thus support the significance of path length for C transfer in *P. abies* (Mencuccini and Hölttä 2010). It appears that C release via soil microbiota (including mycorrhiza) and structural growth in roots, both strongly depend on C pools older than 3 years. The rapid signal appearance in soil CO_2 suggests a strong coupling of sinks and sources, but the low fraction of new C in CO_2 release (maximum of 34 % 2 years after FACE onset) highlights the significance of a large, sustained contribution of old C pools to soil CO_2 release (Steinmann et al. 2004; Keel et al. 2006; Kuptz et al. 2011a).

Soil CO_2 in the control plots had more negative $\delta^{13}C$ than roots, a widely observed pattern in C_3 plants (Klumpp et al. 2005; Zhu and Cheng 2011), reflecting additional ^{13}C fractionation (−1.2 ‰) during root metabolism, root decay, and microbial activity (Boström et al. 2007; Werth and Kuzyakov 2010; Brüggemann et al. 2011).

Conclusions

In line with our hypothesis, we conclude that new C is rapidly (within 12 days) allocated to belowground organs and metabolized in respiratory processes. Yet, the further away from the crown (source) the greater the fraction of old C that contributes to new growth and C release (sink). Our work alludes to the branch C-autonomy hypothesis, and warns at expecting a tight isotopic linking between concurrent photosynthesis and tree ring formation (Gessler et al. 2009), and root growth (and, presumably, root metabolism) depending on several years old, stored carbohydrates. A clear distinction needs to be made between the rapidity of the new C signal appearance and the quantitative contribution of new C to sink activity.

Overall, tree growth and metabolic processes in stems and soils reflect a legacy signal of C pools older than 3 years. It does not seem that the delay of novel C investment in stems and belowground sinks such as fine roots reflects a transport problem. We rather suggest that most novel C passes through a large non-structural C pool closely associated with the phloem conduits, rather than being invested directly, irrespective of whether or not trees are C saturated. Such a large turnover of non-structural C pools from phloem to storage and back to phloem must incur a substantial metabolic cost, the tradeoff of which may be a highly buffered C provision system in the case of C limitation under stress. Our data show that the needle C pool is turned over within a few late-season weeks. It has been shown for leaves in the top of a tropical forest in Panama that it takes less than 6 days to completely replace this mobile C pool (Würth et al. 1998). Our analysis underlines the central role of intermediary mobile C pools in tree growth and metabolism. This strategy may be an evolutionary legacy from a predominantly low CO_2 world (only 180 ppm CO_2 at the end of the last glaciation), and may have lost its functional significance under current and future high CO_2 concentrations. We may speculate that the extra C assimilated by trees exposed to elevated CO_2 may cause an enhanced turnover (in terms of mass of C compounds) along the phloem path and associated parenchyma in both xylem and phloem. Distributed across the entire tree, the signal of the associated metabolism (respiration) may be too small to be detected per unit of tissue, but it may be large enough to explain a substantial part of the 'missing carbon' in trees exposed to elevated CO_2 that do not exhibit a growth rate, which is matching their photosynthetic stimulation.

Acknowledgments We greatly appreciate help from M. Saurer and K. Lötscher at the Paul Scherrer Institute for C isotope analyses, E. Amstutz for crane operation and FACE maintenance, Georges Grun for FACE supervision, Olivier Bignucolo and several student helpers for their support in data collection and sample processing (notably Martin Trischler as part of his Master thesis), and Rigobert Keller and members of the Mycological Association Basel for fungal taxonomic classification. This project was funded by the Swiss National Science Foundation (Grants 31003AB-126028 and 31003A_140753, 31-67775.02, 3100-059769.99, 3100-067775.02, and 3100AO-111914/1). The crane was sponsored by the Swiss Federal Office of the Environment (FOEN).

References

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising CO_2: mechanisms and environmental interactions. Plant Cell Environ 30:258–270

Andersen CP, Ritter W, Gregg J, Matyssek R, Grams TEE (2010) Below-ground carbon allocation in mature beech and spruce trees following long-term, experimentally enhanced O_3 exposure in Southern Germany. Environ Pollut 158:2604–2609

Andrews JA, Harrison KG, Matamala R, Schlesinger WH (1999) Separation of root respiration from total soil respiration using ^{13}C

labeling during free-air carbon dioxide enrichment (FACE). Soil Sci Soc Am J 63:1429–1435

Badeck FW, Tcherkez G, Nogués S, Piel C, Ghashghaie J (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs: a widespread phenomenon. Rapid Commun Mass Spectrom 19:1381–1391

Bader MKF, Hiltbrunner E, Körner C (2009) Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE). Funct Ecol 23:913–921

Bader MKF, Siegwolf RTW, Körner C (2010) Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. Planta 232:1115–1125

Bader MKF, Leuzinger S, Keel SG, Siegwolf RTW, Hagedorn F, Schleppi P, Körner C (2013) Central European hardwood trees in a high-CO_2 future: synthesis of an 8-year forest canopy CO_2 enrichment project. J Ecol. doi:10.1111/1365-2745.12149

Boström B, Comstedt D, Ekblad A (2007) Isotope fractionation and c-13 enrichment in soil profiles during the decomposition of soil organic matter. Oecologia 153:89–98

Bowling DR, Pataki DE, Randerson JT (2008) Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. New Phytol 178:24–40

Brownlee C, Duddridge JA, Malibari A, Read DJ (1983) The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant Soil 71:433–443

Brüggemann N, Gessler A, Kayler Z, Keel SG, Badeck F, Barthel M, Boeckx P, Buchmann N, Brugnoli E, Esperschütz J, Gavrichkova O, Ghashghaie J, Gomez-Casanovas N, Keitel C, Knohl A, Kuptz D, Palacio S, Salmon Y, Uchida Y, Bahn M (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. Biogeosciences 8:3457–3489

Cernusak LA, Tcherkez G, Keitel C, Cornwell WK, Santiago LS, Knohl A, Barbour MM, Williams DG, Reich PB, Ellsworth DS, Dawson TE, Griffiths HG, Farquhar GD, Wright IJ (2009) Viewpoint: why are non-photosynthetic tissues generally ^{13}C enriched compared with leaves in C_3 plants? Review and synthesis of current hypotheses. Funct Plant Biol 36:199–213

Ceulemans R, Mousseau M (1994) Tansley review no-71: effects of elevated atmospheric CO_2 on woody-plants. New Phytol 127:425–446

Comstedt D, Boström B, Marshall JD, Holm A, Slaney M, Linder S, Ekblad A (2006) Effects of elevated atmospheric carbon dioxide and temperature on soil respiration in a boreal forest using $\delta^{13}C$ as a labeling tool. Ecosystems 9:1266–1277

Dannoura M, Maillard P, Fresneau C, Plain C, Berveiller D, Gerant D, Chipeaux C, Bosc A, Ngao J, Damesin C, Loustau D, Epron D (2011) In situ assessment of the velocity of carbon transfer by tracing ^{13}C in trunk CO_2 efflux after pulse labelling: variations among tree species and seasons. New Phytol 190:181–192

Ekblad A, Boström B, Holm A, Comstedt D (2005) Forest soil respiration rate and $\delta^{13}C$ is regulated by recent above ground weather conditions. Oecologia 143:136–142

Endrulat T, Saurer M, Buchmann N, Brunner I (2010) Incorporation and remobilization of ^{13}C within the fine-root systems of individual *Abies alba* trees in a temperate coniferous stand. Tree Physiol 30:1515–1527

Epron D, Ngao J, Dannoura M, Bakker MR, Zeller B, Bazot S, Bosc A, Plain C, Lata JC, Priault P, Barthes L, Loustau D (2011) Seasonal variations of belowground carbon transfer assessed by in situ $^{13}CO_2$ pulse labeling of trees. Biogeosciences 8:1153–1168

Epron D, Bahn M, Derrien D, Lattanzi FA, Pumpanen J, Gessler A, Högberg P, Maillard P, Dannoura M, Gerant D, Buchmann N (2012) Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. Tree Physiol 32:776–798

Etzold S, Zweifel R, Ruehr NK, Eugster W, Buchmann N (2013) Long-term stem CO_2 concentration measurements in Norway spruce in relation to biotic and abiotic factors. New Phytol 197:1173–1184

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the inter-cellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137

Fatichi S, Leuzinger S (2013) Reconciling observations with modeling: the fate of water and carbon allocation in a mature deciduous forest exposed to elevated CO_2. Agric For Meteorol 174:144–157

Flower-Ellis JGK (1993) Dry-matter allocation in Norway spruce branches: a demographic approach. Stud For Suec 191:51–73

Freeland RO (1952) Effect of age of leaves upon the rate of photosynthesis in some conifers. Plant Physiol 27:685–690

Friedlingstein P, Houghton RA, Marland G, Hackler J, Boden TA, Conway TJ, Canadell JG, Raupach MR, Ciais P, Le Quéré C (2010) Update on CO_2 emissions. Nat Geosci 3:811–812

Gessler A, Brandes E, Buchmann N, Helle G, Rennenberg H, Barnard RL (2009) Tracing carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree-ring archive. Plant Cell Environ 32:780–795

Gleixner G, Danier HJ, Werner RA, Schmidt HL (1993) Correlations between the ^{13}C content of primary and secondary plant-products in different cell compartments and that in decomposing basidiomycetes. Plant Physiol 102:1287–1290

Gordon JC, Larson PR (1968) Seasonal course of photosynthesis respiration and distribution of ^{14}C in young *Pinus resinosa* trees as related to wood formation. Plant Physiol 43:1617–1624

Hansen J, Beck E (1994) Seasonal-changes in the utilization and turnover of assimilation products in 8-year-old Scots pine (*Pinus-sylvestris* L.) trees. Trees Struct Funct 8:172–182

Hansen J, Vogg G, Beck E (1996) Assimilation, allocation and utilization of carbon by 3-year-old Scots pine (*Pinus sylvestris* L.) trees during winter and early spring. Trees Struct Funct 11:83–90

Hättenschwiler S, Handa IT, Egli L, Asshoff R, Ammann W, Körner C (2002) Atmospheric CO_2 enrichment of alpine treeline conifers. New Phytol 156:363–375

Hobbie EA, Werner RA (2004) Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis. New Phytol 161:371–385

Hobbie EA, Tingey DT, Rygiewicz PT, Johnson MG, Olszyk DM (2002) Contributions of current year photosynthate to fine roots estimated using a ^{13}C-depleted CO_2 source. Plant Soil 247:233–242

Hoch G (2005) Fruit-bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees. Plant Cell Environ 28:651–659

Hoch G, Keel SG (2006) ^{13}C labelling reveals different contributions of photoassimilates from infructescences for fruiting in two temperate forest tree species. Plant Biol 8:606–614

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26:1067–1081

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792

Högberg P, Högberg MN, Göttlicher SG, Betson NR, Keel SG, Metcalfe DB, Campbell C, Schindlbacher A, Hurry V, Lundmark T, Linder S, Näsholm T (2008) High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. New Phytol 177:220–228

Högberg MN, Keel SG, Metcalfe DB, Campbell C, Midwood AJ, Thornton B, Hurry V, Linder S, Näsholm T, Högberg P (2010) Quantification of effects of season and nitrogen supply on tree

below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytol 187:485–493

Holt CC (1957) Forecasting trends and seasonals by exponentially weighted moving averages. ONR Research Memorandum. Carnegie Institute of Technology 52

Idso SB (1999) The long-term response of trees to atmospheric CO_2 enrichment. Glob Change Biol 5:493–495

Jäggi M, Saurer M, Fuhrer J, Siegwolf RTW (2002) The relationship between the stable carbon isotope composition of needle bulk material, starch, and tree rings in *Picea abies*. Oecologia 131:325–332

Jensen KH, Liesche J, Bohr T, Schulz A (2012) Universality of phloem transport in seed plants. Plant Cell Environ 35:1065–1076

Kagawa A, Sugimoto A, Maximov TC (2006) Seasonal course of translocation, storage and remobilization of ^{13}C pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings. New Phytol 171:1571–1584

Kaufmann MR (1982) Leaf conductance as a function of photosynthetic photon flux-density and absolute-humidity difference from leaf to air. Plant Physiol 69:1018–1022

Keel SG, Siegwolf RTW, Körner C (2006) Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. New Phytol 172:319–329

Keel SG, Siegwolf RTW, Jäggi M, Körner C (2007) Rapid mixing between old and new C pools in the canopy of mature forest trees. Plant Cell Environ 30:963–972

Keeling CD (1958) The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim Cosmochim Acta 13:322–334

Klumpp K, Schäufele R, Lötscher M, Lattanzi FA, Feneis W, Schnyder H (2005) C-isotope composition of CO_2 respired by shoots and roots: fractionation during dark respiration? Plant Cell Environ 28:241–250

Körner C (2000) Biosphere responses to CO_2 enrichment. Ecol Appl 10:1590–1619

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

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

Kuptz D, Fleischmann F, Matyssek R, Grams TEE (2011a) Seasonal patterns of carbon allocation to respiratory pools in 60-year-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling. New Phytol 191:160–172

Kuptz D, Matyssek R, Grams TEE (2011b) Seasonal dynamics in the stable carbon isotope composition ($\delta^{13}C$) from non-leafy branch, trunk and coarse root CO_2 efflux of adult deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees. Plant Cell Environ 34:363–373

Le Quéré C, Raupach MR, Canadell JG, Marland G, Bopp L, Ciais P, Conway TJ, Doney SC, Feely RA, Foster P, Friedlingstein P, Gurney K, Houghton RA, House JI, Huntingford C, Levy PE, Lomas MR, Majkut J, Metzl N, Ometto JP, Peters GP, Prentice IC, Randerson JT, Running SW, Sarmiento JL, Schuster U, Sitch S, Takahashi T, Viovy N, van der Werf GR, Woodward FI (2009) Trends in the sources and sinks of carbon dioxide. Nat Geosci 2:831–836

Leuzinger S, Bader MKF (2012) Experimental vs. modeled water use in mature Norway spruce (Picea abies) exposed to elevated CO2. Front Plant Sci 3:229

Leuzinger S, Luo Y, Beier C, Dieleman W, Vicca S, Körner C (2011) Do global change experiments overestimate impacts on terrestrial ecosystems? Trends Ecol Evol 26:236–241

Lippu J (1994) Patterns of dry matter partitioning and ^{14}C-photosynthate allocation in 1.5-year-old Scots pine seedlings. Silva Fenn 28:145–153

Livingston NJ, Whitehead D, Kelliher FM, Wang YP, Grace JC, Walcroft AS, Byers JN, McSeveny TM, Millard P (1998) Nitrogen allocation and carbon isotope fractionation in relation to intercepted radiation and position in a young *Pinus radiata* D. Don tree. Plant Cell Environ 21:795–803

Marshall JD, Linder S (2013) Mineral nutrition and elevated CO_2 interact to modify $\delta^{13}C$, an index of gas exchange, in Norway spruce. Tree Physiol 33:1132–1144

Maunoury F, Berveiller D, Lelarge C, Pontailler J-Y, Vanbostal L, Damesin C (2007) Seasonal, daily and diurnal variations in the stable carbon isotope composition of carbon dioxide respired by tree trunks in a deciduous oak forest. Oecologia 151:268–279

McCarthy HR, Oren R, Johnsen KH, Gallet-Budynek A, Pritchard SG, Cook CW, LaDeau SL, Jackson RB, Finzi AC (2010) Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric CO_2 with nitrogen and water availability over stand development. New Phytol 185:514–528

Medhurst J, Parsby J, Linder S, Wallin G, Ceschia E, Slaney M (2006) A whole-tree chamber system for examining tree-level physiological responses of field-grown trees to environmental variation and climate change. Plant Cell Environ 9:1853–1869

Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomäki S, Laitat E, Rey A, Roberntz P, Sigurdsson BD, Strassemeyer J, Wang K, Curtis PS, Jarvis PG (2001) Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. New Phytol 149:247–264

Mencuccini M, Hölttä T (2010) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytol 185:189–203

Norby RJ, Zak DR (2011) Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annu Rev Ecol Evol Syst 42:181–203

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci USA 107:19368–19373

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schäfer KVR, McCarthy H, Hendrey G, McNulty SG, Katul GG (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411:469–472

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

Popp M, Lied W, Meyer AJ, Richter A, Schiller P, Schwitte H (1996) Sample preservation for determination of organic compounds: microwave versus freeze-drying. J Exp Bot 47:1469–1473

R Development Core Team (2008–2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Richter A, Wanek W, Werner RA, Ghashghaie J, Jäggi M, Gessler A, Brugnoli E, Hettmann E, Göttlicher SG, Salmon Y, Bathellier C, Kodama N, Nogués S, Søe A, Volders F, Sörgel K, Blöchl A, Siegwolf RTW, Buchmann N, Gleixner G (2009) Preparation of starch and soluble sugars of plant material for the analysis of carbon isotope composition: a comparison of methods. Rapid Commun Mass Spectrom 23:2476–2488

Ritter W, Andersen CP, Matyssek R, Grams TEE (2011) Carbon flux to woody tissues in a beech/spruce forest during summer and in response to chronic O_3 exposure. Biogeosciences 8:3127–3138

Roberntz P, Stockfors J (1998) Effects of elevated CO_2 concentration and nutrition on net photosynthesis, stomatal conductance

and needle respiration of field-grown Norway spruce trees. Tree Physiol 18:233–241

Schädel C, Blöchl A, Richter A, Hoch G (2009) Short-term dynamics of nonstructural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. Tree Physiol 29:901–911

Schädel C, Richter A, Blöchl A, Hoch G (2010) Hemicellulose concentration and composition in plant cell walls under extreme carbon source-sink imbalances. Physiol Plant 139:241–255

Schäfer KVR, Oren R, Ellsworth DS, Lai CT, Herrick JD, Finzi AC, Richter DD, Katul GG (2003) Exposure to an enriched CO_2 atmosphere alters carbon assimilation and allocation in a pine forest ecosystem. Glob Change Biol 9:1378–1400

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

Schlesinger WH, Bernhardt ES, DeLucia EH, Ellsworth DS, Finzi AC, Hendrey GR, Hofmockel KS, Lichter J, Matamala R, Moore D, Oren R, Pippen JS, Thomas RB (2006) The Duke forest FACE experiment: CO_2 enrichment of a loblolly pine forest. In: Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H (eds) Ecological studies 187. Springer, Berlin, pp 197–212

Sigurdsson BD, Medhurst JL, Wallin G, Eggertsson O, Linder S (2013) Growth of mature boreal Norway spruce was not affected by elevated CO_2 and/or air temperature unless nutrient availability was improved. Tree Physiol 33:1192–1205

Spinnler D, Egli P, Körner C (2002) Four-year growth dynamics of beech-spruce model ecosystems under CO_2 enrichment on two different forest soils. Trees Struct Funct 16:423–436

Sprugel DG (2002) When branch autonomy fails: Milton's law of resource availability and allocation. Tree Physiol 22:1119–1124

Sprugel DG, Hinckley TM, Schaap W (1991) The theory and practice of branch autonomy. Annu Rev Ecol Syst 22:309–334

Steinmann KTW, Siegwolf RTW, Saurer M, Körner C (2004) Carbon fluxes to the soil in a mature temperate forest assessed by C-13 isotope tracing. Oecologia 141:489–501

Ubierna N, Marshall JD, Cernusak LA (2009a) A new method to measure carbon isotope composition of CO_2 respired by trees: stem CO_2 equilibration. Funct Ecol 23:1050–1058

Ubierna N, Kumar AS, Cernusak LA, Pangle RE, Gag PJ, Marshall JD (2009b) Storage and transpiration have negligible effects on ^{13}C of stem CO_2 efflux in large conifer trees. Tree Physiol 29:1563–1574

von Felten S, Hättenschwiler S, Saurer M, Siegwolf RTW (2007) Carbon allocation in shoots of alpine treeline conifers in a CO_2 enriched environment. Trees Struct Funct 21:283–294

Warren CR (2006) Why does photosynthesis decrease with needle age in *Pinus pinaster*? Trees Struct Funct 20:157–164

Warren JM, Iversen CM, Garten CT Jr, Norby RJ, Childs J, Brice D, Evans RM, Gu L, Thornton P, Weston DJ (2012) Timing and magnitude of C partitioning through a young Loblolly pine (*Pinus taeda* L.) stand using ^{13}C labeling and shade treatments. Tree Physiol 32:799–813

Watson MA, Casper BB (1984) Morphogenetic constraints on patterns of carbon distribution in plants. Annu Rev Ecol Syst 15:233–258

Werth M, Kuzyakov Y (2010) ^{13}C fractionation at the root-microorganisms-soil interface: a review and outlook for partitioning studies. Soil Biol Biochem 42:1372–1384

Würth MKR, Winter K, Körner C (1998) Leaf carbohydrate responses to CO_2 enrichment at the top of a tropical forest. Oecologia 116:18–25

Zhu B, Cheng W (2011) ^{13}C isotope fractionation during rhizosphere respiration of C_3 and C_4 plants. Plant Soil 342:277–287

Chapter 3

Respiratory fluxes and fine root responses in mature *Picea abies* trees exposed to elevated atmospheric CO_2 concentrations

Manuel Mildner, Martin K.-F. Bader, Christina Baumann and Christian Körner

published in *Biogeochemistry 124 (2015):95-111. DOI 10.1007/s10533-015-0084-5*

Biogeochemistry
DOI 10.1007/s10533-015-0084-5

Respiratory fluxes and fine root responses in mature *Picea abies* trees exposed to elevated atmospheric CO_2 concentrations

Manuel Mildner · Martin Karl-Friedrich Bader · Christina Baumann · Christian Körner

Received: 1 June 2014 / Accepted: 15 February 2015

Abstract With their dominant share in global plant biomass carbon (C), forests and their responses to atmospheric CO_2 enrichment are key to the global C balance. In this free air CO_2 enrichment (FACE) study, we assessed respiratory losses from stems and soil, and fine root growth of ca. 110-year-old *Picea abies* growing in a near-natural forest in NW Switzerland. We anticipated a stimulation of all three variables in response to a ca. 150 ppm higher CO_2 concentration in the tree canopies. During the first 2.5 years of the experiment, stem CO_2 efflux (R_{stem}) remained unresponsive to CO_2 enrichment. This indicates that there is no enhancement of metabolic activity in phloem and xylem of these mature trees. Soil CO_2 efflux (R_{soil}) beneath trees experiencing elevated CO_2 (eCO_2) showed a slight but significant reduction compared to R_{soil} under control trees. High CO_2 trees did not increase their fine root biomass in in-growth cores after 20 months under FACE relative to the fine root fractions collected in undisturbed soil. Tree growth (stem radial increment, not shown here) remained completely unchanged although earlier experiments showed largest responses (if any) during the early years after a step increase in atmospheric CO_2 concentration. The data presented here suggest C saturation of the study trees at the current close to 400 ppm CO_2 ambient concentrations. Together with the high local atmospheric N-deposition rates (ca. 20 kg N ha^{-1} a^{-1}), our findings imply that factors other that C and N supply appear to constrain growth and metabolism of these mature *P. abies* trees under eCO_2.

Keywords Conifers · FACE · Forest · Growth · Soil · Stem · Carbon

Responsible Editor: W. Troy Baisden.

Electronic supplementary material The online version of this article (doi:10.1007/s10533-015-0084-5) contains supplementary material, which is available to authorized users.

M. Mildner (✉) · M. K.-F. Bader · C. Baumann · C. Körner
Institute of Botany, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland
e-mail: manuel.mildner@unibas.ch

M. K.-F. Bader
New Zealand Forest Research Institute (SCION), Te Papa Tipu Innovation Park, 49 Sala Street, 3046 Rotorua, New Zealand

C. Baumann
UTAS AG Büro für Landschaft, Natur und Siedlung, Brünigstrasse 64, 6074 Giswil, Switzerland

Introduction

The rising levels of atmospheric CO_2 are potentially affecting forest biomes not only indirectly via the climatic change, but also directly via potentially enhanced CO_2 uptake by tree canopies. Higher leaf-

Published online: 21 February 2015

level CO_2 uptake of forest trees in response to elevated CO_2 (eCO_2) was repeatedly reported (Bader et al. 2010; Darbah et al. 2010; Ellsworth et al. 2012). However, this additional C uptake mostly resulted in a less-than-anticipated, or no long-term increase in growth or net primary productivity in maturing trees (Körner 2006; Norby and Zak 2011; Leuzinger and Hättenschwiler 2013; Sigurdsson et al. 2013). The direct effect of CO_2 via photosynthesis might be masked by a set of counteracting biotic and abiotic effects on tree growth (Körner 2000; Leuzinger and Hättenschwiler 2013). Soil nutrient availability, stand development, and species identity are influencing the potential CO_2 fertilization effect (Hättenschwiler et al. 1997; De Graaff et al. 2006; Norby et al. 2010; Bader et al. 2013). The imbalance between increased foliar C uptake without corresponding aboveground growth response to eCO_2 might be compensated by a stimulation of fine root growth, or by increased respiratory release of CO_2 to the atmosphere.

Although total fine root mass (<2 % of total tree biomass; Körner 1994) contributes little to ecosystem biomass C-stores (<1 %, including in soil organic matter), the turnover of fine roots provides a significant source for soil humus formation. The rapid turnover of fine roots may, in fact, contribute between 20 and 50 % to annual biomass production (Jackson et al. 2009), and thus, plays a significant role in the global C cycle (Matamala et al. 2003). Some studies on young, expanding systems arrived at ca. 40 % increase in fine root biomass at eCO_2 (Curtis and Wang 1998). These results are most likely due to a faster exploration of 'empty' soil when these young trees grew toward canopy closure (unlikely a steady-state signal for mature forests). The Oak Ridge free air CO_2 enrichment (FACE) study in a plantation of *Liquidambar styraciflua* (10-year-old when the study was initiated in 1997) initially reported several years of increased fine root production (Norby et al. 2004), which led to increased C fluxes to the soil (Jastrow et al. 2005; Iversen et al. 2012). However, this CO_2-induced belowground growth stimulation ceased completely towards the end of the 11-year enrichment period, which was explained by the higher nitrogen (N) demand for greater C uptake (Norby et al. 2010; Garten et al. 2011). Intriguingly, a study with *Pinus taeda* (Duke FACE; initiated in 1996 with then 13-year-old trees) did not reveal increased soil C accumulation (Phillips et al. 2012) despite accelerated belowground C fluxes and higher fine root production belowground (Pritchard et al. 2008; Jackson et al. 2009; Drake et al. 2011). Phillips et al. (2012) highlighted that accelerated microbial activity under eCO_2 not only enhances the mineralization of soil organic matter pools (soil priming; Jenkinson et al. 1985) but also offsets the increased input of root-derived C under eCO_2 (rhizodepositions, exudation, and allocation to mycorrhizal fungi) by enhancing the decomposition of these compounds. Similar results were recently obtained in a CO_2-enriched scrub-oak community (Hungate et al. 2013). Such priming processes can release additional N, which becomes readily available for tree metabolism (increase N-cycling), and might thus slow the natural, progressive N limitation (PNL) as forests mature. Additionally, deeper soil exploration by roots under eCO_2 might further increase the availability of N (at least transitorily; Pritchard et al. 2008; Iversen et al. 2011). Stimulated microbial activity also accelerated the returns of CO_2 from the soil to the atmosphere in this pine forest (Jackson et al. 2009). CO_2-driven priming effects such as described by Phillips et al. (2012) are in accordance with results of the 8-year FACE study on mature deciduous trees growing under near-natural, but N-saturated conditions at our study site (Bader et al. 2013). Here, soil N availability (Schleppi et al. 2012), and microbial biomass increased significantly under eCO_2 (Bader and Körner 2010). However, in contrast to the *P. taeda* results at Duke FACE, no aboveground growth (Bader et al. 2013), no stimulation of soil CO_2 efflux (Bader and Körner 2010), and reduced fine root biomass (Bader et al. 2009) were observed in these deciduous trees despite strong photosynthetic stimulation by eCO_2 (Bader et al. 2010).

Higher respiratory CO_2 release from soils (Drake et al. 2011) would be a consequence of increased belowground C supply under eCO_2 (growth and turnover of roots, rhizodeposition, metabolic activity of roots and mycorrhizal partners), assuming that soil microbes are limited by labile C (Fierer et al. 2009). This might reverse the effect of the often-anticipated eCO_2 'fertilization' on forest ecosystems (Raich and Schlesinger 1992). Higher soil CO_2 efflux (R_{soil}) under trees exposed to eCO_2 has been reported frequently (Spinnler et al. 2002; Bernhardt et al. 2006; Comstedt et al. 2006; Pregitzer et al. 2008; Jackson et al. 2009). However, all these test systems contained young trees

with expanding root spheres. Not surprisingly, these initial effects declined with time, and were not observed under mature trees in mixed forest stands (Bader and Körner 2010), or in monospecific plantations (King et al. 2004).

Stem CO_2 efflux (R_{stem}) can contribute 13–42 % to the total aboveground C budget of trees (Waring and Schlesinger 1985; Hamilton et al. 2002). R_{stem} responses to eCO_2 have mostly been reported in juvenile trees, and they vary considerably (i.e. reductions and increases; Carey et al. 1996; Janouš et al. 2000; Edwards et al. 2002; Hamilton et al. 2002; Zha et al. 2005; Acosta et al. 2010). So far, we do not see any aboveground growth stimulation following CO_2 enrichment in the tall trees examined here (T. Klein and C. Körner, unpublished), despite indications of increased leaf-level C uptake under eCO_2 (Leuzinger and Bader 2012; Bader et al., in prep.; T. Klein, pers. comm.). Therefore, any CO_2-driven stimulation of R_{stem} would reflect higher phloem activity or maintenance respiration, or a signal resulting from enhanced CO_2 release in the rhizosphere, from where the respiratory C may find its way into R_{stem} via the xylem sap.

We used the Swiss Canopy Crane (SCC) web-FACE facility (Pepin and Körner 2002) to expose the canopies of 37-m tall, and ca. 110-year-old *P. abies* to increased levels of atmospheric CO_2. The effectiveness of CO_2 enrichment could be confirmed by C isotope signals (Mildner et al. 2014). Here we report the initial responses (i.e. the first 2.5 years of FACE) of *P. abies* to atmospheric CO_2 enrichment, with a focus on stem and soil CO_2 release, and fine root production. We hypothesized (i) a stimulation in fine root production, (ii) enhanced CO_2 efflux from soils, and (iii) greater stem CO_2 efflux under eCO_2 compared to ambient conditions.

Materials and methods

Study site and experimental setup

The experiment was established in a highly diverse, near-natural forest 12 km south-west of Basel, Switzerland (47°33′N, 7°36′E, 500 m a.s.l), dominated by ca. 100–120-year-old deciduous and coniferous trees (dominant species are i.e. *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl., *Carpinus betulus* L., *Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.; Fig. S4). The site has a mild temperate climate, with seasonal mean temperatures (May–September) of 14.7 °C, and ca. 800 mm a^{-1} precipitation (Bader and Körner 2010). In 2009, five 37 m tall, 110-year-old Norway spruce (*P. abies*) individuals were equipped with an improved web-FACE system (Pepin and Körner 2002; Körner et al. 2005; Mildner et al. 2014) using a 45 m tall canopy crane. CO_2 was released into the tree canopies through non-invasive laser-punched tubes (4 mm diameter) woven around the tree branches, allowing for computer-controlled adjustment of the CO_2 release with regard to wind direction by employing sectional control of CO_2. The web-FACE technique applied here provided the best possible means to enrich mature *P. abies* trees with additional CO_2 given the tree height, and the complexity of the conditions on-site. Those limitations of the web-FACE technique have been discussed in more detail elsewhere (see Pepin and Körner 2002). Our system showed good spatio-temporal performance (Leuzinger, pers. comm.). Median CO_2 concentrations were between 500 and 560 ppm in the canopies (60 sampling points per IRGA reading), with means of 541, 532, and 541 ppm for 2009, 2010, and 2011, respectively (Mildner et al. 2014). We discontinued the FACE treatment if either temperatures were below 4 °C, PPFD was <100 µmol, or wind was above 10 m s^{-1}. So, FACE was largely off during the coldest period from early November until early March (4 months). CO_2 enrichment started on 30 July 2009. Only the tree canopy between 15 and 37 m aboveground was CO_2-enriched, with no downward flow, preventing uncontrolled 'CO_2 pollution' of the understory vegetation and soil surface. Since the CO_2 employed for canopy enrichment carries a constant ^{13}C isotope signal ($\delta^{13}C$ −30 ‰), it was possible to trace the carbon flows in trees and soils. Together with IRGA-based monitoring of CO_2 concentrations in the canopy air, this isotopic C tracing allowed us to assess the effectiveness of the web-FACE system, and to show that there was no contamination of the control trees by extra CO_2 (Mildner et al. 2014). The CO_2-treated trees (eCO_2-trees) formed a group, facilitating CO_2 enrichment and clear association of signals with investigated trees (Fig. S4). Five similarly tall trees under ambient CO_2 (aCO_2), away from the treated trees, served as controls (aCO_2-trees). All but one of these aCO_2-trees were outside the perimeter of the crane's jib.

Climate variables

Hourly temperatures at different heights (10 cm belowground, T_{soil}; at the soil surface in the litter layer, T_{litter}; 2 m aboveground, T_{air}) were recorded next to an eCO_2-tree using a temperature data logger (HOBO TidbiT v2; Onset Computer Corp., Bourne, MA, USA). Technical failure caused incomplete datasets that could not be complemented by statistical interpolation (see Fig. 1). Starting in August 2008 (a year before FACE), soil moisture (vol. %) at 0–10 cm depth was recorded every 6 h around the investigated trees (11 and 18 sensors arranged around the eCO_2- and aCO_2-trees, respectively) using soil moisture probes, connected to a self-contained data logger (10HS and EM50, Decagon Devices Ltd., Pullman, Washington, DC, USA). Precipitation was recorded every 2 min, provided by a weather station situated 2 km from the SCC site (Flüh, Solothurn, Switzerland). Precipitation was summed on a daily basis.

Fine root sampling

On 24 March 2010, 8 months after the onset of FACE, or 4 months of effective canopy CO_2 enrichment, we took 9 soil cores (12 cm in depth × 3.6 cm diameter) per tree in the main rooting sphere (2 m around the tree trunks) to ensure that we captured the fine roots of *P. abies*. The 9 soil cores were organized into three groups of three soil cores (triplets). The triplets were placed at an angle of 120° around each trunk, with 10 cm distance between each soil core in a triplet. The fine root biomass found in the soil cores was averaged per tree to account for microscale heterogeneity. We used these coring holes to install equally sized in-growth cores (cylinders made of a 2 mm stiff mesh), filled with sieved, root-free soil collected on-site. The soil in the in-growth cores was gently compacted to match the in situ bulk soil density (mass to volume ratio). The in-growth cores were extracted 20 months later (6 December 2011) by means of a knife. The soil and in-growth cores were kept frozen

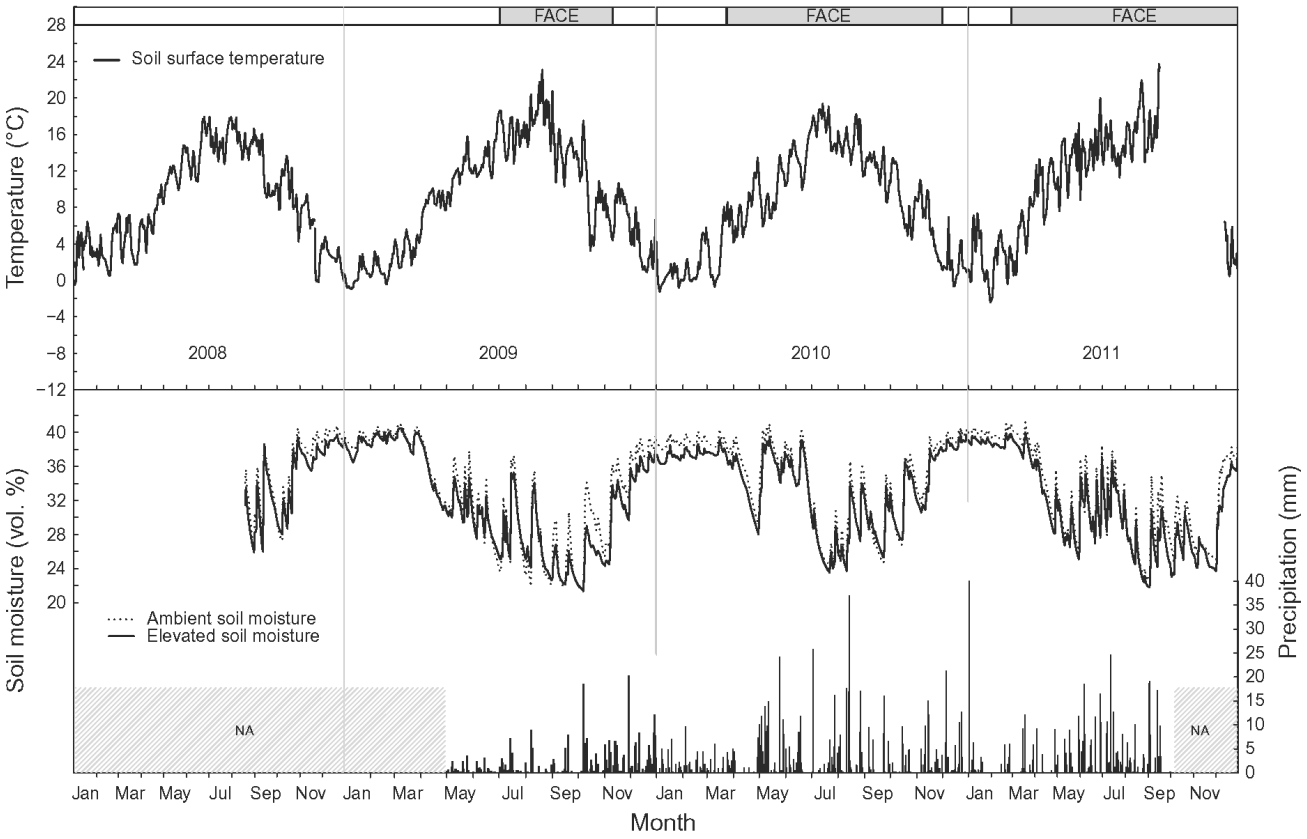

Fig. 1 Seasonal variation of daily soil surface T under litter (*solid black line* in the *upper four panels*), precipitation (*vertical bars*), and soil moisture in the top 10 cm (*lower four panels*) measured either at the swiss canopy crane (SCC) site (T and soil moisture), or taken from a nearby weather station 2 km away from the SCC site (precipitation) in the years 2008 to 2011 (*left* to *right*). Soil moisture was measured either under control *Picea abies* trees (*dashed line*), or under CO_2 treated trees (*solid line*)

at −20 °C until further analysis. The cores were defrosted in cold water for 48 h at 4 °C before processing to slow microbial degradation of fine roots. Fine roots were extracted using a sieve (1 mm mesh) and tweezers. *P. abies* fine roots were selected on the basis of a *P. abies* reference root collection. The distinct morphology of *P. abies* roots warranted the separation of *P. abies* roots from roots of other species (as later confirmed by $\delta^{13}C$ signals; Mildner et al. 2014). We did not quantify the fraction of non-*P. abies* fine roots at the time of harvest. However, we revisited the fine root fraction matter and re-sampled the same location, and weighed the non-*P. abies* fine root fraction in autumn 2014. We found that half of the fine roots were from *P. abies*, and the other half belonged to the surrounding trees of this semi-natural mixed forest. Fine roots were classified into three diameter classes (<0.5, 0.5–1, 1–2 mm), dried at 80 °C for 48 h, and weighed for biomass determination. No differentiation of still intact dead and live fine roots was made.

Soil respiration

We measured CO_2 release from the forest floor, hereafter referred to as soil respiration (R_{soil}; μmol CO_2 m^{-2} s^{-1}), with two identical custom-made, closed, non-steady-state, non-through-flow chambers. The chambers were equipped with open path, non-dispersive infrared gas analysers, and relative humidity/T sensors (GMP343 carbon dioxide probe, HMP75 rH/T probe; Vaisala, Vantaa, Finland; detailed description of the system in Bader and Körner 2010). Polypropylene collars (Ø 20, 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 replicates for each tree. These collars were left in place throughout the course of the experiment. Photosynthetically 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, 1 year before FACE initiation, and were intensified after the onset of FACE on 30 July 2009. During winter, measurements were suspended when snow covered the ground. Chamber recordings were performed at maximum daytime R_{soil} rates (i.e. from 1 to 6 pm), alternating between eCO_2- and aCO_2-trees to reduce any temporal bias. R_{soil} rates were calculated by applying a linear regression to the increase of the CO_2 concentration inside the chamber headspace over 4 min (60 recordings per 5 min, the first minute of each measurement were discarded to account for potential chamber placement effects). Soil temperature at 10 cm soil depth (T_{soil}) was recorded simultaneously adjacent to the collars using a KM20REF thermometer (Comark, Instruments, Norwich, UK).

Stem respiration

Stem CO_2 release, hereafter referred to as stem respiration (R_{stem}; μmol CO_2 m^{-2} s^{-1}), was measured using the LI-COR 6400-09 Soil CO_2 Flux Chamber connected to a LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). The soil chamber operated in a closed system mode, and CO_2 drawdown inside the headspace allowed us to measure multiple cycles. We recorded 2–3 cycles per measurement and calculated the average. Four circular polyethylene collars (Ø 10, 4–5 cm high) were attached to the stem surface of each tree at ca. 1.3 m above ground, facing the cardinal directions (N, E, S, W). We used hot-melt adhesive and sealent (Terostat-IX, Teroson, Ludwigsburg, Germany) to ensure airtight collar connection to the stem surface. These collars served as chamber sockets. We did not install T sensors inside the stem sapwood. Thus, air temperature measured directly on the bark (T_{bark}) served as temperature reference using the LI-COR 6000-09TC Soil Probe Thermocouple (LI-COR, Lincoln, Nebraska, USA). We started measurements prior to the start of FACE (pre-treatment). We regarded R_{stem} signals 7 days after the onset of FACE as pre-treatment signals since the lag between leaf-level C assimilation and signal detection in R_{stem} is ca. 12 days (Mildner et al. 2014). Measurements were taken in 1–3 month intervals in 2009 and 2010, with two final measurements early in 2011.

Data analysis

The T dependency of respiratory fluxes (soil and stem) was modeled using a nonlinear least squares regression following Lloyd and Taylor (1994):

$$R = R_{10}\, e^{E_0\left(\frac{1}{56.02} - \frac{1}{T-227.13}\right)}, \quad (1)$$

where R is the measured respiration rate (either R_{soil} or R_{stem}) and R_{10} the respiration rate at 10 °C (T_{soil} for

soil respiration, and T_{bark} for stem respiration), E_O is the activation energy. The Lloyd and Taylor (LT) approach outperforms conventional R versus T correlation models (Arrhenius, van't Hoff). The T sensitivity of R_{soil} or R_{stem} (Q_{10}) was modeled following:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10^{\circ}C}{T2-T1}\right)}, \quad (2)$$

where R_1 and R_2 are the respiration rates at temperatures of 10 °C (T_1) and 20 °C (T_2) (at 10 cm soil depth or at the bark surface), respectively, derived from the modeled LT regression. Pre-treatment differences in R_{soil} between trees later exposed to eCO_2 and aCO_2 were accounted for by assigning a temperature dependent correction factor to R_{soil} of eCO_2-trees in the FACE period. This correction factor was calculated from the difference of the modeled LT curves (Eq. 1) between eCO_2 and aCO_2 during the pre-treatment years (2008 and 2009). However, since the pre-treatment data for R_{stem} did not allow for modeling a R versus T relationship (insufficient T range), R_{stem} of eCO_2-trees in the FACE period was standardized by the mean pre-treatment aCO_2/eCO_2 difference, thus assuming that the T response of R_{stem} did not change. These corrected respiratory fluxes were used to model the LT regression of eCO_2-trees under FACE (Eq. 1), and further parameters (Q_{10}, R_{10}; Eq. 2). Confidence limits for the modeled Q_{10} and R_{10} values were obtained from bootstrapped 95 % confidence intervals. Annual release of C by stems, or soil, respectively, was calculated based upon the modeled LT regression (Eq. 1) by summing the estimated hourly R rates from continuously logged temperatures for all investigated years. We used either hourly records of T_{air} to calculate the annual C release by stems, or continuously available groundlitter (soil surface) temperature, correlating well with T_{soil} to calculate annual C release by the soil (missing values in the second half of 2011 were reconstructed from T_{air}; see Fig. 1). We know that the T response of R_{soil} does not differ between day and night based on diurnal respiration measurements (Bader et al. in prep.). Data analysis was performed using the software R, version 2.15.0 (R Development Core Team 2011).

Statistical analysis

Linear mixed effects models fitted by restricted maximum likelihood were applied in all statistical analyses using R, version 2.15.0 (R Development Core Team 2011; R package *nlme*). The replicated unit in this project was a single 'tree' (five control trees under aCO_2, and five trees subjected to eCO_2). Therefore, all measurements per tree were averaged prior to analysis. Since both groups of trees were studied before FACE and after the onset of FACE, we defined a 'pre-treatment' factor to account for any change that might have occurred between the pre-treatment and FACE period. We assessed the significance of the main effects using a backwards selection procedure that progressively removes all non-significant terms until the optimal model is attained. This means that all terms not contained in the final model were statistically not significant. Model selection was validated by likelihood ratio tests and the akaike information criterion. The random factor'tree' was included in all models. Where necessary, homogeneity violations were modeled using adequate variance function structures (power, constant power, exponential and constant variance structures, or a combination thereof), and independence violations were corrected by implementing temporal autocorrelation structures. Model assumptions were examined using diagnostic plots (i.e. residual and quantile–quantile plots).

Results

Climatic conditions

Annual T_{air} averaged 8.2 °C in 2010 (min. −8.6, and max. 24.4 °C), and at 10.1 °C in 2011 (min. −5.7, and max. 25.2 °C). The mean T_{litter} was 9.1 °C (min. −1.0, and max. 23.1 °C), and 8.1 °C (min. −1.3, and max. 19.4 °C) for 2009 and 2010, respectively. We fitted linear regression models with T_{litter} as response variable, and either T_{air} or T_{soil} as predictors. The amount of explained variation seen in T_{litter} increased when T_{soil} was used instead of T_{air}. This is reflected in the lower R^2 between T_{litter} and T_{air} ($R^2 = 0.841$) compared to T_{litter} and T_{soil} ($R^2 = 0.967$). The T_{soil} record had some gaps so that T_{litter} could be used for T_{soil}. Given the strong correlation between T_{soil} and T_{litter}, we only show T_{litter} (Fig. 1). Precipitation was normal during the study period (no exceptionally dry period; for daily precipitation records see Fig. 1). Soil moisture was always high and tended to be slightly lower (−1.1 vol. %) under the CO_2-treated spruce

trees compared to control trees before FACE (Fig. 1). This pattern did not change over the course of this experiment (Leuzinger and Bader 2012).

Tree fine root biomass

Irrespective of the later ongoing treatment (eCO_2 or aCO_2), there was significantly more biomass in the finest root fraction (78 ± 8 g m^{-2} in <0.5 mm) compared to the biomass of 0.5-2 mm fine roots (18 ± 3 g m^{-2}) collected in late March 2010 after only three months of late season CO_2-enrichment ('Diameter' effect: $P < 0.001$; Table 2; Fig. 2). These amounts of fine roots, collected from undisturbed soil, are supposed to depict the initial steady-state situation for this forest. Total fine root biomass (all diameter classes combined) of eCO_2-trees and aCO_2-trees did not differ (115 vs. 112 g m^{-2}; no 'site' effect; $P = 0.211$; Table 2). However, we determined 27 % lower fine root biomass under eCO_2 compared to aCO_2 in the <0.5 mm diameter class (99 ± 17 vs. 135 ± 10 g m^{-2}), whereas there was 29–61 % higher biomass under eCO_2 relative to aCO_2 in the 0.5–1 mm (51 ± 8 vs. 39 ± 4 g m^{-2}), and

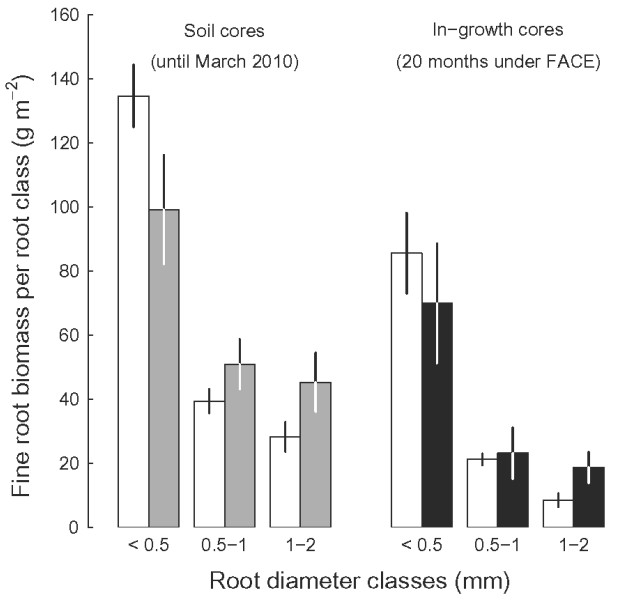

Fig. 2 Fine root biomass under *Picea abies* trees. *White bars* indicate data from trees exposed to ambient CO_2, and *grey bars* specify initial root data for trees exposed to elevated CO_2 (in situ content of boreholes later used for in-growth cores), *black bars* show the fine roots accumulated in in-growth cores after 20 months (ambient CO_2: $n = 5$ trees, elevated CO_2: $n = 5$ trees, mean ± SE). *Left panel*, fine roots in soil cores sampled at the beginning of the experiment. *Right panel*, fine roots in in-growth cores after 20 months of FACE

1–2 mm (45 ± 9 vs. 28 ± 5 g m^{-2}) categories at the start of the experiment (significant 'root thickness × site' interaction at $P = 0.001$; Table 2; Fig. 2).

Generally, there was a high proportionality between in-growth fine root mass and initial mass under in situ conditions (soil cores). The <0.5 mm diameter category had far more fine root biomass than the 0.5–2 mm classes (117 ± 18 vs. 41 ± 5 g m^{-2}; $P < 0.001$; Table 2; Fig. 2). Overall, fine roots (all diameter categories combined) expanding into root-free soil exclusively during FACE (in-growth cores) produced similar biomass under eCO_2 and aCO_2 (202 vs. 195 g m^{-2} under eCO_2 relative to aCO_2; $P = 0.575$; Table 2). However, similar to the soil cores, the in-growth cores showed more fine root biomass under eCO_2 relative to aCO_2 in the 0.5–1 and 1–2 mm diameter fractions (0.5–1 mm: 23 ± 8 vs. 21 ± 2 g m^{-2}; 1–2 mm: 19 ± 5 vs. 8 ± 2 g m^{-2}; Fig. 2). Yet, in the <0.5 mm root fraction we found 18 % lower fine root biomass in eCO_2-trees relative to aCO_2-trees (70 ± 19 vs. 86 ± 13 g m^{-2}; Fig. 2), which was again similar to the initial pattern seen in undisturbed soil cores ('Diameter × CO_2' effect: $P = 0.036$; Table 2). Irrespective of the CO_2 treatment and diameter class, fine root dry mass in in-growth cores (227 g m^{-2}) arrived at only 57 % of the fine root mass previously found in soil cores (397 g m^{-2}; Fig. 2). This finding suggests that the soil space in in-growth cores was not fully explored after 20 months under FACE.

Soil respiration

Pre-treatment measurements (all records collected during the 12 months before the start of FACE on 30 July 2009) revealed 0.6 ± 0.1 µmol CO_2 m^{-2} s^{-1} higher R_{soil} rates under later eCO_2-trees compared to aCO_2-trees (3.5 ± 0.2 vs. 2.9 ± 0.2 µmol CO_2 m^{-2} s^{-1} in later eCO_2-trees vs. aCO_2-trees; Fig. S1). A trend towards slightly lower rates of R_{soil} under eCO_2 relative to aCO_2 was observed when standardizing R_{soil} of eCO_2-trees during FACE by the T-dependent difference during the pre-treatment years (reduction of 0.2 ± 0.1 µmol CO_2 m^{-2} s^{-1} in 2010, and 0.3 ± 0.1 µmol CO_2 m^{-2} s^{-1} in 2011; mean ± SE; Fig. 3). This corresponds to 90 ± 4 and 86 ± 3 % of R_{soil} under aCO_2 in 2010 and 2011, respectively. The annual mean of R_{soil} of aCO_2-trees was 2.4 ± 0.1 (2009), 2.1 ± 0.1 (2010), and 2.2 ± 0.1 µmol CO_2 m^{-2} s^{-1} (2011), whereas R_{soil} of eCO_2-trees averaged at 2.3 ± 0.1 (2009), 1.9 ± 0.1 (2010), and 2.0 ± 0.1 µmol CO_2 m^{-2} s^{-1} (2011).

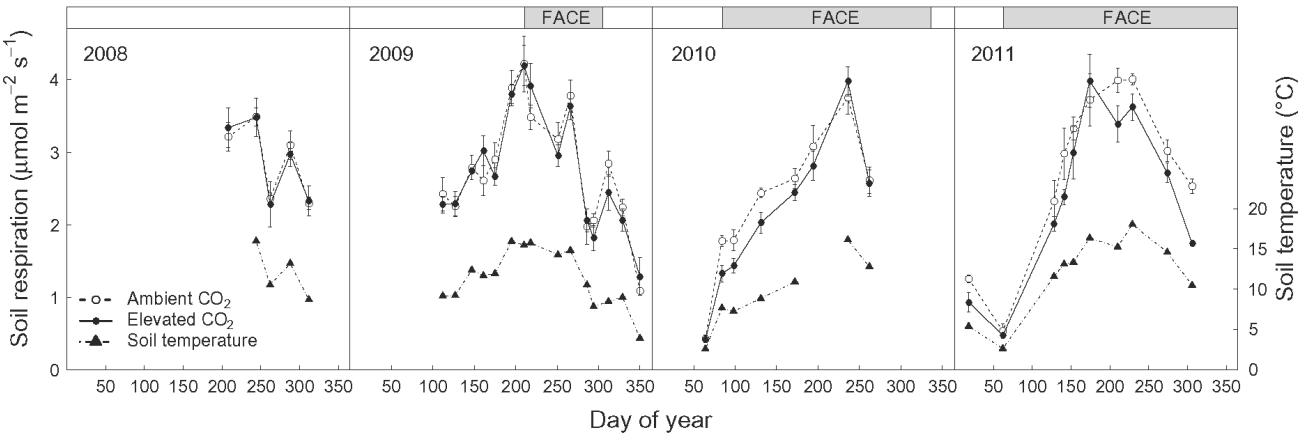

Fig. 3 Soil respiration (R_{soil}) and soil temperature at 10 cm depth of mature *Picea abies* exposed to ambient, or elevated atmospheric CO_2 concentrations in 2008, 2009, 2010, and 2011 (ambient CO_2: $n = 5$ trees; elevated CO_2: $n = 5$ trees; mean ± SE). R_{soil} of trees exposed to elevated CO_2 was corrected for the pre-treatment difference observed between control and treated trees (see materials and methods). Soil temperature under elevated and ambient CO_2 did not differ (*n.s.*). Therefore, the mean of all trees is plotted ($n = 10$). The *grey-shaded areas* on *top of the panels* denote the FACE periods

Hence, the significant main 'CO_2' effect ($P = 0.025$; Table 2), and the 'CO_2 × pre-treatment' interaction ($P < 0.001$) indicate that the pattern observed before the initiation of FACE differed significantly from the pattern observed after the onset of FACE, with significantly lower R_{soil} under eCO_2-trees relative to aCO_2-trees. Also the cumulative annual C release was lower under eCO_2-trees (Fig. 5; Table 1). During the pre-treatment period, designated eCO_2-trees showed 19–23 % higher annual R_{soil} but, after correcting for the pre-treatment differences, this signal reversed, resulting in 8–11 % lower levels during the FACE periods in 2010-2011 after correcting for the pre-treatment differences (Fig. S3; Fig. 5; Table 1). R_{soil} revealed a distinct seasonality that was determined by the seasonal course of T_{soil}. T_{soil} explained 38–88 % of the variation in R_{soil} ($P < 0.001$; Fig. 3; Tables 1, 2). Maximum R_{soil} was measured in July 2009 just before the onset of FACE (eCO_2: 4.2 ± 0; and aCO_2: 4.2 ± 0.2 µmol CO_2 m^{-2} s^{-1} at a T_{soil} of c. 16 °C). Soil moisture influenced R_{soil} only in interaction with T_{soil} ($P = 0.036$). The statistically insignificant two-way interactions (CO_2 × T_{soil} $P = 0.114$; CO_2 × soil moisture $P = 0.287$; Table 2) indicate that the observed CO_2 enrichment effect was independent of these parameters.

Stem respiration

Instantaneous mid-summer rates of R_{stem} were 4.5 ± 0.2 and 3.5 ± 0.3 µmol CO_2 m^{-2} s^{-1} in later eCO_2-trees and in aCO_2-trees, respectively, before the FACE treatment became effective (Fig. S2). Thus, R_{stem} in designated eCO_2-trees was 1.0 µmol CO_2 m^{-2} s^{-1} higher relative to aCO_2-trees during this period of peak R_{stem} (a 29 % higher signal; Fig. S2). Since T_{bark} was similar among the treatments, the different rates of R_{stem} observed before FACE initiation reflects tree-specific differences (Fig. S2; Fig. S3). Accounting for this pre-treatment difference, R_{stem} of eCO_2-trees was slightly but not significantly (0.1 ± 0.1 µmol CO_2 m^{-2} s^{-1}) lower than in aCO_2-trees across all years under FACE (mean ± SE of R_{stem} in eCO_2-trees and aCO_2-trees, respectively: 1.8 ± 0.4 and 1.9 ± 0.4 µmol CO_2 m^{-2} s^{-1}; Table 2; Fig. 4). The mean Q_{10} for 2010 was 1.9 for both treated and control trees (95 % CI: 1.5–2.3 for eCO_2-trees, and 1.6–2.3 for aCO_2-trees; Fig. 5; Fig. S3; Table 1). Q_{10} data for 2009 and 2011 suffered from insufficient sample size (large 95 % CI; Table 1). Accounting for the ca. 29 % higher peak season pre-treatment signal under eCO_2 compared to aCO_2 (Fig. S2), the cumulative C release from stems over the year 2009 was similar (4 % higher) under eCO_2 compared to aCO_2 (n.s.). During FACE, eCO_2-trees respired less than aCO_2-trees (−7 % in 2010, and −14 % in 2011; Table 1). Irrespective of the CO_2 treatment, R_{stem} correlated with T_{bark} ($P < 0.001$), accounting for 60–81 % of the seasonal variation in R_{stem} (Table 1). Accordingly, R_{stem} peaked in late summer and coincided with highest T_{bark} (eCO_2: 4.0 ± 0.6 µmol CO_2 m^{-2} at 29.2 °C, and aCO_2: 4. 4 ± 0.5 µmol CO_2 m^{-2} at 28.9 °C in July 2010; mean ± SE).

Table 1 Nonlinear regression estimates (Lloyd and Taylor 1994) of annual CO_2 efflux rates at 10 °C (R_{10}), temperature sensitivity (Q_{10}) with bootstraped 95 % confidence intervals (CI), and annual cumulative C fluxes from stems and soil of *Picea abies* under elevated or ambient CO_2

CO_2 efflux	Period	Treatment (CO_2)	R_{10}	95 % CI of R_{10}	Q_{10}	95 % CI of Q_{10}	Explained variation (%)	Annual C release (g m^{-2} a^{-1})[a]	Change (%)[c]
Stem	PF 2009[b]	C	1.6	0.8–3.1	2.0	1.2–3.7	35	596	n.a.
	PF 2009[b]	E	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
	F 2009	C	0.7	0.6–0.9	2.7	1.8–4.3	80	318	4
	F 2009	E	0.8	0.5–0.9	2.7	1.7–6.8	63	329	
	F 2010	C	1.3	1.1–1.7	1.9	1.6–2.3	68	450	−7
	F 2010	E	1.3	1.0–1.6	1.9	1.5–2.3	60	418	
	F 2011	C	0.9	0.1–1.6	2.5	1.5–30.0	74	460	−14
	F 2011	E	0.9	0.2–1.2	2.4	1.7–10.1	81	396	
Soil	PF 2008[b]	C	2.5	2.2–2.8	2.0	1.6–2.6	58	887	19
	PF 2008[b]	E	2.9	2.6–3.3	1.7	1.2–2.2	38	1059	
	PF 2009[b]	C	2.4	2.2–2.7	2.2	1.7–2.8	52	906	23
	PF 2009[b]	E	3.0	2.9–3.3	2.0	1.7–2.4	62	1117	
	F 2009	C	2.4	2.2–2.6	2.0	1.7–2.4	66	909	4
	F 2009	E	2.3	2.1–2.5	2.4	2.0–2.9	73	948	
	F 2010	C	2.2	2.0–2.4	2.5	2.2–3.0	84	784	−8
	F 2010	E	2	1.8–2.1	3.3	2.8–3.8	88	723	
	F 2011	C	2.1	1.9–2.3	2.5	2.1–3.0	80	845	−11
	F 2011	E	1.8	1.6–2.0	2.8	2.3–3.5	77	750	

FACE Free air CO_2 enrichment, *PF* pre-FACE, *F* FACE, *C* control trees, *E* CO_2-treated trees

[a] based on temperature records in the litter layer (soil CO_2 efflux), or 2 m above ground (stem CO_2 efflux)

[b] Pre-FACE: uncorrected values

[c] Percentage increase in the annual carbon release from trees subjected to elevated CO_2 versus control trees

Discussion

This project aimed at identifying respiratory and root growth responses in tall *P. abies* trees to elevated atmospheric CO_2 concentration. Stable carbon isotope signals allowed us to trace the fate of new carbon from the tree tops to the soil, and these data confirmed the effectiveness of our CO_2 treatment (Mildner et al. 2014). We expected that these 110-year-old spruce trees have reached a steady-state annual canopy and fine root renewal. A sudden exposure to a 150 ppm higher CO_2 concentration may thus cause strong initial, but declining long-term responses (Leuzinger et al. 2011; Norby and Zak 2011). In fact, we did not observe any significant downward adjustment of leaf-level photosynthesis at eCO_2 in current and previous year needles shortly after the onset of FACE in 2009 (*n.s.*; Fig. S5). In the fifth year of FACE, rates of photosynthesis remained enhanced at eCO_2, and the photosynthetic enhancement ratio was similar in control and CO_2-treated trees (Klein, pers. comm.). This indicates that there is no photosynthetic acclimation to higher levels of CO_2. Also, stomatal conductance remained unchanged (Leuzinger and Bader 2012). These results suggest that more C entered the trees under eCO_2. However, we found no stimulation of fine root accumulation, a reduced CO_2 efflux from soils, and unchanged CO_2 efflux from stems. In the following we will discuss these findings in the light of the results of other CO_2 enrichment experiments.

Fine root biomass

The in-growth core fine root samples reached slightly more than half the initial steady-state biomass, which suggests that 20 months are not enough to arrive at a new steady-state. Therefore, roots in in-growth cores were still in an expanding stage. Fine root growth showed no stimulation by eCO_2 until December 2011

Table 2 Linear mixed effects model results for *Picea abies* fine root biomass (soil cores integrating until 2010; and in-growth cores March 2010 to December 2011), and CO_2 efflux from stems and soil under ambient and elevated CO_2

Factor	Df	*F* value	*P*
Fine root biomass in soil cores (2010)			
Diameter	2,16	86.59	<0.001***
Site	1,8	1.85	0.211
Diameter × site	2,16	14.27	0.001**
Fine root biomass in in-growth cores (2010–2011)			
Diameter	2,16	31.13	<0.001***
CO_2	1,8	0.34	0.575
Diameter × CO_2	2,16	4.14	0.036*
Stem CO_2 efflux 2009–2011			
Bark temperature	1,106	24.99	<0.001***
Pre-treatment	1,106	12.70	0.001**
Soil CO_2 efflux 2008–2011			
CO_2	1,8	7.55	0.025
Pre-treatment	1,308	251.06	< 0.001***
Soil temperature	1,308	1655.21	<0.001***
Soil moisture	1,308	1.29	0.257
CO_2 × pre-treatment	1,308	13.44	<0.001***
CO_2 × soil temperature	1,308	2.51	0.114
Pre-treatment × soil temperature	1,308	0.78	0.378
CO_2 × soil moisture	1,308	1.14	0.287
Pre-treatment × soil moisture	1,308	0.12	0.733
Soil temperature × soil moisture	1,308	4.44	0.036*
Soil moisture × soil temperature × pre-treatment	1,308	9.80	0.002**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

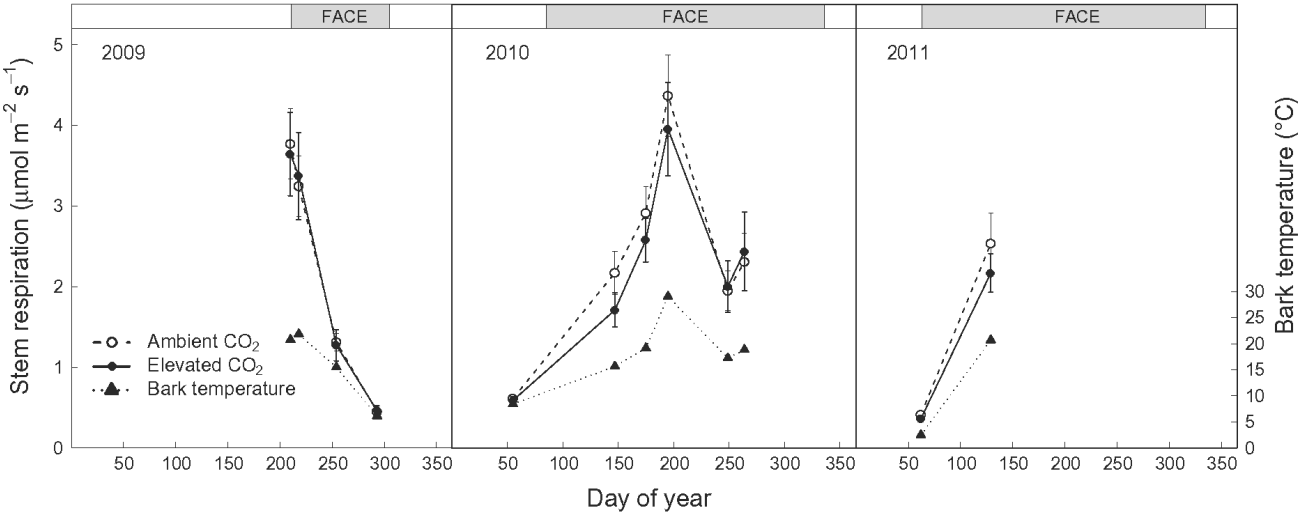

Fig. 4 Stem respiration (R_{stem}) and bark surface temperature of mature *Picea abies* exposed to ambient, or elevated atmospheric CO_2 concentrations in 2009, 2010, and 2011 (ambient CO_2: $n = 5$ trees; elevated CO_2: $n = 5$ trees; mean ± SE). R_{stem} of trees exposed to elevated CO_2 was corrected for the pre-treatment difference observed between control and treated trees (see materials and methods). Bark surface temperature under elevated and ambient CO_2 did not differ (n.s.). Therefore, the mean of all trees is plotted ($n = 10$). The *grey-shaded* areas on *top of the panels* denote the FACE periods

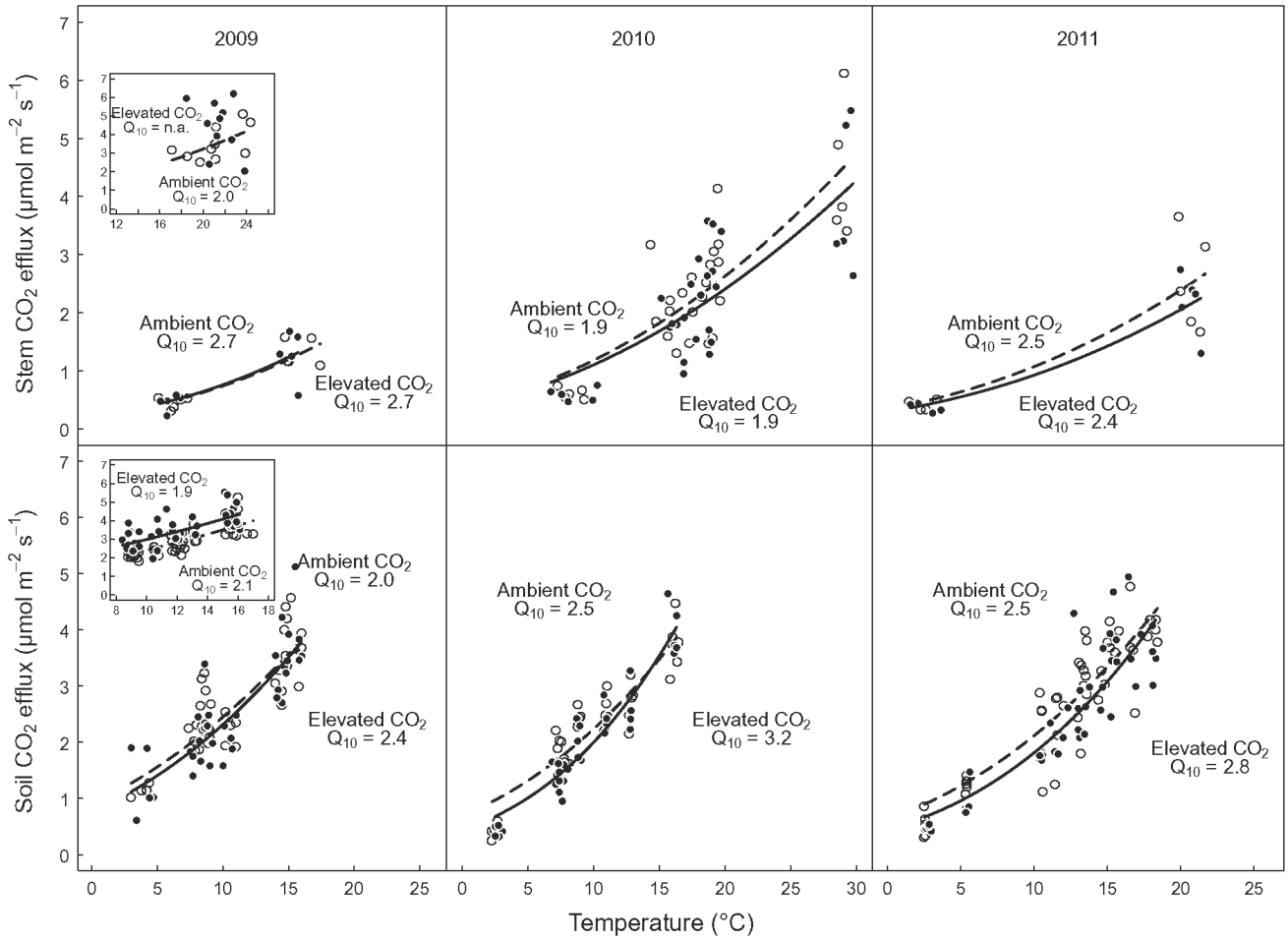

Fig. 5 *Picea abies* stem respiration (R_{stem}) response to bark surface temperature (*upper panels*), and soil respiration (R_{soil}) response to soil temperature 10 cm below ground (*lower panels*) during the FACE periods of the years 2009, 2010, and 2011. R_{stem} and R_{soil} of trees exposed to elevated CO_2 were corrected for the pre-treatment difference observed between control and treated trees (see materials and methods). The *inset* diagrams in 2009 depict the pre-treatment uncorrected R_{stem} (*upper inset*) and R_{soil} (*lower inset*) response in the period before the initiation of FACE in 2009. All respiration measurements were fitted with Lloyd and Taylor (1994) functions. Trees were exposed to ambient (*open symbols, dashed line*), or elevated atmospheric CO_2 concentrations (*filled symbols, solid line*). Each *symbol* represents the mean R_{stem} or R_{soil} rates measured per tree (n = 2–4) and measurement campaign. The Q_{10} values indicate the mean increase in the R_{stem} or R_{soil} rate per 10 °C temperature increase (from 5 to 15 °C)

when compared to the pre-treatment signals. These results contrast observations in juvenile trees where increased fine root production at eCO_2 was found in *P. abies* grown in open top chambers (Lebègue et al. 2004), or in Glass Domes (Pokorný et al. 2013), regenerating birch/aspen stands, three *Populus* species, and young deciduous trees of three species, all grown under FACE (Lukac et al. 2003; Pregitzer et al. 2008; Smith et al. 2013). In the Oak Ridge FACE experiment, a plantation of *Liquidambar styraciflua* (21-year-old in 2009) showed an increase in fine root production and mortality during the first 7 years (Norby et al. 2004), but the signal disappeared after 11 years due to progressive N-limitation (Norby et al. 2010). An initial stimulation of fine root production by eCO_2 was also reported for a young closed-canopy *Pinus taeda* plantation at the Duke FACE (Allen et al. 2000). These studies investigated young trees, which may not have completely explored the available soil volume, and mostly grew under ample nutrient supply (expanding systems; Körner 2006).

The soil space beneath mature trees in a fully-grown forest can be expected to be fully explored by roots and to have arrived at a steady-state fine root turnover, which would prevent stimulation by eCO_2 (Norby et al. 1999; Körner 2006). The 110-year-old trees studied in our

web-FACE experiment operated at constant annual needle renewal rates (unpublished litter production data) and, thus, should also be in a steady-state of fine root renewal, not affected by eCO_2 (Körner 2006; Norby and Zak 2011). A Swiss treeline FACE study on 35-year-old *L. decidua* and *Pinus uncinata*, both in a quasi steady-state development, did not reveal any fine root growth following high CO_2 exposure despite higher soil CO_2 efflux (Handa et al. 2008; Dawes et al. 2013; Hagedorn et al. 2013). Additionally, a CO_2 enrichment experiment in a scrub-oak system in Florida showed an initial burst of fine root production under eCO_2 after disturbances (fire and hurricane), a signal that gradually vanished in the following years (Day et al. 2013) with canopy closure (full LAI recovery; Palmroth et al. 2006). The former web-FACE study at our site on mature deciduous forest trees showed even reduced fine root production after 7 years of eCO_2 (Bader et al. 2009). This was explained by stand maturation, and (stomata driven) reduced canopy transpiration. Thus, soil moisture savings reduced the need for intensified soil exploration by fine roots (Leuzinger and Körner 2007; Bader et al. 2009). In contrast, our spruce trees showed no reduction of sap flow when exposed to eCO_2 and, hence, exhibited no soil moisture savings that might be responsible for the missing fine root growth response (Leuzinger and Bader 2012).

Abundance of soil nutrients, especially the availability of N, determines how fine roots will respond to eCO_2 (Pregitzer et al. 1995; Curtis and Wang 1998; De Graaff et al. 2006; Dieleman et al. 2010), regardless of tree or stand age. Recently two meta-analyses investigated the interactive effects of high CO_2 and N availability in soils, with high soil N fueling the CO_2 effect on fine root growth (De Graaff et al. 2006; Dieleman et al. 2010). This contradicts our results since we found no fine root response to eCO_2 despite decades of N-deposition of ca. 20 kg N ha^{-1} a^{-1} at our site. Additionally, CO_2 enrichment induced soil nitrate release both in the present study (unpublished data), and in the former web-FACE experiment on mature deciduous trees (Schleppi et al. 2012). In *Pinus taeda* at the Duke FACE site, N-fertilization reduced fine root biomass by ca. 12 % compared to unfertilized plots, accompanied by reductions in soil respiration (Jackson et al. 2009; Drake et al. 2011). In 6–8-year-old *P. abies* saplings, N-addition reduced fine root production in comparison to plots without extra N in CO_2-enriched plots (Spinnler et al. 2002). It appears that the trees in this near-natural, mature forest do not exhibit such N-mediated fine root responses to eCO_2.

Given the substantial atmospheric N deposition in the test region, PNL, caused by accelerated soil N withdrawal during long-term CO_2 enrichment (Luo et al. 2004), is unlikely to occur here and stimulate fine root expansion under high CO_2 (see Franklin et al. 2009; Garten et al. 2011 for PNL effects).

CO_2 fertilization may also induce deeper rooting, a phenomenon commonly observed in CO_2 enrichment experiments (Lukac et al. 2003; Norby et al. 2004; Jackson et al. 2009; Iversen 2010; Smith et al. 2013). However, we could not explore this possibility here, because the accessible soil profile at the SCC site is maximal 25 cm deep, with extremely rocky subsoil.

Soil respiration

In the short term, R_{soil} is mainly controlled by soil moisture and soil temperature (Raich and Schlesinger 1992; Davidson et al. 1998). When accounting for these covariates plus pre-treatment signals (Fig. S1), we detected a trend towards reduced R_{soil} in response to FACE. Spruce trees under eCO_2 also showed continuously decreasing annual C returns to the atmosphere compared to control trees (Table 1). The (moderate) reduction of CO_2 release compared to pre-treatment conditions is surprising, given that soil CO_2 efflux carried a clear ^{13}C signal that indicates effective CO_2 enrichment and fast belowground allocation of new C (Mildner et al. 2014). The absolute reduction in R_{soil} in response to eCO_2 might be even more pronounced, had the soil space been fully occupied by *P. abies* fine roots instead of a ca. 50 % fraction of all fine roots, including those from neighboring deciduous trees. The finding of reduced R_{soil} contrasts with many examples for very young stands (mostly obtained in open top chambers, OTC) that showed increased but highly variable R_{soil} under eCO_2 (plus 5–93 %) compared to aCO_2 (Zak et al. 2000). Forest FACE experiments in young plantations initially showed increases in R_{soil} rates at eCO_2, but these signals declined with time (Hamilton et al. 2002; King et al. 2004; Comstedt et al. 2006; Jackson et al. 2009; Norby and Zak 2011). Dieleman et al. (2010) summarized the results for 32 OTC and FACE sites using trees and found an average 19 % increase in R_{soil}, with soil N fertilization enhancing the CO_2 effect. However, a few CO_2 enrichment experiments

showed no stimulation or a decline of R_{soil}, e.g. soil under mature deciduous trees subjected to web-FACE at our study site (N-fertilized soil) did not release more CO_2 under eCO_2. This was attributed to higher soil moisture at eCO_2 that may have impeded soil CO_2 efflux (Bader and Körner 2010). Furthermore, Tingey et al. (2006) reported declining rates of R_{soil} in Ponderosa pine seedlings subjected to eCO_2 in growth chambers, caused by altered R_{soil} sensitivity to soil temperature and soil moisture at eCO_2.

The extent to which R_{soil} responds to eCO_2 has been found to be strongly related to responses of fine roots (Zak et al. 2000; Jackson et al. 2009; Drake et al. 2011). Root respiration (and associated mycorrhizal fungal respiration) can contribute 50–65 % to total R_{soil} (Andrews et al. 1999; Högberg et al. 2001, 2002; Bhupinderpal-Singh et al. 2003), and is fueled by fresh aboveground assimilates (Högberg et al. 2001). Therefore, the relative reduction in R_{soil} is likely to reflect reduced belowground C transfer under eCO_2 (Palmroth et al. 2006; but see Jastrow et al. 2005). Generally, C supply to belowground microorganisms, or fungal symbionts was found to either increase with CO_2 fertilization, or did not change (Fransson 2012). In the short term, extra C is likely to increase the abundance of microorganisms (e.g. fungi and bacteria; Blankinship et al. 2011) which may become competitors for essential plant nutrients (Diaz et al. 1993; Hättenschwiler and Körner 1998; Inauen et al. 2012). Likewise, heterotrophic rhizomicrobial respiration could decline when exudates alter the microbial community (Bader and Körner 2010), its activity (Drake et al. 2011), or species composition (Carney et al. 2007; Drigo et al. 2008; reviewed in Zak et al. 2000). A higher release of nitrate under eCO_2 relative to aCO_2 (Schleppi and Textor, pers. comm.; similar to Schleppi et al. 2012) could also contribute to reduced microbial activity. However, we expected the 'priming effect' (Jenkinson et al. 1985) to dominate, as was found in the Duke FACE study (Drake et al. 2011; Phillips et al. 2012) that reported slowly increasing R_{soil} over the course of 12 years of FACE (Jackson et al. 2009). The tall, 110-year-old trees in our study may either respond more slowly, or have their roots spread over such a large area that R_{soil} signals get diluted.

Stem respiration

During the first 2.5 years of web-FACE, there was no indication of a CO_2-driven decline or increase of R_{stem} in these mature *P. abies* trees, although a strong stable C isotope signal in respiratory CO_2 evidences that the novel C derived from web-FACE (Mildner et al. 2014). The lack of any stem growth stimulation at eCO_2 in these trees (the 2009–2014 mean basal area increment standardized by mean pre-treatment rates was 1.4 ± 0.1 at aCO_2 and 1.5 ± 0.3 at eCO_2; $n = 6$ years, mean $\pm$ SE; Klein and Körner; unpublished), given the assumption that the stem diameter increment largely determines the magnitude of R_{stem} signals under eCO_2 (Zha et al. 2005; Moore et al. 2008), co-explains why we also see no R_{stem} signal in response to web-FACE. In contrast to these results, juvenile trees exposed to a step increase in CO_2 on fertile ground, or with ample soil space, grew faster and their stems respired more. For instance, R_{stem} was 16 % higher in eCO_2 in 16-year-old *P. abies* (Acosta et al. 2010). Similarly, an increase in R_{stem} in response to eCO_2 was observed in 15-year-old *Liquidambar styraciflua* (Edwards et al. 2002), and in 20-year-old *P. sylvestris* (Zha et al. 2005). Stem growth and the associated R_{stem} responses to eCO_2 are largely determined by the developmental stage (age) of a tree, the species investigated, and the nutrient supply (Körner 2006). CO_2 enrichment may also contribute to higher maintenance respiration (Carey et al. 1996; Edwards et al. 2002; Zha et al. 2005), and mature trees exhibit higher maintenance respiration rates than juvenile trees (Ryan and Waring 1992). Hence, mature trees might be expected to yield even greater responses, but this is in contrast to what we found. R_{stem} signals might become diminished by translocation of dissolved CO_2 in sap flow (Negisi 1979; Teskey and McGuire 2002; Moore et al. 2008; Bloemen et al. 2013). However, sap flow measured prior to web-FACE was similar in the trees examined, and this relation did not change at eCO_2 (Leuzinger and Bader 2012). Whatever the reason, these tall trees did not exhibit a greater R_{stem} response under web-FACE.

Conclusions and outlook

Previous and ongoing works revealed that photosynthesis, was, and still is, enhanced under eCO_2, and stomatal conductance remained unaffected by eCO_2 (Leuzinger and Bader 2012). Therefore, we expected strong and positive initial responses to a step increase in CO_2 in both types of respiratory CO_2 release, and in fine root growth in these tall trees. The fact that we did

not detect such a stimulation, despite clear isotopic evidence of successful canopy CO_2 enrichment, by default, suggests other pathways of C-dissipation under eCO_2. We expected such overflow responses because we (seemingly correctly) anticipated no stem growth response for reasons related to tree nutrition (other than by N), and tissue element stoichiometry (ongoing research). It remains to be seen if accelerated root growth will occur at a later stage, as was the case in other FACE works (Allen et al. 2000; Spinnler et al. 2002; Norby et al. 2004). The fine root data should be highly sensitive to CO_2 because fine roots from in-growth cores had not yet arrived at steady-state root density, and the signal should still capture the root expansion process. The data presented here add to the growing evidence that mature trees or trees growing in stands that arrived at steady-state leaf and root turnover are unlikely to take benefit from eCO_2. These trees are likely to be C saturated at current ambient CO_2 concentrations, as has been shown for boreal spruce trees (Sigurdsson et al. 2013). We observed highly homeostatic stem respiratory signals, and soil CO_2 efflux even declined slightly in response to web-FACE.

Acknowledgments We are particularly indebted to E. Amstutz and G. Grun who kept the free air CO_2 enrichment system running. We are also notably obliged to S. Jakob, and several student helpers for their support in data collection and sample processing. We thank T. Klein for providing additional fine root biomass and preliminary stem basal increment data that supported our argumentation. Thanks to T. Baisden and the invaluable comments from three anonymous reviewers that greatly helped in improving earlier drafts. Funding came from the Swiss National Science Foundation (Grant Nos 31003AB-126028 and 31003A_140753, 31-67775.02, 3100-059769.99, 3100-067775.02, and 3100AO-111914/1). The crane was sponsored by the Swiss Federal Office of the Environment (FOEN).

References

Acosta M, Pokorný R, Janouš D, Marek MV (2010) Stem respiration of Norway spruce trees under elevated CO_2 concentration. Biol Plant 54:773–776

Allen AS, Andrews JA, Finzi AC, Matamala R, Richter DD, Schlesinger WH (2000) Effects of free-air CO_2 enrichment (FACE) on belowground processes in a *Pinus taeda* forest. Ecol Appl 10:437–448

Andrews JA, Harrison KG, Matamala R, Schlesinger WH (1999) Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). Soil Sci Soc Am J 63:1429–1435

Bader MKF, Körner C (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Glob Change Biol 16:2830–2843

Bader MKF, Hiltbrunner E, Körner C (2009) Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE). Funct Ecol 23:913–921

Bader MKF, Siegwolf RTW, Körner C (2010) Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. Planta 232:1115–1125

Bader MKF, Leuzinger S, Keel SG, Siegwolf RTW, Hagedorn F, Schleppi P, Körner C (2013) Central European hardwood trees in a high-CO_2 future: synthesis of an 8-year forest canopy CO_2 enrichment project. J Ecol 101:1509–1519

Bernhardt ES, Barber JJ, Pippen JS, Taneva L, Andrews JA, Schlesinger WH (2006) Long-term effects of free air CO_2 enrichment (FACE) on soil respiration. Biogeochemistry 77:91–116

Bhupinderpal-Singh NA, Ottosson-Löfvenius M, Högberg MN, Mellander P-E, Högberg P (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. Plant Cell Environ 26:1287–1296

Blankinship JC, Niklaus PA, Hungate BA (2011) A meta-analysis of responses of soil biota to global change. Oecologia 165:553–565

Bloemen J, McGuire MA, Aubrey DP, Teskey RO, Steppe K (2013) Transport of root-respired CO_2 via the transpiration stream affects aboveground carbon assimilation and CO_2 efflux in trees. New Phytol 197:555–565

Carey EV, DeLucia EH, Ball JT (1996) Stem maintenance and construction respiration in pinus ponderosa grown in different concentrations of atmospheric CO_2. Tree Physiol 16:125–130

Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. P Natl Acad Sci USA 104:4990–4995

Comstedt D, Boström B, Marshall JD, Holm A, Slaney M, Linder S, Ekblad A (2006) Effects of elevated atmospheric carbon dioxide and temperature on soil respiration in a boreal forest using $\delta^{13}C$ as a labeling tool. Ecosystems 9:1266–1277

Curtis PS, Wang XZ (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113:299–313

Darbah JNT, Kubiske ME, Nelson N, Kets K, Riikonen J, Sober A, Rouse L, Karnosky DF (2010) Will photosynthetic capacity of aspen trees acclimate after long-term exposure to elevated CO_2 and O_3? Environ Pollut 158:983–991

Davidson EA, Belk E, Boone RD (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Glob Change Biol 4:217–227

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

Day FP, Schroeder RE, Stover DB, Brown ALP, Butnor JR, Dilustro J, Hungate BA, Dijkstra P, Duval BD, Seiler TJ, Drake BG, Hinkle CR (2013) The effects of 11 yr of CO_2 enrichment on roots in a Florida scrub-oak ecosystem. New Phytol 200:778–787

De Graaff MA, Van Groenigen KJ, Six J, Hungate B, Van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Glob Change Biol 12:2077–2091

Diaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364:616–617

Dieleman WI, Luyssaert S, Rey A, de Angelis P, Barton CV, Broadmeadow MS, Broadmeadow SB, Chigwerewe KS, Crookshanks M, Dufrêne E, Jarvis PG, Kasurinen A, Kellomäki S, Le Dantec V, Liberloo M, Marek M, Medlyn B, Pokorný R, Scarascia-Mugnozza G, Temperton VM, Tingey D, Urban O, Ceulemans R, Janssens IA (2010) Soil [N] modulates soil C cycling in CO_2-fumigated tree stands: a meta-analysis. Plant Cell Environ 33:2001–2011

Drake JE, Gallet-Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB, Johnsen KS, Lichter J, McCarthy HR, McCormack ML, Moore DJP, Oren R, Palmroth S, Phillips RP, Pippen JS, Pritchard SG, Treseder KK, Schlesinger WH, DeLucia EH, Finzi AC (2011) Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. Ecol Lett 14:349–357

Drigo B, Kowalchuk GA, van Veen JA (2008) Climate change goes underground: effects of elevated atmospheric CO_2 on microbial community structure and activities in the rhizosphere. Biol Fert Soils 44:667–679

Edwards NT, Tschaplinski TJ, Norby RJ (2002) Stem respiration increases in CO_2-enriched sweetgum trees. New Phytol 155:239–248

Ellsworth DS, Thomas R, Crous KY, Palmroth S, Ward E, Maier C, DeLucia EH, Oren R (2012) Elevated CO2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. Glob Change Biol 18:223–242

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. Ecol Lett 12:1238–1249

Franklin O, McMurtrie RE, Iversen CM, Crous KY, Finzi AC, Tissue DT, Ellsworth DS, Oren R, Norby RJ (2009) Forest fine-root production and nitrogen use under elevated CO_2: contrasting responses in evergreen and deciduous trees explained by a common principle. Glob Change Biol 15:132–144

Fransson P (2012) Elevated CO_2 impacts ectomycorrhiza-mediated forest soil carbon flow: fungal biomass production, respiration and exudation. Fungal Ecol 5:85–98

Garten CT, Iversen CM, Norby RJ (2011) Litterfall N^{15} abundance indicates declining soil Nitrogen availability in a free-air CO_2 enrichment experiment. Ecology 92:133–139

Hagedorn F, Hiltbrunner D, Streit K, Ekblad A, Lindahl BD, Miltner A, Frey B, Handa IT, Hättenschwiler S (2013) Nine years of CO_2 enrichment at the alpine treeline stimulates soil respiration but does not alter soil microbial communities. Biogeochemistry 57:390–400

Hamilton JG, DeLucia EH, George K, Naidu SL, Finzi AC, Schlesinger WH (2002) Forest carbon balance under elevated CO_2. Oecologia 131:250–260

Handa IT, Hagedorn F, Hättenschwiler S (2008) No stimulation in root production in response to 4 years of in situ CO_2 enrichment at the Swiss treeline. Funct Ecol 22:348–358

Hättenschwiler S, Körner C (1998) Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. Oecologia 113:104–114

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

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792

Högberg P, Nordgren A, Agren GI (2002) Carbon allocation between tree root growth and root respiration in boreal pine forest. Oecologia 132:579–581

Hungate BA, Dijkstra P, Wu Z, Duval BD, Day FP, Johnson DW, Megonigal JP, Brown AL, Garland JL (2013) Cumulative response of ecosystem carbon and nitrogen stocks to chronic CO_2 exposure in a subtropical oak woodland. New Phytol 200:753–766

Inauen N, Körner C, Hiltbrunner E (2012) No growth stimulation by CO_2 enrichment in alpine glacier forefield plants. Glob Change Biol 18:985–999

Iversen CM (2010) Digging deeper: fine-root responses to rising atmospheric CO_2 concentration in forested ecosystems. New Phytol 186:346–357

Iversen CM, Hooker TD, Classen AT, Norby RJ (2011) Net mineralization of N at deeper soil depths as a potential mechanism for sustained forest production under elevated CO_2. Glob Change Biol 17:1130–1139

Iversen CM, Keller JK, Garten CT, Norby RJ (2012) Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of CO_2-enrichment. Glob Change Biol 18:1684–1697

Jackson RB, Cook CW, Pippen JS, Palmer SM (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. Ecology 90:3352–3366

Janouš D, Pokorný R, Brossaud J, Marek MV (2000) Long-term effects of elevated CO_2 on woody tissues respiration of Norway spruce studied in open-top chambers. Biol Plant 43:41–46

Jastrow JD, Miller RM, Matamala R, Norby RJ, Boutton TW, Rice CW, Owensby CE (2005) Elevated atmospheric carbon dioxide increases soil carbon. Glob Change Biol 11:2057–2064

Jenkinson DS, Fox RH, Rayner JH (1985) Interactions between fertilizer nitrogen and soil nitrogen–the so-called 'priming' effect. J Soil Sci 36:425–444

King JS, Hanson PJ, Bernhardt E, De Angelis P, Norby RJ, Pregitzer KS (2004) A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments. Glob Change Biol 10:1027–1042

Körner C (1994) Biomass fractionation in plants: a reconsideration of definitions based on plant functions. In: Roy J, Garnier E (eds) A whole plant perspective on carbon-nitrogen interactions. SPB Acad Publishing, The Hague, pp 173–185

Körner C (2000) Biosphere responses to CO_2 enrichment. Ecol Appl 10:1590–1619

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

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

Lebègue C, Laitat È, Perrin D, Pissard G (2004) Mise en œuvre de carottages de sol et de minirhizotrons pour l'étude à long terme de la réponse des fines racines d'épicéa (*Picea abies* (l.) Karst.) à l'augmentation de la concentration en CO_2 dans l'atmosphère et la nutrition minérale. Biotechnol Agron Soc 8:41–53

Leuzinger S, Körner C (2007) Water savings in mature deciduous forest trees under elevated CO_2. Glob Change Biol 13:2498–2508

Leuzinger S, Bader MKF (2012) Experimental vs. modeled water use in mature norway spruce (*Picea abies*) exposed to elevated CO_2. Front Plant Sci 3:229

Leuzinger S, Hättenschwiler S (2013) Beyond global change: lessons from 25 years of CO_2 research. Oecologia 171:639–651

Leuzinger S, Luo Y, Beier C, Dieleman W, Vicca S, Körner C (2011) Do global change experiments overestimate impacts on terrestrial ecosystems? Trends Ecol Evol 26:236–241

Lloyd J, Taylor JA (1994) On the temperature-dependence of soil respiration. Funct Ecol 8:315–323

Lukac M, Calfapietra C, Godbold DL (2003) Production, turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated CO_2 (popFACE). Glob Change Biol 9:838–848

Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731–739

Matamala R, Gonzàlez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH (2003) Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science 32:1385–1387

Mildner M, Bader MKF, Leuzinger S, Siegwolf RTW, Körner C (2014) Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*. Oecologia 175:747–762. doi:10.1007/s00442-014-2935-5

Moore DJP, Gonzalez-Meler MA, Taneva L, Pippen JS, Kim HS, DeLucia EH (2008) The effect of carbon dioxide enrichment on apparent stem respiration from *Pinus taeda* L. is confounded by high levels of soil carbon dioxide. Oecologia 158:1–10

Negisi K (1979) Bark respiration rate in stem segments detached from young *Pinus densiflora* trees in relation to velocity of artificial sap flow. J Jpn For Sci 61:88–93

Norby RJ, Zak DR (2011) Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annu Rev Ecol Evol Syst 42:181–203

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ 22:683–714

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. P Natl Acad Sci USA 101:9689–9693

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. P Natl Acad Sci USA 107:19368–19373

Palmroth S, Oren R, McCarthy HR, Johnsen KH, Finzi AC, Butnor JR, Ryan MG, Schlesinger WH (2006) Aboveground sink strength in forests controls the allocation of carbon below ground and its CO_2-induced enhancement. Proc Natl Acad Sci USA 103:19362–19367

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

Phillips RP, Meier IC, Bernhardt ES, Grandy AS, Wickings K, Finzi AC (2012) Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecol Lett 15:1042–1049

Pokorný R, Tomášková I, Marek MV (2013) Response of Norway spruce root system to elevated atmospheric CO_2 concentration. Acta Physiol Plant 35:1807–1816

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS (1995) Atmospheric CO_2, soil-nitrogen and turnover of fine roots. New Phytol 129:579–585

Pregitzer KS, Burton AJ, King JS, Zak DR (2008) Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. New Phytol 180:153–161

Pritchard SG, Rogers HH, Davis MA, Van Santen E, Prior SA, Schlesinger WH (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air- CO_2-enrichment: a six-year-minirhizotron study. Glob Change Biol 14:588–602

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Raich JW, Schlesinger WH (1992) The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44:81–99

Ryan MG, Waring RH (1992) Maintenance respiration and stand development in a subalpine lodgepole pine forest. Ecology 73:2100–2108

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

Sigurdsson BD, Medhurst JL, Wallin G, Eggertsson O, Linder S (2013) Growth of mature boreal Norway spruce was not affected by elevated CO2 and/or air temperature unless nutrient availability was improved. Tree Physiol 33:1192–1205

Smith AR, Lukac M, Bambrick M, Miglietta F, Godbold DL (2013) Tree species diversity interacts with elevated CO_2 to induce a greater root system response. Glob Change Biol 19:217–228

Spinnler D, Egh P, Körner C (2002) Four-year growth dynamics of beech-spruce model ecosystems under CO_2 enrichment on two different forest soils. Trees-Struct Funct 16:423–436

Teskey RO, McGuire MA (2002) Carbon dioxide transport in xylem causes errors in estimation of rates of respiration in stems and branches of trees. Plant Cell Environ 25:1571–1577

Tingey DT, Lee EH, Waschmann R, Johnson MG, Rygiewicz PT (2006) Does soil CO_2 efflux acclimatize to elevated

temperature and CO_2 during long-term treatment of douglas-fir seedlings? New Phytol 170:107–118

Waring RH, Schlesinger WH (1985) Forest ecosystems: concepts and management. Academic Press, London

Zak DR, Pregitzer KS, King JS, Holmes WE (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol 147:201–222

Zha TS, Kellomäki S, Wang KY, Ryyppö A (2005) Respiratory responses of scots pine stems to 5 years of exposure to elevated CO_2 concentration and temperature. Tree Physiol 25:49–56

Supplementary Material:

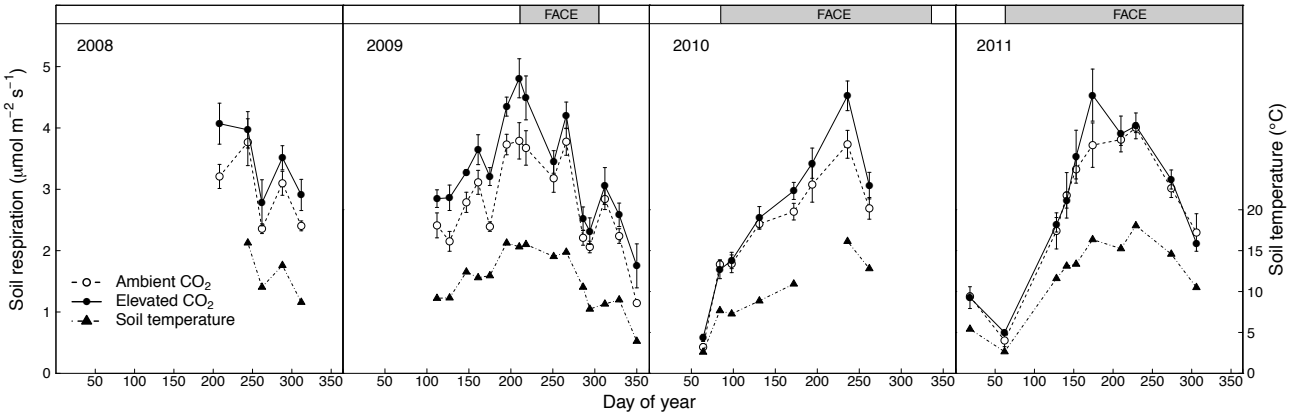

Fig. S1 Raw data of soil respiration (*R* soil) and soil temperature at 10 cm depth of mature *Picea abies* exposed to ambient, or elevated atmospheric CO2 concentrations in 2008, 2009, 2010, and 2011 (ambient CO2: $n = 5$ trees; eCO2: $n = 5$ trees; mean ± SE). Soil temperature under elevated and ambient CO2 did not differ (*n.s.*). Therefore, the mean of all trees is plotted ($n = 10$). The grey-shaded areas on top of the panels denote the FACE periods.

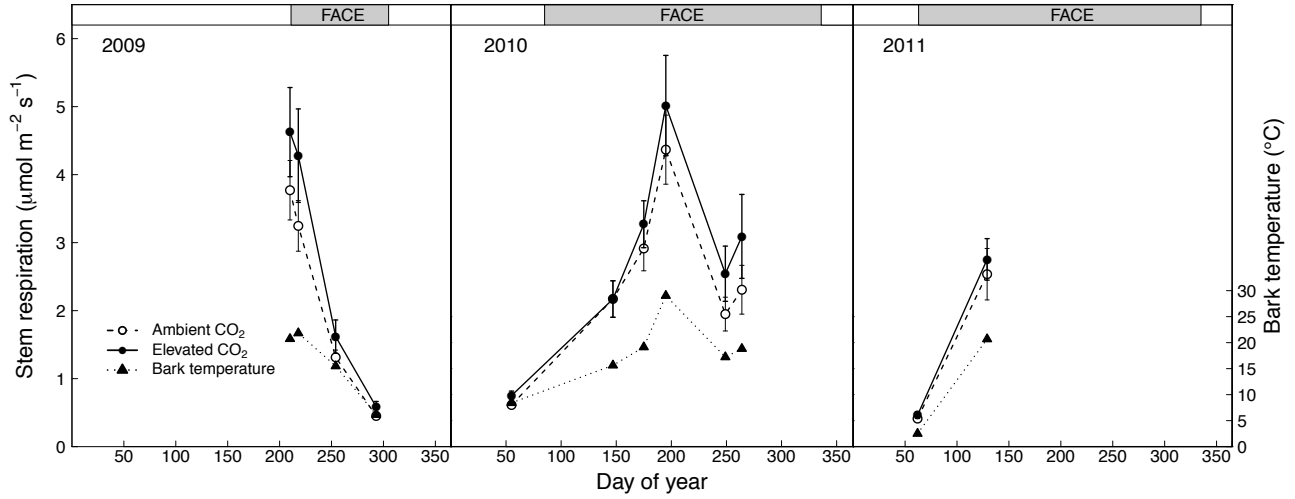

Fig. S2 Raw data of stem respiration (*R* stem) and bark surface temperature of mature *Picea abies* exposed to ambient, or elevated atmospheric CO2 concentrations in 2009, 2010, and 2011 (ambient CO2: $n = 5$ trees; eCO2: $n = 5$ trees; mean ± SE). Bark surface temperature under elevated and ambient CO2 did not differ (n.s.). Therefore, the mean of all trees is plotted ($n = 10$). The grey-shaded areas on top of the panels denote the FACE periods.

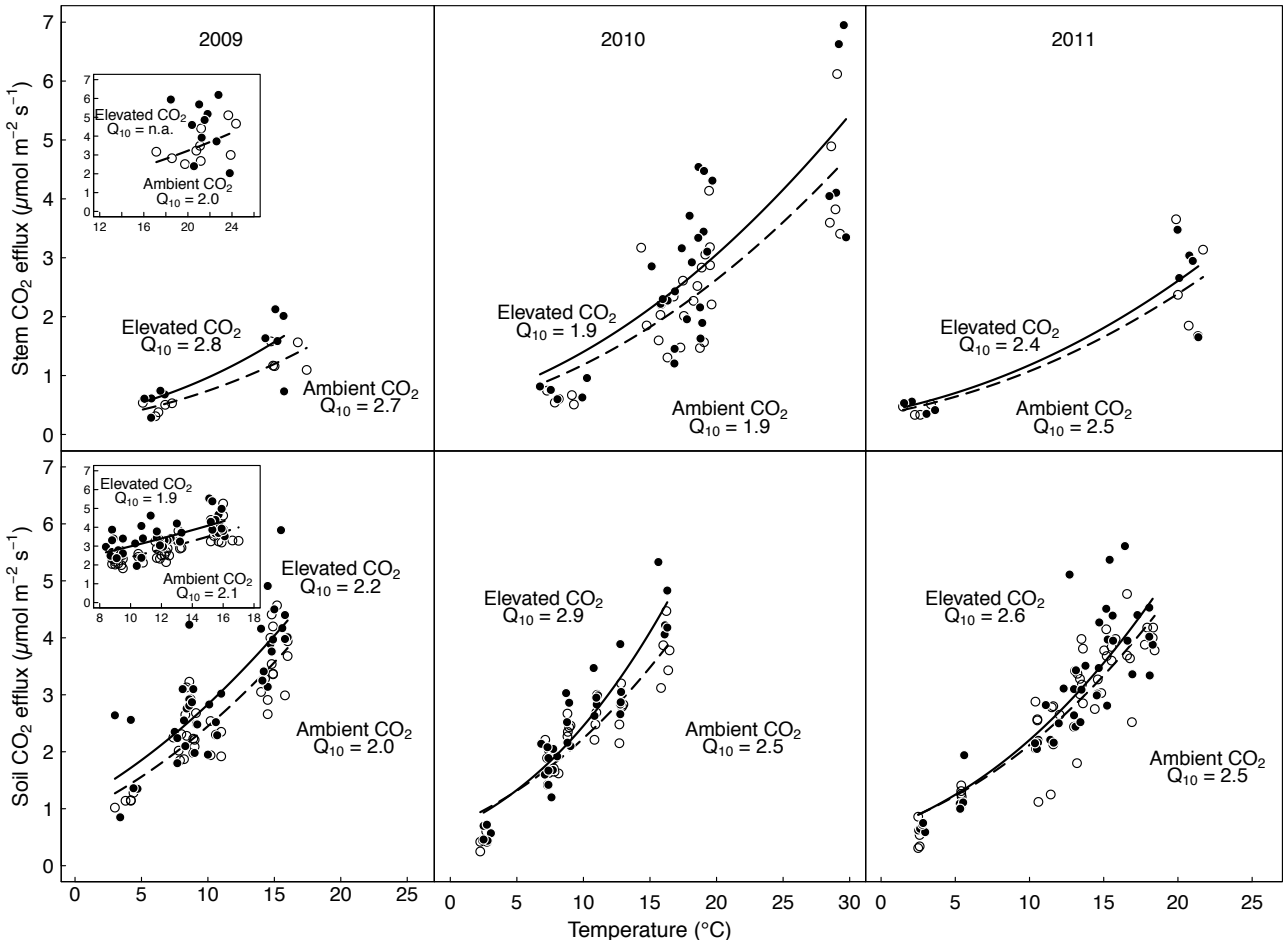

Fig. S3 *Picea abies* stem respiration (*R* stem) response to bark surface temperature (upper panels), and soil respiration (*R* soil) response to soil temperature 10 cm below ground (lower panels) during the FACE periods of the years 2009, 2010, and 2011. Here, we show raw data of *R* stem and *R* soil (i.e. uncorrected for the pre-treatment difference observed between control and treated trees). The inset diagrams in 2009 depict the pre-treatment uncorrected *R* stem (upper inset) and *R* soil (lower inset) response in the period before the initiation of FACE in 2009. All respiration measurements were fitted with Lloyd and Taylor (1994) functions. Trees were exposed to ambient (*open symbols, dashed line*), or elevated atmospheric CO_2 concentrations (*filled symbols, solid line*). Each symbol represents the mean *R* stem or *R* soil rates measured per tree (n = 2–4) and measurement campaign. The *Q 10* values indicate the mean increase in the *R* stem or *R* soil rate per 10 °C temperature increase (from 5 °C to 15 °C).

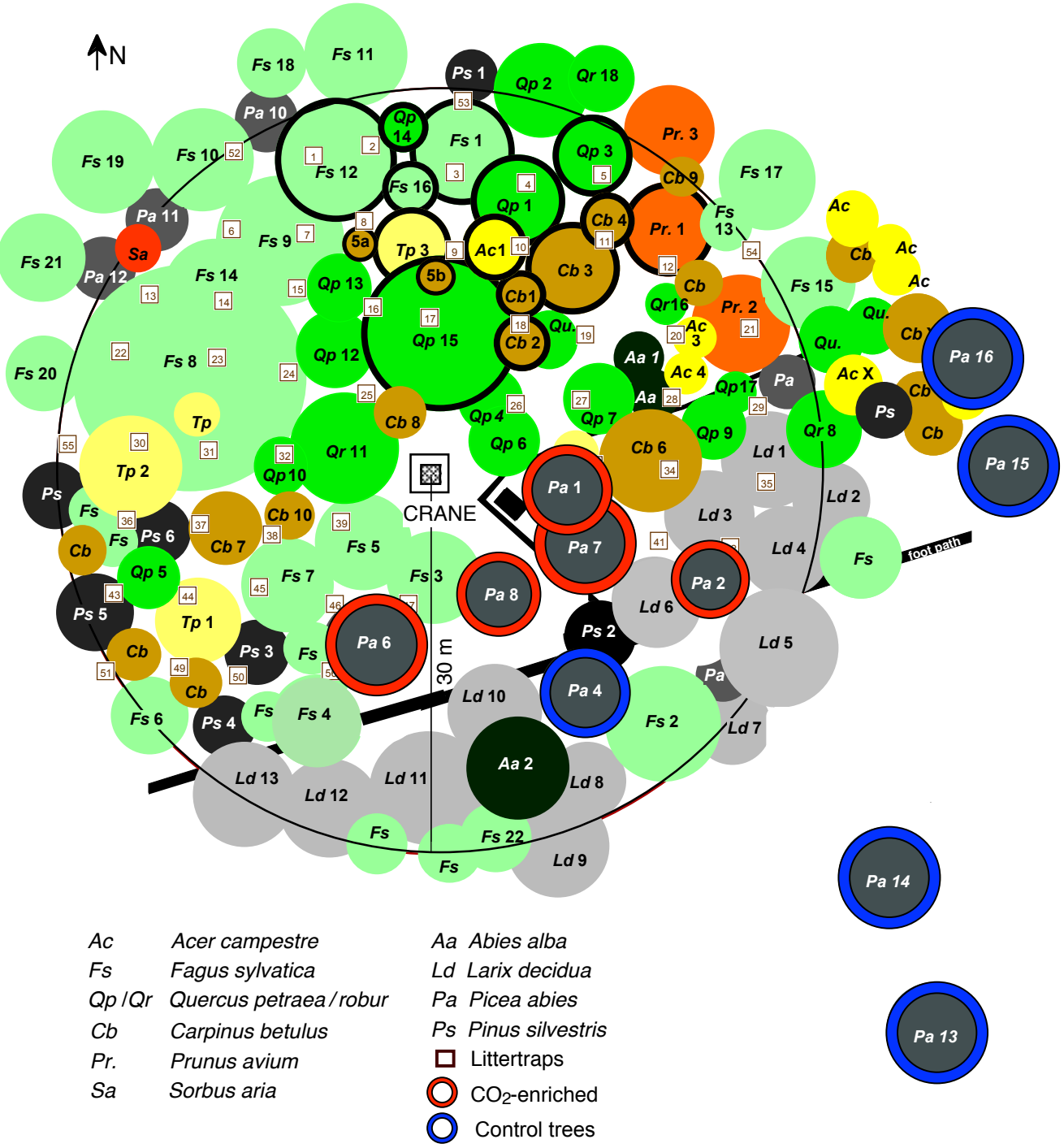

Fig. S4 Tree distribution map at the SCC site near Basel, Switzerland. Outside the perimeter of the crane's jib, only the *Picea abies* control trees are depicted, not the surrounding trees.

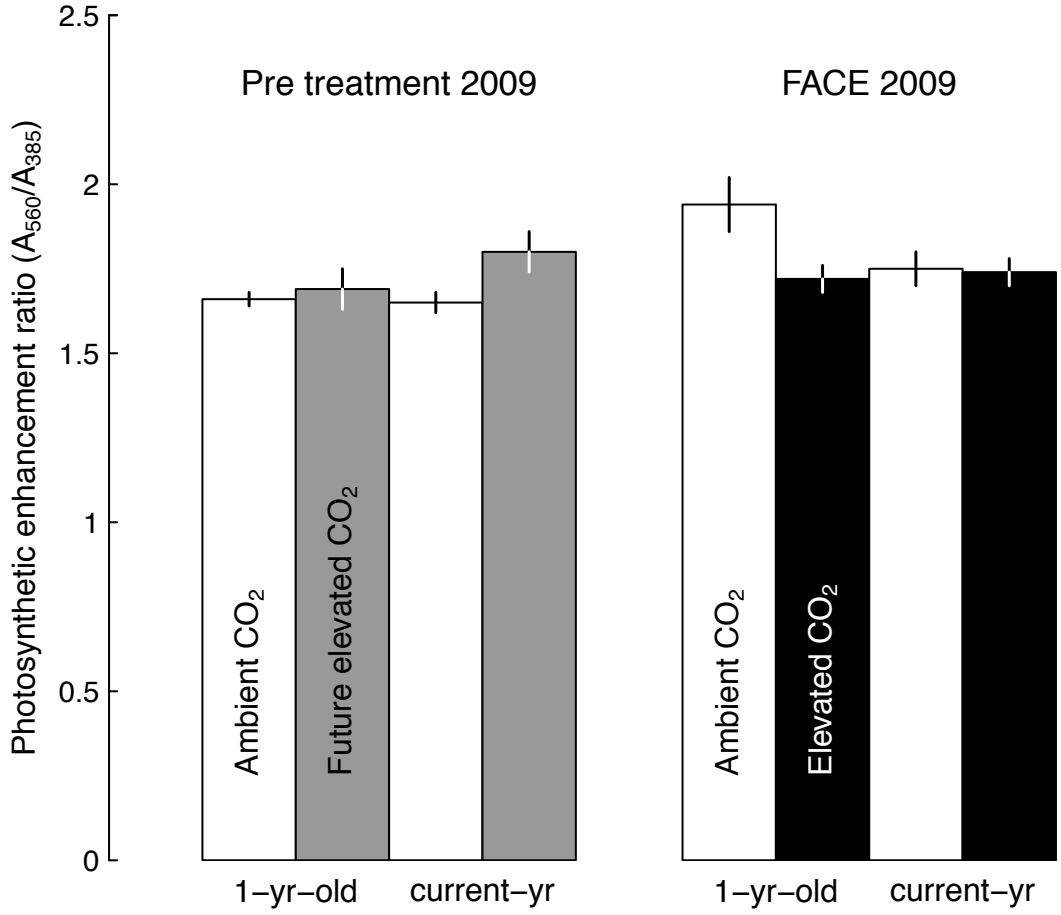

Fig. S5 Photosynthetic enhancement ratio of one-year old (2008) and current-year (2009) needles prior to the start of CO_2 enrichment (pre-treatment, left panel) and 4 weeks after FACE initiation (FACE, right panel). White bars indicate control trees, grey bars indicate trees selected for CO_2 enrichment and black bars denote trees receiving CO_2 enrichment. Mean ± SE (n = 5 per group/treatment).

Chapter 4

Photosynthetic enhancement and diurnal stem and soil carbon fluxes in a mature Norway spruce stand under elevated CO_2

Martin K.-F. Bader, Manuel Mildner, Christina Baumann, Sebastian Leuzinger and Christian Körner

published in *Environmental and Experimental Botany 124 (2016):110-119.*

DOI 10.1016/j.envexpbot.2015.12.005

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Photosynthetic enhancement and diurnal stem and soil carbon fluxes in a mature Norway spruce stand under elevated CO_2

Martin Karl-Friedrich Bader[a,*], Manuel Mildner[b], Christina Baumann[b], Sebastian Leuzinger[c], Christian Körner[b]

[a] New Zealand Forest Research Institute (SCION), Te Papa Tipu Innovation Park, 49 Sala Street, Rotorua 3046, New Zealand
[b] Institute of Botany, University of Basel, Schönbeinstrasse 6, Basel 4056, Switzerland
[c] Institute for Applied Ecology New Zealand, School of Applied Sciences, Auckland University of Technology, 31-33 Symonds Street, Auckland 1142, New Zealand

ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 2 December 2015
Accepted 12 December 2015
Available online 17 December 2015

Keywords:
Global change
Carbon assimilation
Stem respiration
Soil respiration
Free-air CO_2 enrichment (FACE)
Ecosystem carbon cycling

ABSTRACT

Understanding the effects of elevated atmospheric CO_2 on carbon (C) relations of mature forest trees is central to understanding ecosystem C fluxes and pools in a future high-CO_2 world. Here, we investigated the CO_2-induced photosynthetic enhancement and the diurnal variation in shoot carbon assimilation, stem CO_2 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 CO_2 enrichment (FACE) 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 CO_2 enrichment, and one year later. Under controlled leaf chamber conditions, an increase in the atmospheric CO_2 concentration of *ca.* 150 ppm above ambient stimulated light-saturated rates of photosynthesis in previous- and current-year upper-canopy shoots equally by $73 \pm 2\%$. In the course of the day such large differences in C assimilation between trees growing under elevated CO_2 (eCO_2) and ambient conditions (aCO_2) only occurred around midday under non-limiting light conditions. The CO_2 efflux rates from spruce stems (CE_{stem}) and surrounding soil (R_{soil}) shared a similar range during night- and daytime (3–5 $\mu mol\, m^{-2}\, s^{-1}$) but were not stimulated by eCO_2. Both CE_{stem} 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.

1. 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, i.e. 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 and 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

* Corresponding author.
E-mail address: martin.bader@scionresearch.com (M.K.-F. Bader).

http://dx.doi.org/10.1016/j.envexpbot.2015.12.005
0098-8472/

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 and 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 and 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 tunover, no sustained stimulation had been found unless mineral fertilizer was applied (e.g. Norby and 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 CO_2 'fertilization' effects early after an experimental step increase in the atmospheric CO_2 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 CO_2 concentration.

One plausible pathway for the extra C taken up by forests exposed to elevated atmospheric CO_2 concentrations is increased soil respiration. However, the stimulation of belowground C transfer under eCO_2 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 CO_2 efflux (23–54%) than more mature stands (12–16%) under eCO_2 (King et al., 2004). Long-term trends under CO_2 enrichment have been inconclusive because rates of CO_2 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 and Körner, 2010; Mildner et al., 2015). R_{soil} may be indirectly influenced by elevated CO_2 through changes in temperature and soil moisture sensitivity, which may even result in reduced rates of R_{soil} under CO_2 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 and Cheng, 2001). Diurnal variation in R_{soil} is typically low (Buchmann, 2000; Bader and Körner, 2010), and dominated by photosynthesis, with R_{soil} lagging behind by several hours to days (Tang et al., 2005; Wertin and Teskey, 2008; Kuzyakov and 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 and 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, CO_2 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 CO_2 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 and McGuire, 2007), reflecting the transport of dissolved CO_2 in or out of the stem compartment via the xylem stream (Levy et al., 1999; Teskey and McGuire, 2002; Moore et al., 2008; Bloemen et al., 2013; Etzold et al., 2013). Therefore, any changes in sap flow under eCO_2 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 CO_2 concentration, and the contribution of xylem-transported CO_2 also produce sizable effects (Teskey and 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 CO_2 concentrations by means of free air CO_2 enrichment (FACE) technology. We aimed to characterize the main CO_2 fluxes (photosynthetic C uptake, R_{soil} and CE_{stem}) under elevated CO_2 compared to current ambient CO_2 levels on an intra-daily timescale. This was achieved by simultaneously measuring net photosynthetic rates in branchlets using a crane gondola, and respiratory CO_2 release from stems and the soil in the understorey of *P. abies* trees before and after the onset of free air CO_2 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.

2. Material and methods

2.1. Study site and CO_2 enrichment system

This free air CO_2 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., *P. abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.). In forest patch selected for the CO_2 enrichment experiment *Picea abies* is the predominant tree species. The mild temperate climate in this region is characterized by a mean growing season temperature of 14.7 °C (May–September) and around 1000 mm annual precipitation (Bader and Körner, 2010). In 2009, five *ca.* 110-year-old *P. abies* trees of around 37 m height were fitted with a modified web-FACE system (Pepin and Körner, 2002; Mildner et al., 2014) using a construction crane. In brief, the computer-controlled release of pure CO_2 occurred through a fine web of perforated lightweight tubing fixed around tree branches. The target concentration of CO_2 was 550 ppm and an air sampling system comprising multiple sampling points per tree was used to monitor canopy CO_2 concentration using infrared gas analysis. CO_2-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 $<100\ \mu mol\ m^{-2}\ s^{-1}$ or when wind speed exceeded $10\ m\ s^{-1}$. The CO_2 control and monitoring system scanned across four sectors within each tree crown to ensure adequate CO_2 distribution and to maintain the target CO_2 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 CO_2 concentration averaged 541 ppm, 532 ppm, and 541 ppm, respectively; and the median CO_2 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 CO_2-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).

2.2. 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 min under saturating light conditions of 1500 $\mu mol\, m^{-2}\, s^{-1}$ provided by a cold light source (Schott, KL 1500, Mainz, Germany), 25 °C leaf temperature and an air-to-leaf vapor 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.

2.3. 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 and 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 1500 $\mu mol\, m^{-2}\, s^{-1}$ 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 CO_2 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 CO_2 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 CO_2 concentration. Individual response curves were typically completed within 35 min. We used a Farqhar-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 and 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 CO_2 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 (aCO_2:46–57 cm, eCO_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 CO_2 increase in the chamber headspace followed by a CO_2 scrubbing back to ambient CO_2 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 and 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 CO_2 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 and 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).

2.4. 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 modeled using an ordinary least squares model. A/C_i curves recorded under pre-treatment and FACE conditions were analysed using Farquhar-type equations (Long and Bernacchi, 2003) fitted by generalised nonlinear least squares (*gnls*). Model diagnostic plots suggested heteroscedasticity, which was modeled by incorporating exponential or power variance functions using C_i as variance covariate.

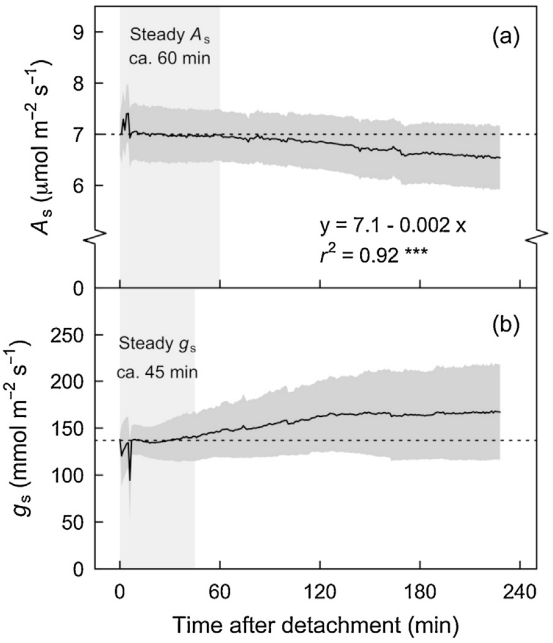

Fig. 1. (a) Net photosynthetic rate and (b) stomatal conductance (lower panel) of one-year old (2008) south-facing branchlets of *Picea abies* as a function of time after detachment. The dashed lines indicate the mean values before branchlet removal. The solid lines show the mean of five branchlets derived from different adult spruce trees ± 1 standard error (grey area). Measurements were conducted in logging mode over a period of 230 min using a 1 min interval under saturating light intensity of 1500 µmol m^{-2} 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.

In order to compare aCO_2 vs. eCO_2 we formulated parameter models allowing separate parameter estimates for each CO_2 treatment. These parameter models were then compared to a base model (assuming common parameter estimates for both CO_2 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, CO_2 treatment and their interaction as fixed term. Model diagnostic plots suggested heteroscedasticity in the pre-treatment and the treatment data, which was modeled 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 'CO_2 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 'CO_2 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 modeled 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 CO_2 treatments (CO_2 × time of day interaction), a model with one common smoother was compared to a model allowing separate smoothers for aCO_2 and eCO_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 and 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*).

3. Results

3.1. 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 ± 0.4 µmol m^{-2} s^{-1} (mean ± SE, n = 5, Fig. 1). After branch detachment, photosynthesis declined at a rate of less than 2% per hour. Nearly 4 h after branchlet detachment the photosynthetic rate had only dropped by 0.5 µmol m^{-2} 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).

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

The photosynthetic enhancement ratio was remarkably similar across designated aCO_2 and eCO_2 trees, needle age and cardinal directions averaging 1.69 ± 0.02 (mean ± 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 aCO_2 and eCO_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 23–36 µmol m^{-2} s^{-1} and J_{max} varied between 95 and 120 µmol m^{-2} s^{-1} (Fig. 3).

Table 1
Results from a generalised least squares model for diurnal gas-exchange of *Picea abies* needles applying individual smoothers for aCO_2 and eCO_2. CO_2 treatment = aCO_2 vs. eCO_2, needle age = previous-year vs. current-year needles, FACE 2009 = 6 August 2009 one week after the start of CO_2 enrichment, FACE 2010 = 14 July 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.

Parameter	DF_{num}	DF_{den}	F	P	
Pre-treatment					
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	
FACE					
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	

*** $P < 0.001$.

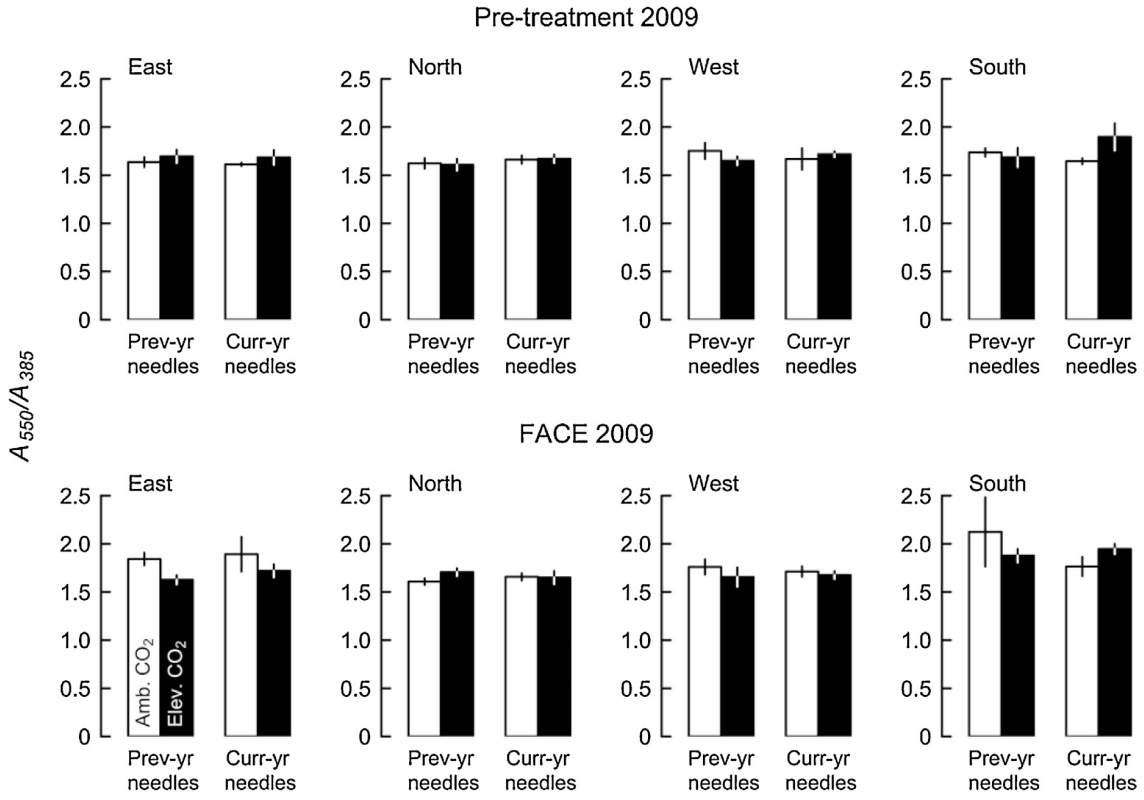

Fig. 2. Instantaneous enhancement of light-saturated photosynthesis measured across cardinal directions in upper-crown branchlets of mature *Picea abies* trees. Measurements were conducted on previous-year and current-year shoot sections one week before the onset of canopy CO_2-enrichment (upper panel)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

3.3. 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 (aCO_2:6–9 μmol m^{-2} s^{-1}, eCO_2:9–12 μmol m^{-2} s^{-1}), followed by a nearly linear decline over the course of the afternoon (Fig. 4a and b). Rates of nighttime shoot respiration ranged from 1 to 3 μmol m^{-2} s^{-1}.

The diurnal courses of gas-exchange showed similar patterns across recording dates, (ΔAIC = 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 (ΔAIC = 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 ppm 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. 4a and 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 eCO_2 compared to trees growing under aCO_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 aCO_2 and eCO_2 (ΔAIC = 30.8 in favor of the model allowing separate smoothing terms for aCO_2 and eCO_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 eCO_2 compared to aCO_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. 4c and d). Night values of CE_{stem} varied on average between 2.8 and 3.8 μmol m^{-2} s^{-1} and nighttime R_{soil} ranged from 2.4 to 3.7 μmol m^{-2} 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 μmol m^{-2} 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 (ΔAIC = 2 in favor 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 (ΔAIC = 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 (ΔAIC = 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

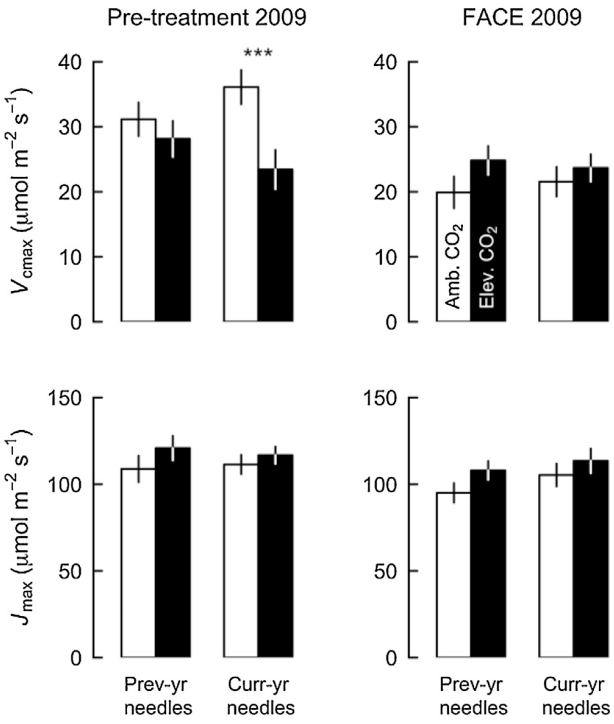

Fig. 3. Maximum rate of Rubisco carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) in south-facing upper-crown branchlets of mature *Picea abies* trees. Measurements were conducted on previous-year and current-year shoot sections one week before the onset of canopy CO_2-enrichment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

pattern across CO_2 treatments (ΔAIC = 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 eCO_2.

4. Discussion

4.1. 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 h following the detachment of the branchlets. 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.

4.2. 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 by Sigurdsson et al. (2002) for *ca.* 30-year-old *Picea abies* trees studied in Sweden using a branch bag CO_2 enrichment technique (+69% instantaneous enhancement). Our findings are also consistent with the photosynthetic enhancement observed in current-year needles of *Pinus taeda* (+67%) growing under elevated CO_2 at the Duke FACE site (Ellswoth 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. aCO_2 and eCO_2 plants measured at both the low and the high CO_2 concentration) but were derived from comparisons between maximal photosynthetic rates at the respective growth CO_2 concentration.

V_{cmax} and J_{max} measured in or study trees were in the range of values observed in *Pinus taeda* exposed to +200 ppm atmospheric CO_2 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 CO_2 in open-top chambers, however, J_{max} was substantially lower reaching values between 50 and 70 µmol m^{-2} s^{-1}.

4.3. 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 CO_2 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 *ca.* 110-year-old and 37 m tall *Picea abies* trees and surrounding soil under eCO_2. Peak daily C assimilation rates in both previous-year and current-year needles were strongly enhanced in trees receiving eCO_2 relative to control trees, but one year after the start of CO_2 enrichment, the photosynthetic stimulation seemed

Table 2
Results from a generalised additive mixed model for diurnal gas-exchange of *Picea abies* needles applying individual smoothers for aCO_2 and eCO_2. CO_2 treatment = aCO_2 vs. eCO_2, needle age = previous-year vs. current-year needles, FACE 2009 = 6 August 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.

Parametric coefficients	Estimate (SE)	*t*	*P*	
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				
	edf	*F*	*P*	
s (time of day):CO_2	2.93	153.9	<0.001	***

** $P < 0.01$.
*** $P < 0.001$.

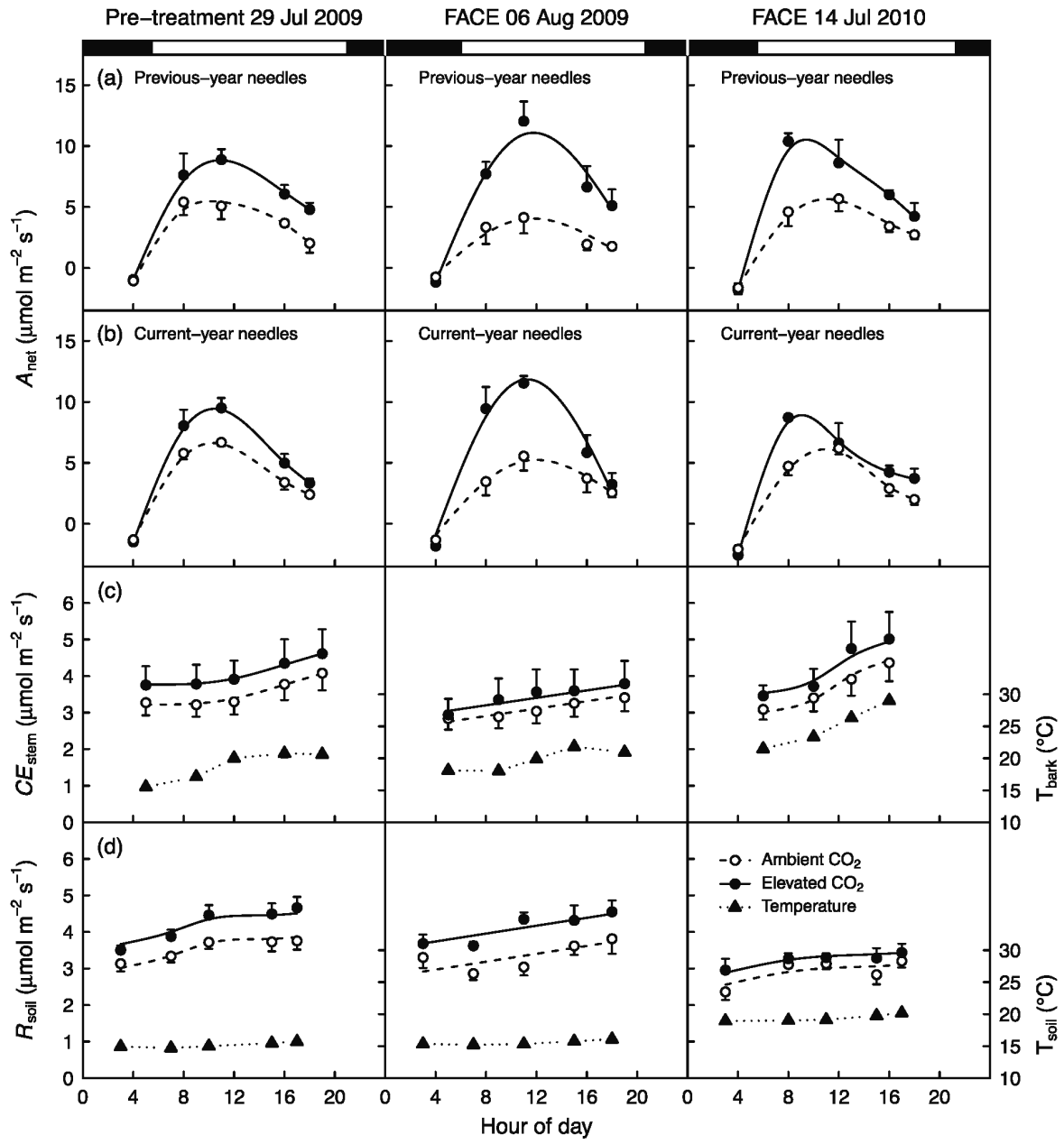

Fig. 4. (a and b) Diurnal courses of net photosynthesis (A_{net}) in previous- and current-year needles, (c) stem CO_2 efflux (CE_{stem}) and bark temperature (T_{bark}), and (d) soil respiration (R_{soil}) along with soil temperature at 10 cm depth (T_{soil}). The measurement campaigns were performed 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 ± 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.

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 eCO_2 enhanced net photosynthesis by 67% in current year needles and 37% in previous year needles (Ellsworth et al., 2012) under ambient atmospheric CO_2 concentration +200 ppm. We did not observe CO_2-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 eCO_2 in open-top chambers. The largest increase in mitchondria 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 eCO_2 (Griffin et al., 2004).

At our FACE site, CE_{stem} remained unaffected by eCO_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, CO_2 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

Table 3
Results from a generalised additive mixed model for diurnal C release from *Picea abies* stems. CO_2 treatment = aCO_2 vs. eCO_2, Diurnal FACE 2009 = recording of 6 August 2009 one week after the start of CO_2 enrichment, Diurnal FACE 2010 = recording of 14 July 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.

Parametric coefficients	Estimate (SE)	*t*	*P*	
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				
	edf	*F*	*P*	
s (time of day)	1.00	32.65	<0.001	***
s (bark temperature)	2.73	6.58	<0.001	***

*** $P < 0.001$.

et al., 2002). Diurnal recordings on 14-year-old *Picea abies* trees subjected to CO_2 enrichment in open-top chambers also showed moderate increases in CE_{stem} of up to 16% over control trees (Acosta et al., 2010). CO_2-related changes in sap flow may confound comparative CE_{stem} readings due to the respiratory CO_2 dissolved and transported in the transpiration stream and the amount of this CO_2 that remains stores internally. CE_{stem} has been shown to correlate negatively with sap flux density implying that a CO_2-induced reduction in whole-tree transpiration would inevitably result in greater CO_2 efflux rates from stems (Bowman et al., 2005). However, such confounding can be ruled out because sap flow in *Picea abies* remained unaffected by eCO_2 at the SCC FACE site (Leuzinger and Bader, 2012). The absence of a CO_2 effect on CE_{stem} in the longer term is corroborated by the lack of a CO_2-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 eCO_2 and high-N conditions in open-top chambers, but not under eCO_2 alone. However, this initial response ceased after 1.25 years, resulting in similar relative growth rates across all eCO_2 and N treatment combinations.

Given the initial increase in photosynthetic C uptake under eCO_2 and the close coupling between photosynthesis and R_{soil} (Tang et al., 2005) we expected a rapid stimulation of soil CO_2 efflux. Stable C isotope tracing revealed a small yet clear signal of the ^{13}C signature of the fossil CO_2 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 CO_2 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 CO_2-enriched trees compared to the controls (Mildner et al., 2015). The lack of a CO_2 driven stimulation of R_{soil} is in contrast to other forest FACE experiments where R_{soil} commonly increased in response to eCO_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 and 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. (2004) had achieved canopy-closure when FACE commenced, their findings suggest that the magnitude of the CO_2-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 CO_2-enrichment (Mildner et al., 2015) than expanding root systems in developing 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 aCO_2 and eCO_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 CO_2 back to atmosphere (Butnor et al., 2003).

Table 4
Results from a generalised additive mixed model for diurnal soil respiration under *Picea abies* trees. CO_2 treatment = aCO_2 vs. eCO_2, Diurnal FACE 2009 = recording of 6 August 2009 one week after the start of CO_2 enrichment, Diurnal FACE 2010 = recording of 14 July 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.

Parametric coefficients	Estimate (SE)	*t*	*P*	
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	***
Approx. significance of smooth terms				
	edf	*F*	*P*	
s (time of day)	1.00	41.85	<0.001	***

*** $P < 0.001$.

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 CO_2 concentrations are common in FACE experiments, however, in reality atmospheric CO_2 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 CO_2 experiments are most likely mitigated due to compensatory effects (Leuzinger et al., 2011; Norby and 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 CO_2-driven stimulation of shoot photosynthesis under controlled leaf chamber conditions across cardinal directions in these tallest and oldest trees ever exposed to eCO_2. There appeared no overall or daytime-dependent stimulation of CO_2 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 CO_2 (Fatichi and 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).

Author contributions

MKFB, SL, MM and CK conceived and designed the experiments. CB, MM, MKFB and SL performed the measurements and analysed the data. MKFB and MM wrote the manuscript, and CK and SL provided input and comments.

Acknowledgements

The authors are indebted to Erwin Amstutz for crane operation and support during measurements campaigns. We thank Rolf Siegwolf from the Paul Scherrer Institute (Switzerland) for the provision of equipment. We would also like to thank four anonymous reviewers, whose valuable comments greatly helped to improve this manuscript. The SCC FACE study was supported by the Swiss National Science Foundation (grant 3100AO-111914/1).

References

Acosta, M., Pokorný, R., Janouš, D., Marek, M., 2010. Stem respiration of Norway spruce trees under elevated CO_2 concentration. Biol. Plant. 54, 773–776.

Amthor, J.S., 1984. The role of maintenance respiration in plant growth. Plant Cell Environ. 7, 561–569.

Atkin, O., Millar, A., Gardeström, P., Day, D., 2000. Photosynthesis, carbohydrate metabolism and respiration in leaves of higher plants. In: Leegood, R., Sharkey, T., von Caemmerer, S. (Eds.), Photosynthesis: Physiology and Metabolism. Kluwer Academic Publishers.

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

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

Bader, M.K.-F., Leuzinger, S., Keel, S.G., Siegwolf, R.T., Hagedorn, F., Schleppi, P., Körner, C., 2013. Central European hardwood trees in a high-CO_2 future: synthesis of an 8-year forest canopy CO_2 enrichment project. J. Ecol. 101, 1509–1519.

Bloemen, J., McGuire, M.A., Aubrey, D.P., Teskey, R.O., Steppe, K., 2013. Transport of root-respired CO_2 via the transpiration stream affects aboveground carbon assimilation and CO_2 efflux in trees. New Phytol. 197, 555–565.

Bowman, W.P., Barbour, M.M., Turnbull, M.H., Tissue, D.T., Whitehead, D., Griffin, K. L., 2005. Sap flow rates and sapwood density are critical factors in within- and between-tree variation in CO_2 efflux from stems of mature *Dacrydium cupressinum* trees. New Phytol. 167, 815–828.

Buchmann, N., 2000. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. Soil Biol. Biochem. 32, 1625–1635.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information—Theoretic Approach. Springer.

Butnor, J.R., Johnsen, K.H., Oren, R., Katul, G.G., 2003. Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands. Global Change Biol. 9, 849–861.

Caprez, R., Niklaus, P., Körner, C., 2012. Forest soil respiration reflects plant productivity across a temperature gradient in the Alps. Oecologia 170, 1143–1154.

Carey, E.V., DeLucia, E.H., Ball, J.T., 1996. Stem maintenance and construction respiration in *Pinus ponderosa* grown in different concentrations of atmospheric CO_2. Tree Physiol. 16, 125–130.

Cernusak, L.A., Hutley, L.B., Beringer, J., Tapper, N.J., 2006. Stem and leaf gas exchange and their responses to fire in a north Australian tropical savanna. Plant Cell Environ. 29, 632–646.

Covey-Crump, E., Attwood, R., Atkin, O., 2002. Regulation of root respiration in two species of *Plantago* that differ in relative growth rate: the effect of short- and long-term changes in temperature. Plant Cell Environ. 25, 1501–1513.

Damesin, C., Ceschia, E., Le Goff, N., Ottorini, J.-M., Dufrène, E., 2002. Stem and branch respiration of beech: from tree measurements to estimations at the stand level. New Phytol. 153, 159–172.

Dieleman, W.I.J., Vicca, S., Dijkstra, F.A., Hagedorn, F., Hovenden, M.J., Larsen, K.S., Morgan, J.A., Volder, A., Beier, C., Dukes, J.S., King, J., Leuzinger, S., Linder, S., Luo, Y., Oren, R., De Angelis, P., Tingey, D., Hoosbeek, M.R., Janssens, I.A., 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature. Global Change Biol. 18, 2681–2693.

Edwards, N.T., Tschaplinski, T.J., Norby, R.J., 2002. Stem respiration increases in CO_2-enriched sweetgum trees. New Phytol. 155, 239–248.

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

Epron, D., Ngao, J., Dannoura, M., Bakker, M., Zeller, B., Bazot, S., Bosc, A., Plain, C., Lata, J., Priault, P., 2011. Seasonal variations of belowground carbon transfer assessed by in situ $^{13}CO_2$ pulse labelling of trees. Biogeosciences 8, 1153–1168.

Etzold, S., Zweifel, R., Ruehr, N.K., Eugster, W., Buchmann, N., 2013. Long-term stem CO_2 concentration measurements in Norway spruce in relation to biotic and abiotic factors. New Phytol. 197, 1173–1184.

Fatichi, S., Leuzinger, S., 2013. Reconciling observations with modeling: the fate of water and carbon allocation in a mature deciduous forest exposed to elevated CO_2. Agric. For. Meteorol. 174, 144–157.

Gielen, B., Scarascia-Mugnozza, G., Ceulemans, R., 2003. Stem respiration of *Populus* species in the third year of free-air CO_2 enrichment. Physiol. Plant. 117, 500–507.

Greenep, H., Turnbull, M.H., Whitehead, D., 2003. Response of photosynthesis in second-generation *Pinus radiata* trees to long-term exposure to elevated carbon dioxid partial pressure. Tree Physiol. 23, 569–576.

Griffin, K.L., Anderson, R.O., Tissue, D.T., Turnbull, M.H., Whitehead, D., 2004. Variations in dark respiration and mitochondrial numbers within needles of *Pinus radiata* grown in ambient or elevated CO_2 partial pressure. Tree Physiol. 24, 347–353.

Hamilton, J.G., DeLucia, E.H., George, K., Naidu, S.L., Finzi, A.C., Schlesinger, W.H., 2002. Forest carbon balance under elevated CO_2. Oecologia 131, 250–260.

Heinemeyer, A., McNamara, N.P., 2011. Comparing the closed static versus the closed dynamic chamber flux methodology: implications for soil respiration studies. Plant Soil 346, 145–151.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Lofvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.

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

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grünwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, Ü., Morgenstern, K., Oltchev, S., Clement, R., Guðmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biol. 7, 269–278.

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

Klein, T., Bader, M.K.-F., Leuzinger, S., Mildner, M., Schleppi, P., Siegwolf, R.T.W., Körner, C., 2015. Growth and carbon relations of mature *Picea abies* trees under five years of free air CO_2 enrichment. J. Ecol. (in review).

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

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol. Biochem. 33, 1915–1925.

Kuzyakov, Y., Gavrichkova, O., 2010. REVIEW: time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biol. 16, 3386–3406.

Leuzinger, S., Bader, M.K.-F., 2012. Experimental vs. modeled water use in mature Norway spruce (*Picea abies*) exposed to elevated CO_2. Front. Plant Sci. 3.

Leuzinger, S., Luo, Y., Beier, C., Dieleman, W., Vicca, S., Körner, C., 2011. Do global change experiments overestimate impacts on terrestrial ecosystems? Trends Ecol. Evol. 26, 236–241.

Levy, P., Meir, P., Allen, S., Jarvis, P., 1999. The effect of aqueous transport of CO_2 in xylem sap on gas exchange in woody plants. Tree Physiol. 19, 53–58.

Malhi, Y., Baldocchi, D., Jarvis, P., 1999. The carbon balance of tropical, temperate and boreal forests. Plant Cell Environ. 22, 715–740.

Mildner, M., Bader, M.K.-F., Leuzinger, S., Siegwolf, R.T., Körner, C., 2014. Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*. Oecologia 1–16.

Mildner, M., Bader, M.K.-F., Baumann, C., Körner, C., 2015. Respiratory fluxes and fine root responses in mature *Picea abies* trees exposed to elevated atmospheric CO_2 concentrations. Biogeochemistry 124, 95–111.

Moore, D.J., Gonzalez-Meler, M.A., Taneva, L., Pippen, J.S., Kim, H.-S., DeLucia, E.H., 2008. The effect of carbon dioxide enrichment on apparent stem respiration from *Pinus taeda* L. is confounded by high levels of soil carbon dioxide. Oecologia 158, 1–10.

Norby, R.J., Zak, D.R., 2011. Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annu. Rev. Ecol. Evol. Syst. 42, 181.

Nowak, R.S., Ellsworth, D.S., Smith, S.D., 2004. Functional responses of plants to elevated atmospheric CO_2—do photosynthetic and productivity data from FACE experiments support early predictions? New Phytol. 162, 253–280.

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

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2014. nlme: Linear and Nonlinear Mixed Effects Models, R package version 3. 1-118 ed.

R Development Core Team, 2014. R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raich, J., Nadelhoffer, K., 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology 70, 1346–1354.

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

Ryan, M.G., Hubbard, R.M., Clark, D.A., Sanford Jr., R.L., 1994. Woody-tissue respiration for *Simarouba amara* and *Minquartia guianensis*, two tropical wet forest trees with different growth habits. Oecologia 100, 213–220.

Ryan, M.G., Gower, S.T., Hubbard, R.M., Waring, R.H., Gholz, H.L., Cropper Jr., W.P., Running, S.W., 1995. Woody tissue maintenance respiration of four conifers in contrasting climates. Oecologia 101, 133–140.

Sigurdsson, B., Roberntz, P., Freeman, M., Naess, M., Saxe, H., Thorgeirsson, H., Linder, S., 2002. Impact studies on Nordic forests: effects of elevated CO_2 and fertilization on gas exchange. Can. J. For. Res. 32, 779–788.

Sigurdsson, B., Medhurst, J., Wallin, G., Eggertson, O., Linder, S., 2013. Growth of mature boreal Norway spruce was not affected by elevated CO_2 and/or air temperature unless nutrient availability was improved. Tree Physiol. 33, 1192–1205.

Tang, J., Baldocchi, D.D., Xu, L., 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Global Change Biol. 11, 1298–1304.
Teskey, R., McGuire, M., 2002. Carbon dioxide transport in xylem causes errors in estimation of rates of respiration in stems and branches of trees. Plant Cell Environ. 25, 1571–1577.
Teskey, R.O., McGuire, M.A., 2005. CO_2 transported in xylem sap affects CO_2 efflux from *Liquidambar styraciflua* and *Platanus occidentalis* stems, and contributes to observed wound respiration phenomena. Trees 19, 357–362.
Teskey, R., McGuire, M., 2007. Measurement of stem respiration of sycamore (*Platanus occidentalis* L.) trees involves internal and external fluxes of CO_2 and possible transport of CO_2 from roots. Plant Cell Environ. 30, 570–579.
Thimonier, A., Graf Pannatier, E., Schmitt, M., Waldner, P., Lorenz, W., Schleppi, P., Dobbertin, M., Kräuchi, N., 2010. Does exceeding the critical loads for nitrogen alter nitrate leaching, the nutrient status of trees and their crown condition at Swiss Long-term Forest Ecosystem Research (LWF) sites? Eur. J. For. Res. 129, 443–461.
Walker, A.P., Zaehle, S., Medlyn, B.E., De Kauwe, M.G., Asao, S., Hickler, T., Parton, W., Ricciuto, D.M., Wang, Y.-P., Warlind, D., Norby, R.J., 2015. Predicting long-term carbon sequestration in response to CO_2 enrichment: how and why do current ecosystem models differ? Global Biogeochem. Cycles 29, 476–495.
Wertin, T.M., Teskey, R.O., 2008. Close coupling of whole-plant respiration to net photosynthesis and carbohydrates. Tree Physiol. 28, 1831–1840.
Wofsy, S., Goulden, M., Munger, J., Fan, S.-M., Bakwin, P., Daube, B., Bassow, S., Bazzaz, F., 1993. Net exchange of CO_2 in a mid-latitude forest. Science 260, 1314–1317.
Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc. B (Statistical Methodology) 73, 3–36.
Zha, T.-S., Kellomäki, S., Wang, K.-Y., Ryyppö, A., 2005. Respiratory responses of Scots pine stems to 5 years of exposure to elevated CO_2 concentration and temperature. Tree Physiol. 25, 49–56.
Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer.

Chapter 5

General summary

General summary

Objectives

The aim of this thesis was to identify pathways and rates of C allocation in tall forest trees, and to identify effects of elevated CO_2 on respiratory processes and root growth. Correspondingly, this thesis is divided into three separate parts:

Chapter 2)
Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies* (published in *Oecologia*)

Chapter 3)
Respiratory fluxes and fine root responses in mature *Picea abies* trees exposed to elevated atmospheric CO_2 concentrations (published in *Biogeochemistry)*

Chapter 4)
Photosynthetic enhancement and diurnal stem and soil carbon fluxes in a mature Norway spruce stand under elevated CO_2 (published in *Environmental and Experimental Botany*)

The work was conducted at the Swiss canopy crane (SCC) research site in Hofstetten near Basel, Switzerland, and explored signals produced by free air CO_2 enrichment (FACE) in 110-year-old, ca. 37m tall *P. abies* trees. **Chapter 2** capitalizes on the isotopic signal carried by the CO_2 gas used for CO_2 enrichment, yet does not address effects of elevated CO_2 as such, but rather deals with basic questions of C transfer in tall trees. **Chapter 3** explores the longer-term CO_2 effects on mature *P. abies* (i.e. 2.5 years), whereas **chapter 4** reports short-term (diurnal) responses to elevated CO_2. In the following, I will provide a summary of the results of the three chapters of my thesis, extended by a conclusion that links these chapters.

Chapter 2) Long-term ^{13}C labeling of *Picea abies*

As a side effect, the FACE technique provided the unique opportunity to study C translocation within the tree body using the stable isotope ^{13}C signal the FACE gas carries. Since control trees are not (can not) be similarly labeled with ^{13}C the tree responses to elevated CO_2 were not the subject of this chapter. Yet, FACE resembles a continuous ^{13}C labeling of new assimilates. Tracking the fate of these assimilates over a period of 2.5 years in tall trees offers new insights in tree C relations under steady state conditions. We tracked ^{13}C signals in mature *P. abies* trees at a high spatial and temporal resolution, i.e. from the canopy (needles and branchlets), down to the tree trunk (year rings and stem CO_2 efflux), and into the soil compartment (fine roots, fungi, soil CO_2 efflux). The following key questions were answered:

1. How long does it take for new C to arrive at a certain tissue type or respiratory flux?
2. What is the proportional contribution of newly assimilated C to concurrent tree tissue production and maintenance?
3. How long does it take until old C is replaced by new C in various tissues?

Generally, we observed a reduction of new assimilate investment with distance from the canopy, which can be explained by a progressive dilution of new C into the existing C storage pools in the tree. New sunlit needles (and adjacent branchlets) exhibited a nearly 100% share of new C, whereas shaded needles also used some older C. Stem wood isotope signals evidenced a complete exchange of old C by new C within 2 years. Fine roots contained only 49-56% new C, hence are using older C pools for a longer period of time. A surprisingly low fraction of novel C (26-43%) was recovered from fungal sporocarps, presumably related to the influence of neighboring trees that were not CO_2 enriched. The first appearance of new C in soil and stem CO_2 release occurred after 12 days, reflecting a lag due to the long transport distances in these 37m tall trees. The CO_2 released by stems was composed of 50% new C already in the first year of FACE. In contrast, only ca. 15% new C contributed to soil CO_2 efflux, reflecting the use of older substrates, and the influence of older roots and litter from neighboring trees blown in by wind.
These findings indicate a rapid contribution of new assimilates to tissue formation, and thus, a fast replacement of mobile C reserves with new C, and a progressive signal dilution from treetop to the bottom. The two-year replacement time in stem xylem shows that the storage pool is contributing substantially to tree ring formation. We speculate that the turnover of mobile C pools might be enhanced by elevated CO_2, and the metabolic costs of this turnover might compensate for some of the extra C taken up at elevated CO_2 concentrations, and thus, may explain the 'missing C' at the whole tree level. These metabolic costs are unlikely to produce measurable signals at tissue level, given the large heterotrophic volume of such trees.

Chapter 3) Responses of *Picea abies* to elevated CO_2

Most FACE experiments revealed strong initial growth responses to elevated CO_2 that diminished over the first 3

years (Körner 2006). Since growth in natural undisturbed systems is commonly not showing a continued stimulation under altered CO_2 for reasons of nutrient supply, a step increase in CO_2 concentration should induce overflow responses in terms of enhanced respiration and fine root expansion, the latter in order to forage for nutrients to balance the additional C input. In this web-FACE experiment, established in a natural Central European forest, we investigated mature ca. 110-year-old *P. abies* trees in their steady state of growth (C cycle coupled to the nutrient cycle; Körner 2006). In this publication we were particularly interested in:

1. Seasonal shifts in assimilate allocation;
2. Locations of C-investment;
3. Residence times (turnover) of mobile C pools.

We tracked the respiratory and fine root growth responses of these trees before and directly after the start of FACE, and for further 2.5 years. The CO_2 concentration in the canopy (e.g. 540 ppm) was about twice the pre-industrial level. We anticipated a stimulation of CO_2 release, and faster root expansion into root-free soil space (in-growth core method), but we also expected a weaker signal in these mature trees compared to young trees. Seasonal stem CO_2 efflux did not show any sign of increase during the 2.5 years under elevated CO_2. This result lines up with the lack of any stem radial growth response (ongoing work). Fine roots (<0.5-2 mm) did not accumulate more dry matter in the course of 2.5 years of CO_2 fertilization. Interestingly, we observed a slight but significant reduction of CO_2 release from the soil despite clear evidence by isotopic signals that novel assimilates arrived in the soil.
These data suggest that such mature trees do not even show a transient stimulation of respiration to a step increase of CO_2, as was observed in other FACE experiments using much younger trees (Norby et al. 2010). Other growth-limiting factors appear to prevent more vigorous tree growth and thus, metabolism at high CO_2 (Norby & Zak 2011). N limitation can be excluded at our site because of high N-deposition. A part of the extra C taken up by needles at elevated CO_2 might have been allocated belowground, however, not to fine roots. Conversely, slightly reduced rather than increased rates of soil CO_2 efflux implies that respiration of roots and/or soil organisms declined under elevated CO_2, implying an overall reduced C allocation into the rhizosphere. We assume that extra C absorbed by foliage is either retained within the tree body (stored carbohydrates), recycled by respiration rates below detection limit across all heterotrophic plant tissues, or lost through enhanced leaching of dissolved organic or inorganic carbon (DIC/DOC).
In summary, we conclude that mature *P. abies* trees at our site are roughly C saturated at current CO_2 concentrations. We find no indication of stimulated belowground metabolic activity (fine roots and soil CO_2 efflux).

Chapter 5) Diurnal courses in *P. abies* under elevated CO_2

Leaf-level photosynthetic stimulation in trees following a step increase of atmospheric CO_2 was commonly observed in CO_2 enrichment experiments, however, mostly without corresponding growth stimulation. Hence, the fate of this additional C input in tree still is not fully resolved, but C overflow mechanisms such as respiratory C losses might account for this C surplus. Since these potential variations in C fluxes might not be detectable on a daily basis, a response may emerge on shorter timescales (i.e. on a diurnal basis). This chapter (co-authorship) explored the diurnal variations in C fluxes (i.e. net photosynthesis, and CO_2 efflux from the forest floor and the from stem) in mature *P. abies* trees exposed to elevated CO_2 in the SCC web-FACE experiment. We tracked the diurnal variations of these fluxes on a summer day shortly before the onset of FACE, and twice during the FACE periods in summer 2009 and 2010.
Results from this study confirmed a CO_2-induced photosynthetic stimulation shortly after the onset of FACE, and a change in magnitude throughout the day. Intriguingly, this stimulation of A_{net} diminished in the second year under FACE, indicating photosynthetic downregulation in these trees. The respiratory fluxes from *P. abies* stems, as observed on a seasonal basis (chapter 4), were not affected by high levels of CO_2 whereas soil CO_2 efflux decreased slightly with prolonged exposure to elevated CO_2. Further, the diurnal patterns of CO_2 release (stems and soil) were not altered by CO_2 enrichment.
In conclusion, despite larger C input into the tree system in the first year of FACE, respiratory overflow mechanisms could not be observed even on a diurnal basis, corroborating our results obtained in chapter 4. Additionally, the photosynthetic downregulation observed at high CO_2 confirms the assumption that these trees are C saturated.

Final conclusions

Stimulatory effects of elevated CO_2 on tree growth are constrained by several growth-limiting factors, mainly availability of nutrients and other resources, and the developmental stage (age) of a tree. This thesis for the first time illuminates the current (**chapter 2**) and future (**chapters 3 and 4**) C balance of mature evergreen conifers subjected to prospective CO_2 levels of 540 ppm in a near-natural forest in Switzerland. Isotopic labeling of fresh assimilates successfully depicted the pathways of C in these trees, thus provided basic insights into how *P. abies* trees handle the distribution of assimilates. We observed remarkable tree-specific variations in all pre-treatment measurements, emphasizing the importance of recording baseline conditions prior to any experiment. At current CO_2 levels, all investigated tissues (except for needles in the sun), and respiratory fluxes depended only partly on new assimilates. The further away from the upper tree canopy, the greater the role of old C stores for new tissue formation and respiration. Since no aboveground growth

stimulation was observed (ongoing works) despite higher but transient rates of photosynthesis, and since stem CO_2 efflux remained unaffected by elevated CO_2, we assume that the extra C assimilated in the first year is dissipated via respiration associated with C turnover (phloem) at rates below detection limit. These processes seem to be too small to be detectable but their accumulated rate along the entire phloem system might account for the unresolved 'missing C' at elevated CO_2. We found no evidence for increased C investment belowground at elevated CO_2 that might also account for some of the higher leaf-level C input at elevated CO_2.

References

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

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proceedings of the National Academy of Sciences of the United States of America 107:19368-19373.

Norby RJ, Zak DR (2011) Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annual Review of Ecology, Evolution, and Systematics 42:181-203

Chapter 6

Curriculum vitae

Curriculum vitae

Personal data

Name	Manuel Mildner
Address	Erwinstr. 41A 79102 Freiburg i. Br. Germany
Date and place of birth	08.12.1976, Hannover
Contact	E-Mail: mmildner_de@yahoo.de

Education

2009-2014	PhD candidate at the Botanical Institute, University of Basel, Switzerland.
Thesis title	*Response of mature Norway spruce (Picea abies) to elevated atmospheric CO_2*
2007-2008	Master of Science in Environmental Management at the University of Stirling, Scotland, UK.
Thesis title	*Assessing the distribution of a rare bumblebee species (Bombus monticola Smith) in a region in Scotland and depicting suitable habitats using a Geographic Information Systems approach*
2007	Trainee at the Stiftung Natur und Umwelt Rheinland-Pfalz, Mainz, Germany.
2006-2007	Trainee at the Gesellschaft Mensch und Natur (GMN) e.V. Rheinland-Pfalz, Hahnheim, Germany
1999-2005	Diploma in Biology at the Albert-Ludwigs University of Freiburg, Germany.
Thesis title	*Analysis of extracellular and intercellular proteins in embryogenic and non-embryogenic tissue of Cyclamen persicum Mill.*
1997-1999	Undergraduate Degree in Biology at the University of Osnabrück, Gemany.
1996-1997	Community service at Tagesförderstätte für Behinderte, Hannover, Germany
1987-1996	Secondary School at the Carl-Friedrich-Gauss Schule Hemmingen, Germany
1983-1987	Primary School at the Grundschule Wettbergen, Germany

Publications

Klein T, Bader MKF, Leuzinger S, **Mildner M**, Schleppi P, Siegwolf RTW, Körner C. Growth and carbon relations of mature Picea abies trees under five years of free air CO_2 enrichment. *Submitted to Journal of Ecology.*

Voelker SL, Brooks JR, Meinzer FC, Anderson R, Bader MKF, Battipaglia G, Becklin KM, Beerling D, Bert D, Betancourt J, Dawson TE, Domec J-C, Guyette R, Körner C, Leavitt SW, Linder S, Marshall JD, **Mildner M**, Ogée J, Panyushkina I, Plumpton H, Pregitzer KS, Saurer M, Smith A, Siegwolf RTW, Stambaugh MC, Talhelm AF, Tardif JC, Van de Water P, Ward JK, Wingate L. A dynamic leaf gas-exchange strategy is conserved in woody plants under changing ambient CO_2:

evidence from carbon isotope discrimination in paleo and CO_2 enrichment studies. *Global Change Biology 2015 Sep 22. DOI 10.111/gcb.13102.*

Bader MKF, Baumann C, Leuzinger S, **Mildner M**, Körner C (2016) Photosynthetic enhancement and diurnal stem and soil carbon fluxes in a mature Norway spruce stand under elevated CO_2. *Environmental and Experimental Botany 126:110-119. DOI 10.1016/j.envexpbot.2015.12.005.*

Mildner M, Bader MKF, Baumann C, Körner C (2015) Respiratory fluxes and fine root responses in mature *Picea abies* trees exposed to elevated atmospheric CO_2 concentrations. *Biogeochemistry 124:95-111. DOI 10.1007/s10533-015-0084-5.*

Mildner M, Bader MKF, Leuzinger S, Siegwolf RTW, Körner C (2014) Long-term ^{13}C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*. *Oecologia 175:747-762. DOI 10.1007/s00442-014-2935-5.*

Hohe A, Rensing SA, **Mildner M**, Lang D, Reski R (2002) Day length and temperature strongly influence sexual reproduction and expression of a novel MADS-box gene in the moss *Physcomitrella patens*. *Plant Biol. 4, 595-602.*

Teaching experience

2011-2013 Lecture for Blockkurs Ökologie: *Carbon pools and fluxes in trees*, Botanical Institute, University of Basel, Switzerland.

2012 Assistent for Blockkurs Ökologie in Torgon, Switzerland – part: *carbon balance*, 1 week, Botanical Institute, University of Basel, Switzerland.

2011 Assistent for Blockkurs Ökologie in Rueun, Switzerland – part: *carbon balance*, 1 week, Botanical Institute, University of Basel, Switzerland.

Conferences

2012 Ecological Society of America (ESA) Annual Meeting in Portland, USA. Mildner M, Bader MKF, Leuzinger S, Körner C. *Response of mature Norway spruce (Picea abies) to elevated atmospheric CO_2.* Talk.

2011 German Association for Stable Isotope Research (GASIR) Meeting, Villigen, Switzerland. Without contribution.