Carbon fluxes in a mature deciduous forest under elevated CO_2

INAUGURALDISSERTATION

zur Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

PHILOSOPHISCH-NATURWISSENSCHAFTLICHEN FAKULTÄT

der Universität Basel

von

MARTIN BADER

aus Steinwenden (Deutschland)

Basel, 2010

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

Prof. Dr. Christian Körner
PD Dr. Michael Bahn

Basel, den 8. Dezember 2009

Prof. Dr. Eberhard Parlow
Dekan

Acknowledgements

Firstly, I would like to thank Christian Körner, for the opportunity to undertake a challenging PhD in such a relevant field of research. With enthusiasm and encouragement and his fast and helpful proof-reading he provided an motivating work environment.

Further, I am grateful to Michael Bahn for various methodological discussions and for co-examining my thesis. Many thanks to Erwin Amstutz not only for day and night crane operations but also for technical advice, for helping out during measuring campaigns, and for cheering me up. I am indebted to Erika Hiltbrunner for sharing her huge stock of knowledge with me during inspiring discussions. Great thanks to Rolf Siegwolf for his advice and the fruitful discussions on stable isotopes. I wish to thank my dear friends and colleagues from the Botanical Institute for creating an inspring and stimulating work environment, which also allowed the formation of a 'Töggeli Team'. I would like to acknowledge Sebastian Leuzinger, Christina Schädel and Daniel Scherrer for their patient help and advice on data analysis in R. Thanks to Pascal Niklaus and Andy Hector for valuable statistical advice.

This research was funded through the Swiss National Science Foundation grant 3100AO-111914/1.

Finally, I thank my family and you my lovely Sonja for all your patience and the great support you provided during these years.

Contents

Chapter 1

General Introduction

General Introduction

A short history of the CO_2 problem

The endeavours of mankind to "subdue the Earth" entered a new era at the beginning of the industrial revolution that provided the basis for the rapid growth of the world population. Indelibly tied together, both resource exploitation and population growth have increased exponentially during the last 200 years and have meanwhile reached dimensions that are causing drastic changes in the Earth system (Vitousek, 1994; IPCC, 2007). The term 'Global Change' was coined to capture all human-caused alterations to the planet's atmosphere, hydrosphere, pedosphere, and biosphere (Körner, 2003). Besides extensive changes in land use, the rapid accumulation of CO_2 and other greenhouse gases in the atmosphere is one of the most dramatic global changes affecting the planet's climate, ecosystem functioning and biodiversity (Körner, 2003; Houghton, 2007; IPCC, 2007). CO_2 is the most important anthropogenic greenhouse gas, accountable for over half of the radiative forcing induced by heat-trapping gases other than water vapour. Because of its role as the principal substrate for photosynthesis, the rise in atmospheric CO_2 is also the most important global change issue from a plant perspective (Long et al., 2004). The increasing use of fossil fuel and large-scale deforestation since the industrial revolution led to the ongoing rise in atmospheric CO_2 from pre-industrial 280 ppm to 387 ppm today (IPCC, 2007; Tans, 2009). At present, the Earth's vegetation thrives under atmospheric CO_2 concentrations that have been unprecedented over the last 650,000 years and probably even during the past 15 million years (Petit, 1999; Siegenthaler et al., 2005; Tripati et al., 2009). Since 2000, accelerated global economic growth and an increasing carbon intensity of the world economy caused fossil fuel CO_2 emissions to rise even more dramatically (Canadell et al., 2007; Raupach et al., 2008). This increase, together with a decline in the efficiency of the Earth's land and ocean sinks to absorb anthropogenic emissions, produced the strongest acceleration of atmospheric CO_2 enrichment since the beginning of continuous atmospheric monitoring in 1958 (1.93 ppm y^{-1} from 2000-2006, Canadell et al., 2007).

Forests and the global carbon cycle

Today, land ecosystems take up about 30% of the yearly anthropogenic CO_2 emissions from fossil fuel combustion, cement production and land use change that currently amount to 9.1 Pg C y^{-1} (Canadell et al. 2007). Forests are major potential contributors to the C land sink. They cover 30% of the planet's land surface and account for 50% of the terrestrial net primary productivity (NPP; Sabine et al., 2004). Globally, forest trees harbour $> 80\%$ of the terrestrial biomass C corresponding to about 540 Pg C and the forest soils contain another 1104 Pg C (to a depth of 3 m, Jobbágy & Jackson, 2000; Saugier et al., 2001). The total (1640 Pg C) is roughly twice the amount of CO_2 held in the atmosphere (Houghton, 2007). Therefore, managing the biosphere C reservoir is inevitably tied to trees and the forests they are growing in. If and to what extent forests can be carbon sinks or sources strongly depends on tree age, stand demography, disturbance and management activities (Hyvönen et al., 2007; Körner, 2006, 2009; Canadell & Raupach, 2008). Young aggrading forests grow faster than mature forests, but they stock relatively little C and can only contribute to a net C removal from the atmosphere if they were established on previously nonforested land (Körner, 2000). Mature forests on the other hand, may exhibit slow growth but harbour vast amounts of biomass C (up to 500-700 t C ha^{-1}) for prolonged periods (Körner, 2006, 2009; Luyssaert et al., 2008). In terms of mean residence time, old-growth forests have therefore greater importance for C storage unless they are harvested or show accelerated senescence under elevated CO_2. Hence, their replacement by young forests would translate into a net C loss per unit land if the harvested C would rapidly return to the atmosphere (Körner, 2000; Guo & Gifford, 2002; Canadell & Raupach, 2008). Because of the long-standing belief that old-growth forests are carbon neutral (carbon uptake has been thought to be balanced by respiration; Odum, 1969) they have been considered irrelevant to the global carbon budget, but in recent years, compelling evidence has accumulated that primary forests are in fact important carbon sinks (Carey et al., 2001; Zhou et al., 2006; Luyssaert et al., 2008). According to recent estimates of net ecosystem productivity (NEP), old-growth forests (200 yr and older) sequester on average 2.4 t C ha^{-1} yr^{-1} (Luyssaert et al. 2008), while younger boreal and temperate forests ($<$ 200 yr) sequester only 0.3 and 1.7 t C ha^{-1} yr^{-1}, respectively (Pregitzer & Euskirchen, 2004). Old forests accumulate 54% of their NEP in roots and soil organic matter (SOM), 17% in stem biomass, and 29% in coarse woody debris (Luyssaert et al., 2008). It should be noted though, that at the stand and landscape scale, the magnitude as well as the residence time of C stored in biomass per unit land are determined by tree demography rather than growth rate (Carey et al., 2001; Körner, 2006). Hence at larger scales, accounting for all age classes of trees or parcels, the net effect of regrowth and mortality inevitably is close to zero (Körner, 2009).

Carbon fluxes in forests under elevated atmospheric CO$_2$

Given their prominent role in the global carbon cycle, the responses of trees and forests to rising atmospheric CO$_2$ are important to understand how C cycling will change in a future high CO$_2$ world. However, it was not until the mid-to-late 1990's when technological advances and governmental funding finally allowed stand-scale free air CO$_2$ enrichment (FACE) experiments. At that time, the global network of FACE sites agreed on a setpoint of 550 ppm CO$_2$ according to projections for the year 2050 published by the Intergovernmental Panel for Climate Change (IPCC). Although FACE experiments impose a 'step increase' in CO$_2$ and lack temperature control (i.e. the predicted rise in global mean temperature cannot be accounted for), these studies provide valuable insight in tree and ecosystem responses to future levels of atmospheric CO$_2$ under otherwise natural settings. In trees growing under CO$_2$ enrichment, photosynthesis has been shown to be persistently stimulated by *c.* 30-60% and even when acclimation occurred carbon uptake was still substantially enhanced (Noormets et al., 2001; Rogers & Ellsworth, 2002; Sholtis et al., 2004, Ellsworth et al., 2004, Zotz et al., 2005; Liberloo et al., 2006, Crous et al., 2008). Across four forest FACE sites covering a broad range of productivity, the photosynthetic stimulation by elevated CO$_2$ at the leaf-level translated into 23% higher stand NPP (Duke-, Oak Ridge-, Aspen- and POP-FACE, Norby et al., 2005). However, NPP only represents the net amount of that can be captured as biomass but it does not allow a clear statement in terms of the fate of this C (Norby et al., 2005; Körner, 2006). The question lingers whether CO$_2$-driven increases in NPP will result in greater C storage in pools with long mean residence time (wood, soil organic matter) or will the extra C be largely allocated to pools that turn over more rapidly (e.g. fine roots, foliage), and will thus be soon respired back to the atmosphere?The following overview will focus on the three existing steady-state forest systems that had reached canopy closure, constant fine root turnover and were coupled to the natural nutrient cycle when FACE was initiated (Duke-, Oak Ridge- and the Swiss Canopy Crane-FACE). In these forests CO$_2$ enrichment caused a strong initial growth response in tree basal area or NPP that attenuated after 1-2 years (Körner, 2006), while stand LAI (leaf area index) consistently remained unaffected. In the pine plantation at the Duke-FACE site, the abatement was least pronounced and half or more of the remaining stimulation was preserved in wood production (DeLucia & Moore, 2005). Above-ground total litter production was stimulated by 12% (9-yr average, Lichter et al. 2008) and also below-ground C allocation was enhanced under elevated CO$_2$ resulting in a 24% increase in fine root biomass and a 23% increase in soil respiration (12-yr average, Jackson et al., 2009). In the deciduous sweetgum plantation at the Oak Ridge-FACE site, the initial stimulation in stem wood production (35%) lasted only one year and was replaced by a more than two-fold increase in fine root production and standing crop biomass that persisted throughout the experiment (DeLucia & Moore, 2005; Norby et al., 2004, 2006). During the first four years of the study, soil respiration in the CO$_2$-enriched plots was on average 12% higher compared to control plots (King et al., 2004), which is a surprisingly small stimulation given the enormous fine root increment in this stand. Unfortunately, more recent soil respiration data are not available at this time. Because much of the increase in fine root production occurred relatively deep in the soil profile (below 30 cm) the resultant C inputs might at least in part translate into more recalcitrant forms of C in soil humus (Iversen et al., 2008). As a consequence of reduced specific leaf area (SLA), annual leaf mass production was 7.5% higher under elevated CO$_2$ despite unaltered LAI (Norby et al., 2003, 2006). In the near-natural mature forest at the Swiss Canopy Crane, the initial growth stimulation of *c.* 35-m-tall trees of four broad-leaved species had disappeared after 4 years of CO$_2$ enrichment (Körner et al., 2005; Asshoff et al., 2006). The lack of a consistent above-ground biomass response suggested that growth in these trees is not C-limited under current atmosperic CO$_2$ concentrations. Since leaf and fruit litter production also remained largely unaffected, the additional C assimilated in the CO_2-enriched canopy was assumed to be channeled to the soil (Körner et al., 2005). Indeed, ^{13}C tracing of recently assimilated CO_2 and higher CO_2 concentrations in the soil pore space both indicated enhanced C flux belowground under CO_2-enriched trees (Steinmann et al., 2004; Keel et al., 2006, and newer unpublished data). Hence, this PhD study aimed chiefly to throw light on the responses of fine roots and soil respiratory processes to elevated CO_2. Another goal was to assess whether the stimulation of leaf photosynthesis observed during the early years of this experiment was sustained without reductions in photosynthetic capacity after eight years of CO_2 enrichment.This doctoral thesis comprises three core chapters represented by scientific papers, which have been published in the following peer-reviewed Journals *Functional Ecology*, *Global Change Biology* and *Planta*. Three additional articles (published in *Environmental and Experimental Botany*, *Tree Physiology* and *Canadian Journal of Forest Research*) that I co-authored during my PhD are also included in this thesis but are not an official part of my doctoral work and are therefore not considered in the general summary (**chapter eight**). The following two chapters focus on below-ground responses at the mixed deciduous forest at the Swiss Canopy Crane (SCC). **Chapter two** is devoted to fine roots and reports data on biomass, new growth into unexplored soil volume and the quality (C:N ratio) of fine roots of mature deciduous forest trees exposed to elevated atmospheric CO_2 at the SCC site. The **third chapter** deals with soil respiration, which sensitively integrates the complex belowground carbon cycle processes in the study stand. Further, this chapter provides information on root respiration and soil microbial biomass. The **fourth chapter** reports on leaf-level carbon uptake and biochemical as well as morphological leaf characteristics of mature deciduous forest trees after 8 years of exposure to elevated atmospheric CO_2 at the SCC stand.

The three additional publications stem from an invited research on tree responses to drought stress at the forest-steppe ecotone in northern Mongolia (**chapters five, six and seven**).

References

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

Canadell, J.G., Le Quéré, C., Raupach, M.R., Field, C.B., Buitenhuis, E.T., Ciais, P., Conway, T.J., Gillett, N.P., Houghton, R.A. & Marland, G. (2007) Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 18866- 18870.

Canadell, J.G. & Raupach, M.R. (2008) Managing forests for climate change mitigation. *Science*, **320**, 1456-1457.

Carey, E.V., Sala, A., Keane, R. & Callaway, R.M. (2001) Are old forests underestimated as global carbon sinks? *Global Change Biology*, **7**, 339- 344.

Crous, K.Y., Walters, M.B. & Ellsworth, D.S. (2008) Elevated CO_2 concentration affects leaf photosynthesis-nitrogen relationships in *Pinus taeda* over nine years in FACE. *Tree Physiology*, **28**, 607-614.

Ellsworth, D.S., Reich, P.B., Naumburg, E.S., Koch, G.W., Kubiske, M.E. & Smith, S.D. (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated $p\mathrm{CO}_2$ across four free-air CO_2 enrichment experiments in forest, grassland and desert. *Global Change Biology*, **10**, 2121-2138.

Guo, L.B. & Gifford, R.M. (2002) Soil carbon stocks and land use change: a meta analysis. *Global Change Biology*, **8**, 345-360.

Houghton, R.A. (2007) Balancing the global carbon budget. *Annual Review of Earth and Planetary Sciences*, **35**, 313-347.

Hyvönen, R., Agren, G.I., Linder, S., Persson, T., Cotrufo, M.F., Ekblad, A., Freeman, M., Grelle, A., Janssens, I.A., Jarvis, P.G., Kellomaki, S., Lindroth, A., Loustau, D., Lundmark, T., Norby, R.J., Oren, R., Pilegaard, K., Ryan, M.G., Sigurdsson, B.D., Stromgren, M., Van Oijen, M. & Wallin, G. (2007) The likely impact of elevated $[CO_2]$, nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist*, **173**, 463-480.

IPCC (2007) Impacts, adaptations and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change, (eds M.L. Parry, O.F. Canziani, J.P. Palutikof, P .J. van der Linden & C.E. Hanson), Cambridge University Press, Cambridge UK, 976 pp.

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.

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

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

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

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

Körner, C. (2003) Ecological impacts of atmospheric CO_2 enrichment on terrestrial ecosystems. *Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences*, **361**, 2023-2041.

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

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

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

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

Lichter, J., Billings, S.A., Ziegler, S.E., Gaindh, D., Ryals, R., Finzi, A.C., Jackson, R.B., Stemmler, E.A. & Schlesinger, W.H. (2008) Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. *Global Change Biology*, **14**, 2910-2922.

Long, S.P., Ainsworth, E.A., Rogers, A. & Ort, D.R. (2004) Rising atmospheric carbon dioxide: Plants face the future. *Annual Review of Plant Biology*, **55**, 591-628.

Luyssaert, S., Schulze, E.D., Borner, A., Knohl, A., Hessenmoller, D., Law, B.E., Ciais, P. & Grace, J. (2008) Old-growth forests as global carbon sinks. *Nature*, **455**, 213-215.

Noormets, A., McDonald, E.P., Dickson, R.E., Kruger, E.L., Sober, A., Isebrands, J.G. & Karnosky, D.F. (2001) The effect of elevated carbon dioxide and ozone on leaf- and branch- level photosynthesis and potential plant-level carbon gain in aspen. *Trees-Structure and Function*, **15**, 262-270.

Norby, R.J., Delucia, E.H., Gielen, B., Calfapietra, C., Giardina, C.P., King, J.S., Ledford, J., McCarthy, H.R., Moore, D.J.P., Ceulemans, R., De Angelis, P., Finzi, A.C., Karnosky, D.F., Kubiske, M.E., Lukac, M., Pregitzer, K.S., Scarascia-Mugnozza, G.E., Schlesinger, W.H. & Oren, R. (2005) Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 18052- 18056.

Norby, R.J., Ledford, J., Reilly, C.D., Miller, N.E. & O'Neill, E.G. (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, R.J., Sholtis, J.D., Gunderson, C.A. & Jawdy, S.S. (2003) Leaf dynamics of a deciduous forest canopy: no response to elevated CO_2. *Oecologia*, **136**, 574-584.

Norby, R.J., Wullschleger, S.D., Hanson, P.J., Gunderson, C.A., Tschaplinski, T.J. & Jastrow, J.D. (2006) CO_2 enrichment of a deciduous forest: The Oak Ridge FACE Experiment. Managed ecosystems and CO_2: Case studies, processes, and perspectives (eds J. Nösberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendry & H. Blum), pp. 231-251. Springer, Berlin.

Odum, E.P. (1969) Strategy of ecosystem development. *Science*, **164**, 262-264.

Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V.M., Legrand, M., Lipenkov, V.Y., 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.

Pregitzer, K.S. & Euskirchen, E.S. (2004) Carbon cycling and storage in world forests: biome patterns related to forest age. *Global Change Biology*, **10**, 2052-2077.

Raupach, M.R., Canadell, J.G. & LeQuéré, C. (2008) Anthropogenic and biophysical contributions to increasing atmospheric CO_2 growth rate and airborne fraction. *Biogeosciences*, **5**, 1601-1613.

Rogers, A. & Ellsworth, D.S. (2002) Photosynthetic acclimation of Pinus taeda (loblolly pine) to long-term growth in elevated $p\mathrm{CO}_2$ (FACE). *Plant Cell and Environment*, **25**, 851-858.

Roy, J., Saugier, B. & Mooney, H.A. (2001) Terrestrial global productivity. Academic Press, San Diego.

Sabine, C.L., Heimann, M., Artaxo, P., Bakker, D.C.E., Chen, C.-T.A., Field, C.B., Gruber, N., C., L.Q., Prinn, R.G., Richey, J.E., Romero Lankao, P., Sathaye, J.A. & Valentini, R. (2004) Current status and past trends of the global carbon cycle. In: Global carbon cycle - Integrating humans, climate, and the natural world (eds. C.B. Field & M.R. Raupach), 17-44. Island Press.

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

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

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

Tans, P. (2008) Trends in atmospheric carbon dioxide. Web page NOAA/ESRL www.esrl.noaa.gov/gmd/ccgg/trends/.

Tripati, A., Roberts, C.D. & Eagle, R.A. (2009) Coupling of CO_2 and ice sheet stability over major climate transitions of the last 20 million years. *Science*, 326, 1394-1397.

Vitousek, P.M. (1994) Beyond global warming - ecology and global change. *Ecology* , **75**, 1861- 1876.

Zhou, G.Y., Liu, S.G., Li, Z., Zhang, D.Q., Tang, X.L., Zhou, C.Y., Yan, J.H. & Mo, J.M. (2006) Old-growth forests can accumulate carbon in soils. *Science*, **314**, 1417-1417.

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

Chapter 2

Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE)

Functional Ecology
British Ecological Society

Functional Ecology 2009, **23**, 913–921 doi: 10.1111/j.1365-2435.2009.01574.x

Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE)

Martin Bader*, Erika Hiltbrunner and Christian Körner

Institute of Botany, University of Basel, Schönbeinstrasse 6, CH-4056 Basel, Switzerland

Summary

1. Elevated atmospheric carbon dioxide (CO_2) concentrations have often been reported to increase carbon allocation below-ground, particularly to fine root production. However, for trees these responses have primarily been studied in young expanding systems while the evidence for late successional systems that have reached steady state above- and below-ground is very limited.

2. At the Swiss Canopy Crane (SCC) experimental site, we assessed whether elevated CO_2 affects fine root biomass, fine root expansion and fine root C and N concentration under mature deciduous trees (*c*. 100 years) exposed to 7 years of free air CO_2 enrichment (FACE) in a typical near-natural central European forest.

3. After 5 and 6 years of CO_2 enrichment, both, the soil core and ingrowth core method yielded similar reductions in biomass of *c*. –30% under elevated CO_2 for live fine roots < 1 mm diameter. In year 7 of the experiment, when fine root biomass was re-assessed at peak season, there was no significant CO_2-effect detectable. C and N concentrations in newly produced fine roots remained unaffected by elevated CO_2. Soil moisture under CO_2-exposed trees was significantly increased during rainless periods.

4. The isotopic label introduced into the system by canopy enrichment with ^{13}C-depleted CO_2 allowed us to trace the newly assimilated carbon. After 6 years of growth at 550 ppm CO_2, recent fine roots (< 1 mm, ingrowth cores) of CO_2-enriched trees consisted of 51% new carbon, suggesting a rather slow root turnover and/or slow mixing of old and new carbon in these trees.

5. Reduced tree water consumption under elevated CO_2 and resultant soil water savings might cause these trees to reduce their fine root investments in a future CO_2-enriched atmosphere.

6. Our findings and those from other multi-year experiments indicate that fine root mass in late successional systems may also be unaffected or even suppressed instead of being stimulated by elevated CO_2.

Key words: carbon cycle, carbon sequestration, elevated CO_2, fine root turnover, soil moisture

Introduction

Fossil fuel burning and rigorous land use change caused the atmospheric carbon dioxide (CO_2) concentration to rise from pre-industrial 280 ppm to its current 385 ppm and this increase is projected to exceed 700 ppm in the late 21st century (IPCC 2007; Tans 2008). Thus, plant life on earth is directly challenged by carbon enriched nutrition in addition to various indirect CO_2 effects via the climate system (e.g. warming).

Given the role of forests as major terrestrial biomass carbon stores, tree responses to the ongoing rise in atmospheric CO_2 are crucial for the future global carbon cycle (Schimel 1995; Schlesinger 1997). A considerable number of studies suggest that carbon assimilation of woody plants is substantially enhanced in response to increasing atmospheric CO_2 (Rogers *et al.* 1994; Curtis & Wang 1998; Norby *et al.* 1999; Sholtis *et al.* 2004; Zotz, Pepin & Körner 2005). However, this stimulation in photosynthesis does not necessarily result in above-ground biomass increment (Körner & Arnone 1992; Drake, Gonzalezmeler & Long 1997; Körner *et al.* 2005), but often leads to increased carbon allocation to roots (Norby & O'Neill 1991; Körner & Arnone 1992; Rogers *et al.* 1994; Curtis & Wang 1998; Hättenschwiler & Körner 1998; Matamala *et al.* 2003; Norby *et al.* 2004). According to the functional equilibrium concept (Brouwer 1962) this carbon investment in root systems under elevated CO_2 is presumably driven by the need to acquire more nutrients in order to match the enhanced atmospheric C supply.

*Corresponding author. E-mail: Martin.Bader@unibas.ch

Fine roots (≤ 2 mm) link autotrophic plant parts with the rhizosphere and provide the principal pathway for both water and nutrient uptake from the soil, and the input of carbon and nutrients via exudates and turnover to the soil. Although fine roots contribute less than 1·5% to the total biomass in forests (Körner 1994; Perruchoud 1999; Brunner & Godbold 2007), up to one-third of the global annual net primary production may flow below-ground for maintenance and new growth of fine roots (assuming < 1 year turnover, Jackson, Mooney & Schulze 1997). As a consequence, fine roots may supply equal or even larger annual carbon and nutrient inputs to the soil than leaves (Nadelhoffer & Raich 1992; Hendricks, Nadelhoffer & Aber 1993).

At the Swiss Canopy Crane FACE site we did not observe consistent above-ground biomass responses to elevated CO_2 – neither in basal area nor in leaf litter (LAI) – while light-saturated photosynthesis per unit leaf area was 40–52% enhanced during years 3 and 8 of CO_2 enrichment (Körner *et al.* 2005; Zotz *et al.* 2005; Bader *et al.* unpublished data). This paradox was reported previously for various ecosystems exposed to elevated CO_2 and is not yet fully resolved (Pitelka 1994; Niklaus *et al.* 2001; Nowak, Ellsworth & Smith 2004; Körner 2006). However, enhanced soil air CO_2 concentration and stable isotope data both indicated increased carbon flux to the soil under elevated CO_2 during the early years of this experiment (Steinmann *et al.* 2004; Keel, Siegwolf & Körner 2006). Therefore, we hypothesized greater below-ground C allocation under elevated compared to ambient CO_2 assessable in enhanced fine root biomass and fine root expansion into previously unexplored soil volume. Furthermore, we asked whether elevated CO_2 affects fine root quality through shifts in C and N concentrations.

Materials and methods

STUDY SITE

The Swiss Canopy Crane (SCC) site is situated in a diverse mixed forest roughly 15 km south of Basel, Switzerland (47°28′ N, 7°30′ E, 550 m a.s.l.). The *c.* 100-year-old forest reaches canopy heights ranging from 30 to 35 m and the leaf area index at peak season (LAI) is around 5. Tree density is 415 trees ha^{-1} (breast height diameter ≥ 0·1 m) and stem basal area amounts to 46 m^2 ha^{-1}. *Fagus sylvatica* L. (European beech), *Quercus petraea* (Matt.) Liebl. (Sessile oak) and *Carpinus betulus* L. (Hornbeam) dominate the stand, which is interspersed with less abundant tree species such as *Tilia platyphyllos* Scop. (Largeleaf linden), *Acer campestre* L. (Field maple), *Prunus avium* L. (cherry) and 4 species of conifers (*Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.).

The understorey vegetation (which does not receive CO_2 enrichment) is highly diverse and dominated by tree seedlings and saplings, the liana *Hedera helix* and shrubs such as *Rubus fruticosus* agg., and *Lonicera periclymenum*. Among the most abundant herb species appear *Galium odoratum*, *Anemone nemorosa*, *Mercurialis perennis*, *Paris quadrifolia*, *Circaea lutetiana* and *Sanicula europaea*. The forest grows on a Rendzic Leptosol (WRB) (Rendzina (FAO), Lithic Rendoll (USDA)) with a very low accessible profile depth between 10 and 20 cm (maximal 25 cm) followed by extremely rocky subsoil merging into the calcareous bedrock at depths of 20–90 cm. As Swiss forests growing on deeper soils had largely been converted to farmland centuries ago, this type of shallow soil is typical for many Swiss and other European forests, which nevertheless prosper vigorously. According to finger probe estimates we classified the soil as silty loam and the pH determination in 1 molar KCl yielded a value of 5·8 ± 0·2 (Mean ± SE, $n = 25$) in the top 10 cm of the profile.

The temperate climate at the study site is characterized by mild winters and moderately warm summers with mean air temperatures in January and July of 2 °C and 19 °C, respectively. Long-term mean annual precipitation at the study site is 990 mm, two-thirds of which falls during the 6-month growing season (Pepin & Körner 2002).

PRECIPITATION AND SOIL MOISTURE

Precipitation was recorded at a nearby weather station (Flüh, 2 km air-line distance from the SCC) at 2-min intervals and was averaged on a weekly basis. Soil volumetric water content during the growing seasons 2004–2005 was recorded continuously at hourly intervals using eight (ambient CO_2: $n = 5$, elevated CO_2: $n = 3$) TDR probes (ML2x probes, Delta-T, Cambridge, UK) complemented by spot measurements with a hand-held TDR device (Trime-FM, Imko, Ettlingen, Germany). Due to technical failure, there was only discontinuous data available for 2006, which was not sufficient for statistical analysis. In March 2007, we started recording soil water content at 0–10 cm depth 4 times daily at 6-h intervals using 'ECH_2O Probes' (EC-10, Decagon Devices Ltd., Pullman, Washington, DC; ambient CO_2: $n = 20$, elevated CO_2: $n = 15$). Each of the 'ECH_2O Probe' sensors was standardized to its own maximal value. We also used a hydrological model (input variables: daily precipitation sum, daily mean temperature, potential radiative energy input, potential evapotranspiration, and transpiration estimated from sapflow measurements under ambient and elevated CO_2) to estimate soil moisture differences under ambient and elevated CO_2 (Leuzinger & Körner 2009, in press).

FREE AIR CO_2 ENRICHMENT SYSTEM

At the SCC site, a future, CO_2-enriched atmosphere in tree canopies was realized by means of a particular free air CO_2 enrichment (FACE) technique called web-FACE (Pepin & Körner 2002). In this experiment we applied a step increase from ambient to 550 ppm atmospheric CO_2, corresponding to an elevation to approximately twice the pre-industrial CO_2-level. In brief, pure CO_2 was pulse-released through a fine web of perforated tubes woven into tree crowns (20–35 m above-ground) with the aid of a construction crane. Computer-controlled magnetic valves governed the CO_2-supply to the canopies to sustain the setpoint of 550 ppm as accurately as possible. The understorey vegetation was not exposed to elevated CO_2, hence any isotopic signal originated exclusively from the forest canopy (Keel *et al.* 2006). Canopy CO_2-concentration served as the main control signal, which was monitored by an air sampling system consisting of several suction heads per tree feeding canopy air through sampling lines into infra-red gas analysers (LI-800 GasHound and LI-820, Licor, Lincoln, NE). We also used isometers (C_4 grass, *Echinochloa crus-galli*) growing in 50 mL containers (sand-clay mixture) fixed in the tree crowns to monitor the abundance of ^{13}C in the canopy CO_2 (Keel *et al.* 2006; Körner *et al.* 2005). CO_2 enrichment was confined to daylight hours (PPFD > 100 µmol m^{-2} s^{-1}) of the growing season and was discontinued from the time of leaf shedding until next season's bud break (end of October to mid-April). Out of the 62 trees growing in the operating range of the crane (60 m), 12 deciduous trees (three *F. sylvatica*, three *Q. petraea*, three *C. betulus*, one *T. platyphyllos*, one *A. campestre* and one *P. avium*) received elevated atmospheric CO_2 since autumn 2000. A larger number of control trees were available in the remaining crane area at sufficient distance to the CO_2-enriched zone.

FINE ROOT BIOMASS

Given the shallow and rocky soil at the SCC site, we chose soil cores and root ingrowth cores rather than minirhizotrons for assessing fine root biomass. In our experiment, the methodological drawbacks involved with the use of ingrowth cores were of minor concern as this method may allow relative comparisons of new fine root growth between CO_2-enriched and control trees. Moreover, under CO_2 enrichment, the expansion of new roots into unexplored soil volume is likely to produce a signal more sensitive to increased C supply than bulk fine root sampling using soil corers. This is because soil sampled with corers, includes a substantial fraction of older (and dead) fine roots, which perhaps originated prior to the start of CO_2 enrichment and thus, may conceal potential CO_2-induced signals in fine root biomass.

In March 2005, a total of 84 soil cores were taken to a depth of 15 cm using a soil corer (3·5 cm diameter). Soil cores were taken in triplicate at each study tree (trees under ambient CO_2: $n = 16$, trees under elevated CO_2: $n = 12$). In the remaining holes left from the soil core removal, we immediately installed ingrowth cores (12 cm high and 3·6 cm in diameter, 2 mm mesh), filled with root-free soil from fresh molehills in the respective plots. At the time of installation, bulk soil density in ingrowth cores was similar to bulk soil density (on-site adjustment of the mass to volume ratio of freshly stuffed ingrowth cores to that of soil cores). The ingrowth cores were inserted 3 cm below the soil surface, covered with topsoil and their locations were labelled for recovery. At the end of the growing season, 6 months after installation, the ingrowth of freshly formed fine roots was checked in six ingrowth cores that were harvested and directly analysed. These cores showed little ingrowth of fine roots from the surrounding soil. Therefore, the remaining 78 ingrowth cores were harvested after two growing seasons in November 2006, 20 months after installation. In July 2007, we re-assessed fine root biomass but restricted the sampling to the three replicated dominant tree species in this FACE experiment (*F. sylvatica*, *C. betulus*, *Q. petraea*). There were three trees of each species available in both CO_2 treatments ($n = 9$) and we collected 3 soil cores per tree. Soil cores were taken at 1–2 m distance (depending on the penetrability of the soil due to the large number of rocks) around the stem of a given sample tree to a depth of 18 cm using a soil corer (5 cm diameter). Attempts to core beyond this soil depth were not successful.

All soil samples were stored on ice until arrival at the lab where they were kept at 4 °C. Soil cores from 2005 were frozen and processed in autumn 2006 while all other samples were processed within 3 weeks after removal from the forest soil. Fine roots were picked by hand, separated from bulk soil using tweezers and then rinsed with water. By means of a fine root reference collection, shrub and herbal roots were distinguishable from tree roots and were excluded from further analysis as CO_2 enrichment was confined to the tree crowns. In soil cores taken in 2007, a refined reference collection of roots sampled from trees growing on site, allowed us to distinguish between fine roots of different tree species. Roots from soil cores in 2005 and ingrowth cores were sorted into two diameter size classes < 1 mm and $1 \leq 2$ mm, whereas roots from soil cores in 2007 were separated in three size classes $< 0{\cdot}5$ mm, $0{\cdot}5 < 1$ mm and $1 \leq 2$ mm. Tensile strength and white vascular tissue served as vitality indicators to distinguish between live and dead roots (Matamala *et al.* 2003). The sorted fine roots were dried at 80 °C for at least 48 h and then weighed for biomass determination.

C AND N ANALYSIS OF FINE ROOTS

Washed live fine roots (< 1 mm diameter) obtained from ingrowth cores were oven-dried as described above and ground using a steel ball mill (Retsch MM 2000, Haan, Germany). Samples of 2–3 mg of the dry fine root powder were analysed for C and N using a CHN-analyser (Vario EL *III*, Elementar Analysensysteme GmbH, Hanau, Germany).

CARBON—ISOTOPE ANALYSIS OF FINE ROOTS

Aliquots of 0·6–0·8 mg of the dried and ground fine root powder were filled in tin capsules for $\delta^{13}C$ analysis. After combustion in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy) the gas samples passed a variable open-slit interface (Conflo II, Thermo Finnigan Mat, Bremen, Germany) leading to the mass spectrometer (Delta S, Thermo Finnigan Mat, Bremen, Germany), which was operated in continuous flow mode. The precision of $\delta^{13}C$ analyses was < 0·1‰. The isotope values are expressed in the δ-notation: $\delta^{13}C = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ (‰) where R is the molar ratio of ^{13}C to ^{12}C for the sample and standard, respectively. The difference in $\delta^{13}C$ between isometers grown in ambient and elevated CO_2 (Mean ± SE for 2001–2006: 5·7 ± 0·6‰) was assumed to reflect the isotopic signal imposed on the canopy, because the C_4 grass biomass was exclusively formed of C that originated from newly assimilated CO_2, free of the influence of old C reserves. The fraction of new C in fine roots was calculated using the rule of proportion where the isometer signal of 5·7‰ refers to 100%.

STATISTICAL ANALYSIS

As our experiment ran on a tree scale, individual trees growing under ambient and elevated CO_2 were the statistical units of replication. Each replicate consisted of three soil samples (soil cores, ingrowth cores), which were averaged prior to statistical testing to give a robust value for each study tree.

For the analysis of fine root biomass data from soil cores taken in 2005 and ingrowth cores we applied a linear mixed effects-model including the fixed factors 'CO_2', 'living status' (dead or alive), and 'diameter' and the random factor 'tree'. We used Student's *t*-test to analyse soil water content 2007 as well as fine root C and N and stable isotope data obtained from ingrowth cores (only live fine roots < 1 mm diameter).

Analysis of covariance (ANCOVA) was performed to test the influence of the previous fine root biomass estimated from soil cores (covariate) on newly produced fine root biomass in ingrowth cores. In soil cores taken in 2007, a reference collection of roots sampled from plants growing on-site, allowed us to distinguish between fine roots of different species. Therefore, fine root biomass data from these soil cores as well as the related stable isotope data were analysed in a linear mixed effects-model, which included the fixed factors 'CO_2', 'living status', 'diameter', and 'species' and the random factor 'tree'. Heteroscedasticity of within-group errors was modelled using power and constant variance functions. Quantile–quantile plots were applied for normality testing of residuals and random effects. Homogeneity of variances was checked with Bartlett's test. All statistical computations were performed using R, version 2·8·0 <www.r-project.org>.

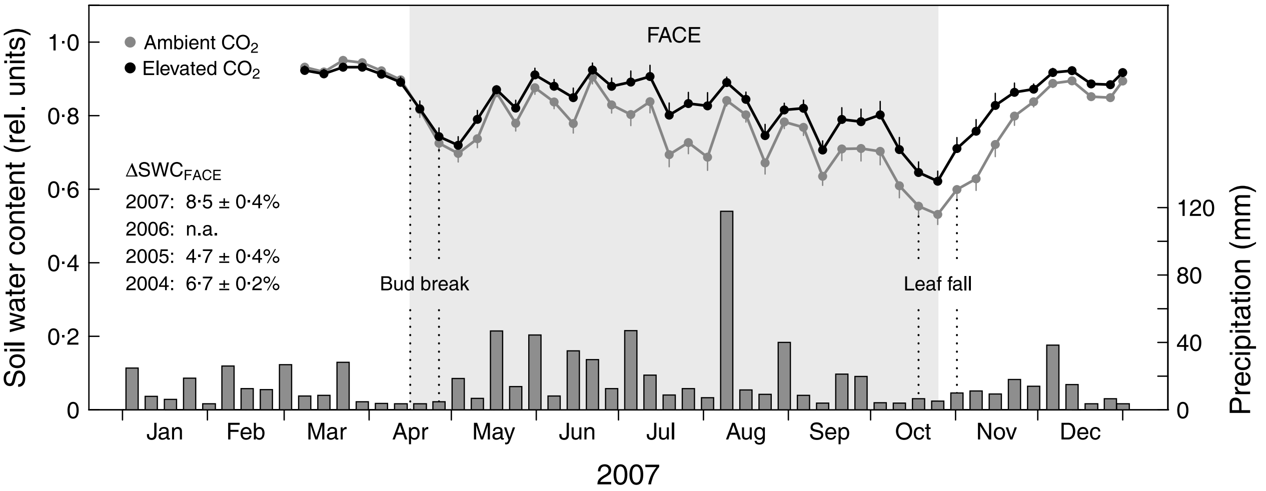


Fig. 1. Precipitation and soil water content (SWC) under trees exposed to ambient or elevated CO_2 in 2007. The CO_2 enrichment period is grey-shaded and the dotted lines indicate the bud break or leaf fall period, respectively. ΔSWC_{FACE} values give the mean difference in SWC between the elevated (E) and the ambient CO_2 area (A) during the FACE period (growing season) from 2004, 2005 and 2007, calculated as E/A × 100% – 100. Due to technical failure there was insufficient data for 2006 (n.a. = not available). Hydrological model data for the study years: 2004: 5·5%, 2005: 5·9%, 2006: 5·6%, 2007: 10·1% (Leuzinger, in revision).

Results

SOIL MOISTURE

Mean volumetric water content during growing seasons was significantly higher in soil under trees exposed to elevated CO_2 than under control trees, resulting from reduced sap flow (i.e. reduced transpiration) in CO_2-enriched trees, which positively fed back to soil water supplies ($t = -10{\cdot}27$, $P < 0{\cdot}001$, Cech, Pepin & Körner 2003; Leuzinger & Körner 2007; Fig. 1). In 2007, prior to bud break and during early leaf development when transpirative demands were low, soil moisture in the control and CO_2 enriched plot was identical (Fig. 1). Parallel to progressing leaf maturation and thus, increasing canopy transpiration, ambient soil moisture was reduced compared to soil moisture under elevated CO_2 and remained lower throughout the entire growing season. During replenishment at high or continuous precipitation, soil water resources in the control and CO_2-enriched area transiently approached each other but rapidly diverged again afterwards. Following leaf abscission, soil moisture in control soil gradually converged with soil moisture in the CO_2-enriched plot towards the end of the year.

TREE FINE ROOT BIOMASS

The original soil cores removed for the installation of ingrowth cores showed less fine root biomass of *c.* –30% in live fine roots < 1 mm diameter in sample areas under trees receiving 550 ppm CO_2 compared to conspecific control trees (Fig. 2). Statistically this is supported by a significant CO_2 effect and a $CO_2 \times$ diameter interaction (Table 1). Similarly, the biomass of new fine roots < 1 mm that had expanded into ingrowth cores over two growing seasons,

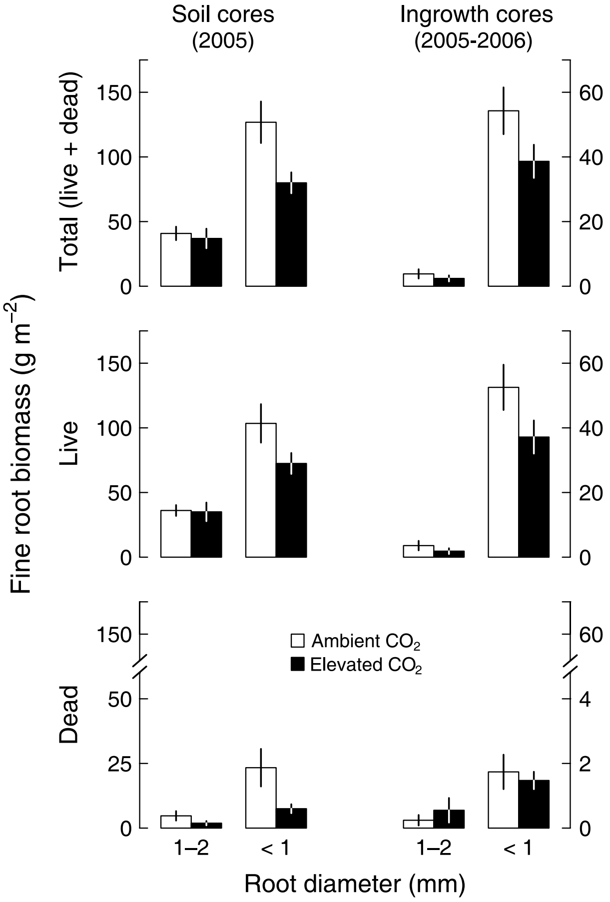


Fig. 2. Fine root biomass of mature deciduous forest trees growing under elevated and ambient CO_2. Soil cores taken in winter 2005 (left panel) represent the soil volume removed for the installation of the ingrowth cores (right panel). Means ± SE, $n = 14$ in ambient and $n = 12$ in elevated CO_2.

Table 1. Linear mixed effects model results for fine root biomass in soil cores and ingrowth cores and ^{13}C signatures of fine roots (soil cores 2007) under ambient and elevated CO_2

Factor	Df	*F*-value	*P*
Soil cores (2005)			
CO_2	1, 24	5·27	0·031*
Diameter	1, 74	38·95	< 0·001***
Living status	1, 74	133·33	< 0·001***
CO_2 × diameter	1, 74	4·78	0·032*
Diameter × living status	1, 74	17·18	< 0·001***
Ingrowth cores (2005–2006)			
CO_2	1, 24	3·58	0·071$^{(*)}$
Diameter	1, 74	92·40	< 0·001***
Living status	1, 74	100·54	< 0·001***
CO_2 × living status	1, 74	3·62	0·061$^{(*)}$
Diameter × living status	1, 74	82·62	< 0·001***
Soil cores (2007)			
CO_2	1, 14	1·02	0·33
Species	2, 14	0·84	0·45
Diameter	2, 83	30·21	< 0·001***
Living status	1, 83	8·30	0·005**
Species × living status	2, 83	16·75	< 0·001***
Diameter × living status	2, 83	8·25	< 0·001***
$\delta^{13}C$ (soil cores 2007)			
CO_2	1, 15	9·71	0·007**
Diameter	2, 86	45·09	< 0·001***
Species	2, 15	0·31	0·740
CO_2 × diameter	2, 86	6·89	0·002**
Diameter × species	4, 86	2·59	0·042*

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

was about 30% lower under elevated compared to ambient CO_2 (Fig. 2). However, this reduction was only marginally significant (Table 1). Although both methods revealed similar differences in fine root biomass between ambient and elevated CO_2, the fine root biomass in soil cores had no significant influence on newly produced fine root biomass in ingrowth cores (ANCOVA, $P > 0·05$). Thus, ingrowth cores installed at the same locations where soil cores showed high fine root biomass did not necessarily yield high fine root biomass. Live fine roots in soil cores contributed 92% and 83% to total fine root biomass (TFRB = live + dead fine root biomass across all diameters ≤ 2 mm) under elevated and ambient CO_2, respectively. Irrespective of CO_2 treatment, live fine roots in ingrowth cores made up around 95% of TFRB. In 2007, fine root biomass at peak season did not differ significantly between CO_2-treatments regardless of root diameter and living status of fine roots (Fig. 3, TFRB: 290 ± 23 g m^{-2} under ambient vs. 256 ± 36 g m^{-2} under elevated CO_2, mean ± SE). Also, fractions of root size classes were similar across CO_2 treatments with root diameters < 0·5 mm contributing 43–45% while 0·5 < 1 mm diameters contributed 23–25% and 1 ≤ 2 mm diameters added 30–34% to TFRB. Irrespective of the CO_2 treatment, live fine roots made up *c.* 60% of TFRB.

TFRB in 2007 was 54% and 42% higher in ambient and elevated CO_2, respectively, compared to TFRB in 2005, which is probably due to the interannual variability and the different sampling dates in the respective years (peak season vs. spring).

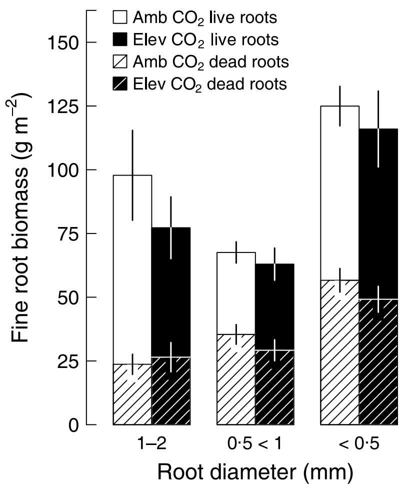


Fig. 3. Fine root biomass of three dominant deciduous forest trees (*F. sylvatica*, *Q. petraea*, *C. betulus*) growing under elevated and ambient CO_2 based on a peak season harvest in July 2007. Means ± SE, *n* = 9.

Table 2. Carbon and nitrogen concentrations in live fine roots (< 1 mm, ingrowth cores) formed within two growing periods (2005–2006) under ambient (*n* = 14) and elevated CO_2 (*n* = 12)

	Ambient CO_2	Elevated CO_2	*P*
	Mean ± SE	Mean ± SE	
Fine root C concentration (mg g^{-1})	421·7 ± 9·5	422·0 ± 9·3	0·98
Fine root N concentration (mg g^{-1})	15·7 ± 0·5	16·5 ± 0·6	0·33
Fine root C : N	27·0 ± 0·8	25·9 ± 0·8	0·32

C AND N CONCENTRATIONS IN FINE ROOTS

Elevated CO_2 had no effect on C and N concentration and C : N ratio in live fine roots (< 1 mm) of deciduous forest trees harvested from ingrowth cores that were installed for 20 months, including two full growing seasons during years 5 and 6 of CO_2 enrichment (Table 2).

FINE ROOT ^{13}C SIGNALS

Growth in elevated CO_2 and root diameter had significant effects on the isotopic signal in fine roots (soil cores 2007), while living status and species identity did not influence fine root $\delta^{13}C$ (Fig. 4, Table 1). The isotopic label in fine roots sampled from CO_2-enriched trees was strongest in the finest diameters (< 0·5 mm) that presumably represented the most recent fraction of the fine root system (significant CO_2 × root diameter interaction, Table 1, Fig. 4). In fine roots (< 1 mm) exclusively formed during years 5 and 6 of CO_2 enrichment (ingrowth cores), 51% of the carbon carried the isotopic signature of the fossil CO_2 released in the tree canopies.

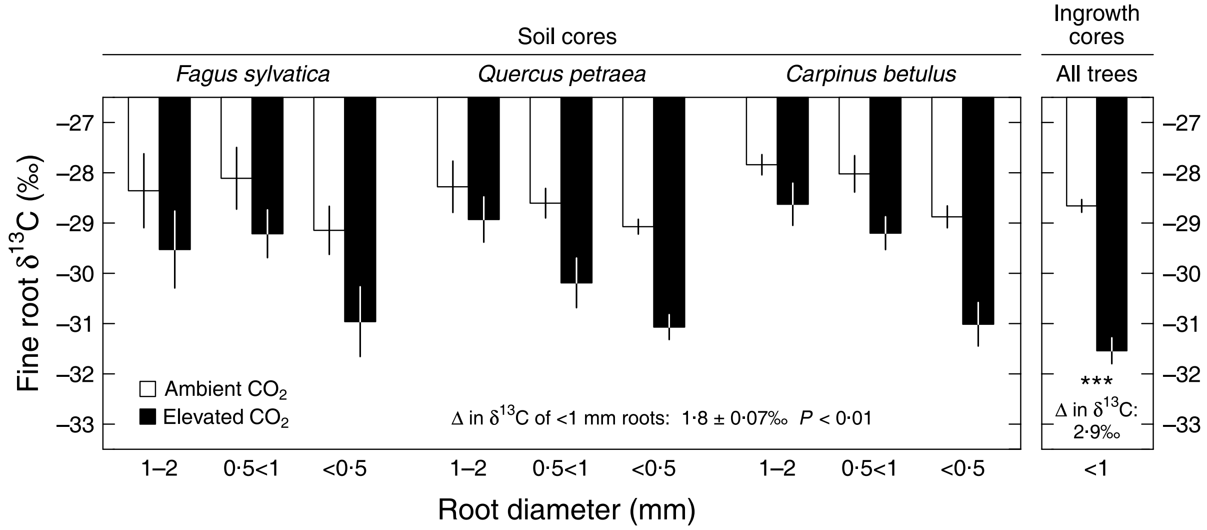


Fig. 4. Left panel, $\delta^{13}C$ of fine roots from the three dominant forest trees (*F. sylvatica*, *Q. Petraea*, *C. betulus*) exposed to ambient or elevated ^{13}C-depleted CO_2 in year 7 of the FACE experiment (soil cores). Means ± SE, $n = 9$. Right panel, $\delta^{13}C$ of fine roots (all species pooled) exclusively formed during years 5 and 6 of the CO_2 enrichment (ingrowth cores). Means ± SE, $n = 14$ in ambient and $n = 12$ in elevated CO_2. Differences in $\delta^{13}C$ ± SE between fine roots < 1 mm collected in the control and CO_2-enriched areas are shown by numbers in graph. ***$P < 0{\cdot}001$.

Discussion

TREE FINE ROOT BIOMASS

The Swiss web-FACE experiment is the only study worldwide where mature deciduous trees growing in a near-natural forest have been exposed to elevated CO_2. After 7 years of CO_2 enrichment our data suggest unaltered (soil cores 2007, Fig. 3) or even reduced fine root compartments (soil cores 2005, Fig. 2) in these trees which comes as a surprise, given we had expected a CO_2-induced increase rather than no change or a reduction in fine root biomass. We also anticipated elevated CO_2 to foster fine root growth but the declining trend observed in ingrowth cores rather suggests a CO_2-driven reduction in fine root growth of CO_2-enriched trees (Fig. 2).

Earlier stable isotope data and soil air CO_2 concentration together with the lack of above-ground growth stimulation while leaf-level photosynthesis was enhanced implied increases in the flow of carbon to below-ground sinks under elevated CO_2 (Steinmann *et al.* 2004; Körner *et al.* 2005; Zotz *et al.* 2005; Asshoff, Zotz & Körner 2006; Keel *et al.* 2006). Soil N limitation may offset potential CO_2 effects on fine root growth (Pregitzer *et al.* 1995, 2000; Oren *et al.* 2001; Spinnler *et al.* 2002) but can be ruled out as an explanation for the negative or lacking fine root responses because the calcareous forest soils in this region are nutrient-rich (Walthert *et al.* 2004) and there is substantial wet nitrogen deposition in this area of 20–25 kg N ha^{-1} a^{-1}, feeding plenty of N to trees and soils (Swiss Federal Office for the Environment 2000). Consequently, nitrate concentrations in the soil solution are rather higher-than-average, often exceeding the threshold value of 25 mg L^{-1} given in the Swiss Water Protection Ordinance (Gschv 1998; Bucher-Wallin *et al.* 2003; P. Schleppi, personal communication). Given that mycorrhizal colonization in most ecosystems increases substantially under elevated CO_2 (+47%, Treseder 2004), the mycorrhizal network may offer an avenue for dissipation of excess carbon. Mycorrhization has not been studied on the mature trees of our site but very rapid transfer of new carbon from these trees to mycorrhizal fungi had been shown previously (Steinmann *et al.* 2004; Keel *et al.* 2006). At the SCC site we measured consistently enhanced soil moisture resulting from reduced tree water consumption under elevated CO_2 (Cech *et al.* 2003; Leuzinger & Körner 2007, Fig. 1). These soil water savings offer a more likely explanation for decreases in fine root biomass as soil water supply *per se* and thus, nutrient availability is improved. This CO_2-induced facilitation in water and nutrient uptake might diminish the need for extensive fine root systems.

Contrary to our findings, below-ground biomass of both broad-leaved and coniferous trees had often been reported to increase under elevated CO_2 (Rogers *et al.* 1994; Curtis & Wang 1998; Tingey, Phillips & Johnson 2000; Zak *et al.* 2000; Nowak, Ellsworth & Smith 2004). However, as emphasized in these reviews, plant developmental stage, study duration and environmental factors, particularly soil conditions may have strongly biased the observed CO_2-responses. Most of the studies were of short duration (≤ 3 years) and tested young trees in 'decoupled' or expanding systems ('coupling' refers to the linkage between the carbon and the nutrient cycle, Körner 2006), the responses of which may not be extrapolated to mature trees (Loehle 1995; Körner 2006).

Data from multi-year FACE experiments conducted in 'coupled' systems with constant leaf area index and (near to) steady state nutrient cycle are still scarce. Among the few steady state systems the well-documented Oak Ridge FACE site is exceptional insofar as the pronounced initial above-ground growth stimulation of sweetgum (*Liquidambar styraciflua*) rapidly ceased while fine root production remained enhanced during 9 years of CO_2 enrichment (Norby *et al.* 2004; Iversen, Ledford & Norby 2008). Both fine root production and mortality were roughly doubled resulting in significantly greater peak season fine root biomass, but declining turnover rates (Norby *et al.* 2004; Iversen *et al.* 2008). Surprisingly, the largest increase in fine root production in this system occurred below 30 cm soil depth (Iversen *et al.* 2008). As the continuous positive fine root response was attributed to previously unexplored soil volume, it will be interesting to observe how long fine root proliferation will persist in this system, once soil exploration by roots has reached a new steady state.

At the Duke FACE site, loblolly pine (*Pinus taeda*) initially showed substantial increases in fine root increment under elevated CO_2, but the effects on fine root biomass were rather modest (Matamala & Schlesinger 2000). Subsequent minirhizotron observations yielded a mean annual (insignificant) increase of 23% in fine root biomass during 6 years of CO_2-exposure matching the 13–27% stimulation in annual tree basal area increment (Moore *et al.* 2006; Pritchard *et al.* 2001, 2008). Elevated CO_2 progressively increased soil moisture during the early years of enrichment. Unlike the soil water savings observed at our site, enhanced soil moisture was largely attributed to enhanced needle litter accumulation, which restricted evaporation from the soil (Schäfer *et al.* 2002).

After 7 years of CO_2 enrichment in a scrub oak system in Florida, the initial stimulation of fine root growth and mortality had completely ceased and, unexpectedly, like in our case, the biomass of surface roots < 0·25 mm diameter was even significantly reduced by 32% (Dilustro *et al.* 2002; Day *et al.* 2006; Brown *et al.* 2007). Similar to what we propose for our deciduous forest trees, the reduction in finest roots had been ascribed to improved soil moisture and/or enhanced nutrient availability under high CO_2 (Hungate *et al.* 2002; Johnson *et al.* 2003).

Consistent with the current study, 4 years of CO_2-exposure failed to stimulate fine root growth in a tree line ecosystem in the Swiss Central Alps, dominated by 30-year-old European larch (*Larix decidua*) and mountain pine (*Pinus uncinata*; Handa, Hagedorn & Hättenschwiler 2008). Similarly, at the Nevada desert FACE facility, elevated CO_2 did not affect fine root dynamics of shrub communities, except for community transects where fine root biomass and turnover were even significantly lower under high CO_2 (Phillips *et al.* 2006). Unlike our site, soil moisture was not measurably increased under elevated CO_2 at the desert site, suggesting that improved water-use efficiency compensates for smaller fine root systems.

C AND N CONCENTRATION OF TREE FINE ROOTS

Tree fine root C and N concentrations remained unaffected under elevated CO_2. This illustrates that not even relative increase in C allocation to the fine roots of our mature trees occurred under elevated CO_2. Plant tissues developed under elevated CO_2 frequently show lower nitrogen and protein concentrations and therefore increases in the C : N-ratio (Cotrufo *et al.* 1998; Norby *et al.* 1999). However, irrespective of life form, fine roots seem to be more variable in their nutrient response to elevated CO_2 as increases as well as decreases but mostly no changes were observed.

In line with our results, other steady state systems also reported no effects of elevated CO_2 on fine root C or N concentrations, including unaltered C : N-ratios in fine roots of *Pinus taeda* at the Duke FACE site, unchanged root nitrogen concentrations in *Larix decidua* and *Pinus uncinata* at the Swiss tree line FACE (Handa *et al.* 2008), and unaltered fine root N concentration in sweetgum at the Oak Ridge FACE site (Iversen *et al.* 2008). Even in the longest CO_2 experiment worldwide, where sour orange trees grew 17 years under elevated CO_2 with orchard-like irrigation and nutrient supply, the elemental composition, including C and N, remained largely unaffected (Kimball *et al.* 2007). In the Florida scrub-oak ecosystem, C and N concentration of surface roots < 0·25 mm decreased significantly after 7 years of CO_2 enrichment, but no differences in deeper soil or larger root diameters were found (Brown *et al.* 2007). Soil N availability can have a greater impact on fine root N than elevated CO_2 (Pregitzer *et al.* 2000; King *et al.* 2005) and in CO_2-enriched spruce and beech trees growing on N-rich soils even declines in fine root N have been observed (Hagedorn *et al.* 2002).

Given the prolonged CO_2 enrichment in some of the studies (≥ 5 years), it is unlikely that initially lacking CO_2 effects on fine root C and N concentration will emerge in the long-term.

ROOT ^{13}C SIGNALS

At the SCC the isotopic signature of the fossil CO_2 used for canopy enrichment, was rapidly traceable within the study trees and also within soil compartments and mycorrhizal fungi, providing evidence for effective CO_2 enrichment and enabling us to estimate the fraction of labelled C (new C) in plant organs, including fine roots (Steinmann *et al.* 2004; Keel *et al.* 2006). After 6 years of CO_2-exposure, half of the carbon in newly produced fine roots (< 1 mm, ingrowth cores) of CO_2-enriched trees was isotopically labelled, suggesting a C-pool turnover of *c.* 12 years which is likely to reflect a slow root turnover as well. This is in line with the findings of Keel *et al.* (2006) who found 38% new C in fine roots (< 1 mm) of the same CO_2-enriched trees after four growing seasons of enrichment implying a C turnover > 10 years. However, we do not believe that such data permit fully reliable root turnover estimates. In a pulse labelling experiment, an intense mixing of new and existing C was shown at branch-level for *F. sylvatica* and *Q. petraea* (Keel *et al.* 2007), and the slow replacement of old by new C in recent fine roots (ingrowth cores) after 6 years of continuous labelling provides strong evidence for slowly diluting C pools on a whole-tree level in these species. Root ^{13}C signals, thus reflect a combination of a near to endless dilution process of old by new mobile C-pools and new tissue formation.

Soil core sampling was likely to include fine roots that originated from periods prior to the start of the FACE experiment, which would further dilute the isotopic signal. In fact, the magnitude of the isotopic label (difference in

$\delta^{13}C$ between ambient and elevated CO_2) was substantially lower in soil core samples compared to samples derived from ingrowth cores in which all roots were exclusively formed during CO_2 enrichment in 2005–2006 (Fig. 4). Therefore, fractions of new C in soil core samples were not estimated.

Similarly long C turnover times were observed at the Swiss tree line FACE experiment with *Pinus* and *Larix*. After 5 years of CO_2-exposure, fine roots (pooled across all species including understorey vegetation) consisted of 25% (< 0·1 mm diameter) and 15% (0·1–2 mm) new C (Handa *et al.* 2008).

Conclusions

Our findings and those from several other CO_2 enrichment experiments studying woody plants suggest that unaltered or even reduced fine root biomass may meet plant resource demands under elevated CO_2. Improved water and thus, probably nutrient supply, through reduced transpiration and resultant soil water savings might cause trees to reduce their fine root investments in this late successional temperate forest in a future CO_2-enriched atmosphere. Whether such soil water savings will persist will depend on atmospheric feedback and future climatic trends. To the degree that soil humus formation (carbon sequestration) depends on enhanced fine root mass or accelerated turnover, our data do not provide any evidence that greater carbon deposits will be formed under such types of tree stands in the future.

Acknowledgements

We thank Rolf Siegwolf and Matthias Saurer (Paul Scherrer Institute) for stable isotope analysis, Erwin Amstutz for on-site technical support and Debbie Coldwell, Andrea Trebs, Nicole Inauen, Chantal Thouvay and Nicolas Brancucci for their help with root sampling and extraction. This research was funded through the Swiss National Science Foundation grant 3100AO-111914/1.

References

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

Brouwer, R. (1962) Distribution of dry matter in the plant. *Netherlands Journal of Agricultural Science*, **10**, 361–376.

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

Brunner, I. & Godbold, D.L. (2007) Tree roots in a changing world. *Journal of Forest Research*, **12**, 78–82.

Bucher-Wallin, I.K., Schleppi, P., Hagedorn, F. & Bucher, J. (2003) Soil nutrients in a mature mixed forest exposed to elevated CO_2. *Ekológia (Bratislava)*, **22**, 62–65.

Cech, P., Pepin, S. & Körner, C. (2003) Elevated CO_2 reduces sap flow in mature deciduous forest trees. *Oecologia*, **137**, 258–268.

Cotrufo, M.F., Briones, M.J.I. & Ineson, P. (1998) Elevated CO_2 affects field decomposition rate and palatability of tree leaf litter: importance of changes in substrate quality. *Soil Biology and Biochemistry*, **30**, 1565–1571.

Curtis, P.S. & Wang, X.Z. (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia*, **113**, 299–313.

Day, F.P., Stover, D.B., Pagel, A.L., Hungate, B.A., Dilustro, J.J., Herbert, B.T., Drake, B.G. & Hinkle, C.R. (2006) Rapid root closure after fire limits fine root responses to elevated atmospheric CO_2 in a scrub oak ecosystem in central Florida, USA. *Global Change Biology*, **12**, 1047–1053.

Dilustro, J.J., Day, F.P., Drake, B.G. & Hinkle, C.R. (2002) Abundance, production and mortality of fine roots under elevated atmospheric CO_2 in an oak–scrub ecosystem. *Environmental and Experimental Botany*, **48**, 149–159.

Drake, B.G., Gonzalez-Meler, M.A. & Long, S.P. (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 609–639.

GSchV (1998) *Gewässerschutzverordnung (Swiss Water Ordinance) 814–201*, Der Schweizer Bundesrat, Bern, Switzerland.

Hagedorn, F., Landolt, W., Tarjan, D., Egli, P. & Bucher, J.B. (2002) Elevated CO_2 influences nutrient availability in young beech-spruce communities on two soil types. *Oecologia*, **132**, 109–117.

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

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

Hendricks, J.J., Nadelhoffer, K.J. & Aber, J.D. (1993) Assessing the role of fine roots in carbon and nutrient cycling. *Trends in Ecology and Evolution*, **8**, 174–178.

Hungate, B.A., Reichstein, M., Dijkstra, P., Johnson, D., Hymus, G., Tenhunen, J.D., Hinkle, C.R. & Drake, B.G. (2002) Evapotranspiration and soil water content in a scrub–oak woodland under carbon dioxide enrichment. *Global Change Biology*, **8**, 289–298.

IPCC (2007) Impacts, adaptations and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change, (eds M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden & C.E. Hanson), Cambridge University Press, Cambridge UK, 976 pp.

Iversen, C.M., Ledford, J. & Norby, R.J. (2008) CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. *New Phytologist*, **179**, 837–847.

Jackson, R.B., Mooney, H.A. & Schulze, E.D. (1997) A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 7362–7366.

Johnson, D.W., Hungate, B.A., Dijkstra, P., Hymus, G., Hinkle, C.R., Stiling, P. & Drake, B.G. (2003) The effects of elevated CO_2 on nutrient distribution in a fire-adapted scrub oak forest. *Ecological Applications*, **13**, 1388–1399.

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

Keel, S.G., Siegwolf, R.T.W., Jaggi, 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.

Kimball, B.A., Idso, S.B., Johnson, S. & Rillig, M.C. (2007) Seventeen years of carbon dioxide enrichment of sour orange trees: final results. *Global Change Biology*, **13**, 2171–2183.

King, J.S., Pregitzer, K.S., Zak, D.R., Holmes, W.E. & Schmidt, K. (2005) Fine root chemistry and decomposition in model communities of north-temperate tree species show little response to elevated atmospheric CO_2 and varying soil resource availability. *Oecologia*, **146**, 318–328.

Körner, C. (1994) Biomass fractionation in plants: a reconsideration of definitions based on plant functions. *A Whole Plant Perspective on Carbon–Nitrogen Interactions* (eds J. Roy & E. Garnier), pp. 173–185. SPB Academic Publishing, The Hague.

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

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

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

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

Leuzinger, S. & Körner, C. (2009) Rainfall distribution is the main driver of runoff under future CO_2-concentration in a temperate deciduous forest. *Global Change Biology* (in press).

Loehle, C. (1995) Anomalous responses of plants to CO_2 enrichment. *Oikos*, **73**, 181–187.

Matamala, R. & Schlesinger, W.H. (2000) Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology*, **6**, 967–979.

Matamala, R., Gonzalez-Meler, M.A., Jastrow, J.D., Norby, R.J. & Schlesinger, W.H. (2003) Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science*, **302**, 1385–1387.

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

Nadelhoffer, K.J. & Raich, J.W. (1992) Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology*, **73**, 1139–1147.

Niklaus, P.A., Wohlfender, M., Siegwolf, R. & Körner, C. (2001) Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant and Soil*, **233**, 189–202.

Norby, R.J. & O'Neill, E.G. (1991) Leaf-area compensation and nutrient interactions in CO_2-enriched seedlings of yellow-poplar (*Liriodendron tulipifera* L.). *New Phytologist*, **117**, 515–528.

Norby, R.J., Ledford, J., Reilly, C.D., Miller, N.E. & O'neill, E.G. (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, R.J., Wullschleger, S.D., Gunderson, C.A., Johnson, D.W. & Ceulemans, R. (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. *Plant Cell and Environment*, **22**, 683–714.

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 Phytologist*, **162**, 253–280.

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

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

Perruchoud, D., Kienast, F., Kaufmann, E. & Braker, O.U. (1999) 20th century carbon budget of forest soils in the Alps. *Ecosystems*, **2**, 320–337.

Phillips, D.L., Johnson, M.G., Tingey, D.T., Catricala, C.E., Hoyman, T.L. & Nowak, R.S. (2006) Effects of elevated CO2 on fine root dynamics in a Mojave Desert community: a FACE study. *Global Change Biology*, **12**, 61–73.

Pitelka, L.F. (1994) Ecosystem response to elevated CO_2. *Trends in Ecology and Evolution*, **9**, 204–207.

Pregitzer, K.S., Zak, D.R., Curtis, P.S., Kubiske, M.E., Teeri, J.A. & Vogel, C.S. (1995) Atmospheric CO_2, soil-nitrogen and turnover of fine roots. *New Phytologist*, **129**, 579–585.

Pregitzer, K.S., Zak, D.R., Maziasz, J., Deforest, J., Curtis, P.S. & Lussenhop, J. (2000) Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. *Ecological Applications*, **10**, 18–33.

Pritchard, S.G., Rogers, H.H., Davis, M.A., Van Santen, E., Prior, S.A. & Schlesinger, W.H. (2001) The influence of elevated atmospheric CO_2 on fine root dynamics in an intact temperate forest. *Global Change Biology*, **7**, 829–837.

Pritchard, S.G., Strand, A.E., Mccormack, M.L., Davis, M.A., Finz, A.C., Jackson, R.B., Matamala, R., Rogers, H.H. & Oren, R. (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air-CO_2-enrichment: a six-year-minirhizotron study. *Global Change Biology*, **14**, 588–602.

R Development Core Team (2008) R: A language and environment for statistical computing, R foundation for Statistical Computing. Vienna Austria. <http://www.R-project.org>.

Rogers, H.H., Runion, G.B. & Krupa, S.V. (1994) Plant-responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, **83**, 155–189.

Schäfer, K.V.R., Oren, R., Chun-Ta, L. & Katul, G.G. (2002) Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO_2 concentration. *Global Change Biology*, **8**, 895–911.

Schimel, D.S. (1995) Terrestrial ecosystems and the carbon-cycle. *Global Change Biology*, **1**, 77–91.

Schlesinger, W.H. (1997) *Biogeochemistry: An Analysis of Global Change*, 2nd edn. Academic Press, New York.

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

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 – Structure and Function*, **16**, 423–436.

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

Swiss Federal Office for the Environment (2000) Nitrogen deposition 2000. Web page http://stratus.meteotest.ch/lugis/map/presentation/lugis_style/map.asp?Cmd=INIT&lang=d&Schadstoff=NDEP_00&idx=29&Zoomfaktor=1.

Tans, P. (2008) Trends in atmospheric carbon dioxide. Web page NOAA/ESRL www.esrl.noaa.gov/gmd/ccgg/trends/.

Tingey, D.T., Phillips, D.L. & Johnson, M.G. (2000) Elevated CO_2 and conifer roots: effects on growth, life span and turnover. *New Phytologist*, **147**, 87–103.

Treseder, K.K. (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist*, **164**, 347–355.

Walthert, L., Zimmermann, S., Blaser, P., Luster, J. & Lüscher, P. (2004) *Waldböden der Schweiz. Band 1. Grundlagen und Region Jura. (Forest soils of Switzerland. Vol. 1. Basics and the Jura Region)*. Birmensdorf, Eidgenössische Forschungsanstalt WSL. Bern, Hep Verlag, 768 pp.

Zak, D.R., Pregitzer, K.S., King, J.S. & Holmes, W.E. (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, **147**, 201–222.

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

Received 6 January 2009; accepted 26 March 2009
Handling Editor: Ken Thompson

Chapter 3

No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment

Global Change Biology

Global Change Biology (2010), doi: 10.1111/j.1365-2486.2010.02159.x

No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment

MARTIN K.-F. BADER and CHRISTIAN KÖRNER
Institute of Botany, University of Basel, Schönbeinstrasse 6, CH-4056 Basel, Switzerland

Abstract

The anthropogenic rise in atmospheric CO_2 is expected to impact carbon (C) fluxes not only at ecosystem level but also at the global scale by altering C cycle processes in soils. At the Swiss Canopy Crane (SCC), we examined how 7 years of free air CO_2 enrichment (FACE) affected soil CO_2 dynamics in a ca. 100-year-old mixed deciduous forest. The use of ^{13}C-depleted CO_2 for canopy enrichment allowed us to trace the flow of recently fixed C. In the 7th year of growth at $\sim$550 ppm CO_2, soil respiratory CO_2 consisted of 39% labelled C. During the growing season, soil air CO_2 concentration was significantly enhanced under CO_2-exposed trees. However, elevated CO_2 failed to stimulate cumulative soil respiration (R_s) over the growing season. We found periodic reductions as well as increases in instantaneous rates of R_s in response to elevated CO_2, depending on soil temperature and soil volumetric water content (VWC; significant three-way interaction). During wet periods, soil water savings under CO_2-enriched trees led to excessive VWC ($>45\%$) that suppressed R_s. Elevated CO_2 stimulated R_s only when VWC was $\leq 40\%$ and concurrent soil temperature was high ($>15\,^{\circ}C$). Seasonal Q_{10} estimates of R_s were significantly lower under elevated ($Q_{10} = 3.30$) compared with ambient CO_2 ($Q_{10} = 3.97$). However, this effect disappeared when three consecutive sampling dates of extremely high VWC were disregarded. This suggests that elevated CO_2 affected Q_{10} mainly indirectly through changes in VWC. Fine root respiration did not differ significantly between treatments but soil microbial biomass (C_{mic}) increased by 14% under elevated CO_2 (marginally significant). Our findings do not indicate enhanced soil C emissions in such stands under future atmospheric CO_2. It remains to be shown whether C losses via leaching of dissolved organic or inorganic C (DOC, DIC) help to balance the C budget in this forest.

Keywords: carbon cycle, elevated CO_2, FACE, fine root respiration, soil CO_2 efflux

Received 28 August 2009 and accepted 29 October 2009

Introduction

Globally, soils accommodate the greatest terrestrial organic carbon (C) pools with turnover times ranging from days to millennia (Dixon *et al.*, 1994; Amundson, 2001). Heterotrophic soil organisms metabolize C from these (labile to recalcitrant) pools whereas autotrophs, comprising plant roots, their symbionts, and the microorganisms of the rhizosphere, metabolize photosynthetically assimilated C (Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003). The release of this auto- and heterotrophic CO_2 from soils, commonly referred to as soil respiration (R_s), dominates terrestrial ecosystem respiration and may account for up to 80% of the CO_2 emitted by temperate hardwood and coniferous forests (Wofsy *et al.*, 1993; Goulden *et al.*, 1996; Lavigne *et al.*, 1997; Law *et al.*, 1999; Malhi *et al.*, 1999; Janssens *et al.*, 2001). On a global scale, R_s represents the second largest pathway in the C cycle, surpassed only by photosynthesis. Annually, soils release 75–80 Pg of C, which exceeds the current rate of fossil fuel combustion more than 11-fold and implies that roughly 10% of the CO_2 held in the atmosphere, cycles through soils each year (Raich & Potter, 1995; Schlesinger & Andrews, 2000; Raich *et al.*, 2002). Slight changes in soil C and soil C cycling are therefore highly relevant to global C exchange between the land surface and atmosphere. Integrating the various biotic and abiotic processes operating and interacting in soils renders R_s a sensitive indicator of alterations in soil C cycling that may follow from human-induced environmental changes. The rapid rise in atmospheric CO_2 concentration can directly affect the production and composition of plant litter that drives the C cycle belowground (Liu *et al.*, 2009). Elevated atmospheric CO_2 has been shown to commonly increase R_s in a variety of ecosystems ranging from natural to agricultural stands on timescales from <1 to several years (Körner & Arnone, 1992; Luo *et al.*, 1996; Zak *et al.*, 2000; Kimball *et al.*, 2002; King *et al.*, 2004; Bernhardt *et al.*, 2006; Pregitzer *et al.*, 2008; Jackson *et al.*, 2009). However, the magnitude of the reported stimulation was strongly dependent on species composition, plant/stand age, soil conditions, and study dura-

Correspondence: Martin K.-F. Bader, tel. + 41 61 267 3511, fax + 41 61 267 3504, e-mail: Martin.Bader@unibas.ch

tion. The exposure of forest stands to elevated CO_2 using free air CO_2 enrichment (FACE) technology caused significant increases in R_s that persisted throughout the entire study period (>5 years) in two aggrading (POP-FACE, ASPEN-FACE) and two more advanced forests (Duke-FACE, ORNL-FACE; King *et al.*, 2004; Pregitzer *et al.*, 2008). On average, the young, expanding stands exhibited a greater stimulation in forest soil CO_2 efflux (23–54%) than the more advanced stands that were close(r) to steady state litter production, above- and belowground (Duke: 16%, ORNL: 12%). In the more advanced forests, the stimulation in R_s below CO_2-enriched trees declined during the study years and seemed to converge towards ambient rates (King *et al.*, 2004; Suwa *et al.*, 2004; Bernhardt *et al.*, 2006). An attenuation of the respiratory signal could be a consequence of initially accelerated above- and belowground resource exploitation under trees exposed to elevated CO_2 which is expected to decrease over time (King *et al.*, 2004; Körner, 2006). However, at one site (Duke forest), this attenuation was transitory because over the course of 12 study years, R_s was on average 23% higher under elevated CO_2 due to a recovery of rates in more recent years (Jackson *et al.*, 2009).

Since the availability of labile C compounds limits autotrophic and heterotrophic activity in the pedosphere, R_s is closely linked to plant metabolism, photosynthesis, and litter production (Raich & Nadelhoffer, 1989; Raich & Schlesinger, 1992; Högberg *et al.*, 2001). Above- and belowground net primary production (NPP) drives annual R_s by providing the metabolic substrate while the instantaneous rate at which this substrate is decomposed is largely controlled by soil temperature (Raich & Nadelhoffer, 1989; Raich & Schlesinger, 1992; Janssens *et al.*, 2003).

Soil water availability also modulates R_s by restricting soil CO_2 evolution if soils are too dry or too wet (Skopp *et al.*, 1990; Davidson *et al.*, 1998; Vincent *et al.*, 2006). When soil water becomes scarce, substrate transport and microbial activity as well as root respiration become constricted and rates of R_s decline. Increasing soil volumetric water content (VWC) facilitates substrate diffusion while gas diffusion becomes progressively impaired resulting in limiting oxygen supply and the retention of respiratory CO_2 at the sites of metabolic activity (Skopp *et al.*, 1990). Thus, changes in VWC, that may also occur in response to elevated CO_2 (Hungate *et al.*, 2002; Leuzinger & Körner, 2007) can counteract or enhance temperature-induced changes in the CO_2 efflux from soils.

At the Swiss Canopy Crane (SCC) FACE site, the crowns of 11 mature deciduous trees (*Fagus sylvatica*, *Quercus petraea*, *Carpinus betulus*, *Acer campestre*, and *Tilia platyphyllos*) growing in a near-natural forest were exposed to an atmosphere containing $\sim$550ppm CO_2 since autumn 2000. Trees receiving CO_2 enrichment showed reduced sap flow and higher rates of leaf-level C uptake ($+40\%$–50%) than control trees but did not produce more leaf or fine root litter (Körner *et al.*, 2005; Zotz *et al.*, 2005; Leuzinger & Körner, 2007; Bader *et al.*, 2009). While reduced tree water consumption under elevated CO_2 led to enhanced VWC throughout the growing seasons, the 'extra' C assimilated in the CO_2-enriched canopy did not translate into enhanced above- or below-ground growth or biomass in the overstorey trees (Körner *et al.*, 2005; Asshoff *et al.*, 2006; Bader *et al.*, 2009). The fate of the 'extra' C assimilated under elevated CO_2 remained unresolved. Higher CO_2 concentrations and a consistent stable C isotope signal in the soil pore space under CO_2-exposed trees rather suggested greater C flux through these trees to the soil throughout the study period (Steinmann *et al.*, 2004; Keel *et al.*, 2006 and newer unpublished data). Therefore, we hypothesized that the majority of the additional C assimilated under elevated CO_2 was respired back to the atmosphere by greater root respiration and respiratory processes linked to the rhizosphere and by microbial decomposition of increased root derived exudates. Consequently, we anticipated: (1) higher rates of R_s under CO_2-enriched trees compared with control trees growing under ambient conditions, (2) higher respiratory rates of fine roots from CO_2-enriched relative to control trees, and (3) greater soil microbial biomass under elevated CO_2.

Materials and methods

Study site

The SCC FACE facility comprises roughly 3000 m^2 of a highly diverse mixed forest, located ca. 15 km south of Basel, Switzerland (47°28′7.75″N, 7°30′8.75″E, 550 m a.s.l.). The stand has an age of about 100 years and dominant trees vary between 30 and 35 m in height. Tree density is 415 trees ha^{-1} (breast height diameter $\geq$0.1m), stem basal area amounts to 46 $m^2\,ha^{-1}$, and leaf area index (LAI) at peak season is around 5. The closed forest canopy is dominated by *Fagus sylvatica* L. (European beech), *Quercus petraea* (Matt.) Liebl. (Sessile oak) and *Carpinus betulus* L. (Hornbeam), interspersed with subdominant tree species such as *Tilia platyphyllos* Scop. (Largeleaf linden), *Acer campestre* L. (Field maple), *Prunus avium* L. (cherry) and four species of conifers [*Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.]. The understorey vegetation (which is excluded from CO_2 enrichment) is also highly diverse and dominated by tree seedlings and saplings, the evergreen liana *Hedera helix* and shrubs such as *Rubus fruticosus* agg., and *Lonicera periclymenum*. The herb layer is predominantly composed of *Galium odoratum*, *Anemone nemorosa*,

Table 1 Soil classification and properties at the Swiss Canopy Crane site (top 10 cm of the profile, means ± SE, pH: $n = 25$, field capacity: $n = 10$)

Soil type	Texture	$pH_{(KCl)}$	Field capacity (vol. %)
Lithic Rendoll	Clay loam	5.8 ± 0.2	56.5 ± 3

Mercurialis perennis, *Paris quadrifolia*, *Circaea lutetiana* and *Sanicula europaea*. The LAI of the understorey vegetation is <1. The finely textured forest soil (Table 1) is characterized by a very low accessible profile depth between 10 and 20 cm (maximum 25 cm) followed by a rocky subsoil merging into the calcareous bedrock at depths between 20 and 90 cm.

The temperate climate at the study site is characterized by mild winters and moderately warm summers with mean air temperatures in January and July of 2 and 19 °C, respectively. Precipitation was recorded at 2 min intervals at a nearby weather station situated in the village of Flüh (2 km air-line distance from the SCC). Long-term mean annual precipitation at the site is 990 mm, two-thirds of which falls during the 6-month growing season (Pepin & Körner, 2002).

FACE system

At the SCC site, the web-FACE technique allowed us to restrict FACE to the tree crowns (Pepin & Körner, 2002). The food-grade CO_2 used in this experiment was of fossil origin and therefore strongly depleted in ^{13}C (Steinmann *et al.*, 2004; Keel *et al.*, 2006).

In brief, pulses of pure CO_2 were released through a dense web of tubes installed in the tree canopies by means of a 45 m tall construction crane. Computer-triggered magnetic valves controlled the CO_2-supply to the canopies to sustain the target value of 550 ppm as accurately as possible. Monitoring canopy CO_2 concentration was achieved by an air sampling system composed of several suction heads per tree feeding canopy air through sampling lines into infra-red gas analysers (LI-800 GasHound and LI-820; Licor, Lincoln, NE, USA). Additionally, we used isometers (C_4 grass, *Echinochloa crus-galli*) growing in 50 mL containers (sand–clay mixture) fixed in the tree crowns to monitor the abundance of ^{13}C in the canopy CO_2 (Körner *et al.*, 2005; Keel *et al.*, 2006). CO_2 enrichment was confined to daylight hours ($PPFD > 100\,\mu mol\,m^{-2}\,s^{-1}$) of the growing season and was discontinued from the time of leaf shedding until next season's bud break (end of October to mid-April). From the total of 62 trees available in the 60 m operating range of the crane, 12 deciduous trees (three *F. sylvatica*, three *Q. petraea*, three *C. betulus*, one *T. platyphyllos*, one *A. campestre* and one *P. avium*) were subjected to elevated atmospheric CO_2 since autumn 2000. Owing to storm damage in 2006, the single *P. avium* tree was excluded from the FACE treatment. A larger number of control trees were selected in the remaining crane area at sufficient distance to the CO_2-enriched zone.

Restricting the CO_2 enrichment to the 30–35 m tall canopy allowed precise isotopic tracing of the C flux from the tree crowns to the soil without confounding C-fluxes originating from air above soil and understorey vegetation (Keel *et al.*, 2006). The successful exclusion of the understorey vegetation from CO_2 enrichment was demonstrated by the lack of an isotopic signal originating from the application of ^{13}C-depleted CO_2 (Keel *et al.*, 2006). Since the understorey LAI is <1 and the closed forest canopy (LAI ~ 5) permits $<4\%$ of above canopy irradiance to reach the forest floor (Hättenschwiler & Körner, 2000), the understorey vegetation represents a near to negligible component of total stand productivity and its exclusion from CO_2 enrichment will thus not confound the respiratory signal in soil CO_2 evolution.

Soil temperature and soil water content

Soil temperature and soil VWC in the top 10 cm of the soil were measured concurrently to R_s in close vicinity to soil rings using a portable platinum PT-100 thermometer and a hand-held time domain reflectometry (TDR) probe (KM20REF; Comark Ltd., Hitchin, UK and Trime-FM; Imko, Ettlingen, Germany). We randomly sampled four soil cores (diameter: 5 cm, soil depth: 0–10 cm) in the CO_2-enriched and the control area each and then determined their VWC, which was used to calibrate the corresponding TDR measurements taken in the field [$VWC = (VWC_{TDR} - 47.5)/2.3$, $r^2 = 0.99$, $P < 0.01$]. In addition to the spot measurements, we continuously recorded soil temperature at 10 cm depth using a waterproof miniaturized temperature logger (HOBO Tidbit v2; Onset Computer Corp., Bourne, MA, USA).

Soil air $\delta^{13}C$ and soil air CO_2 concentration

The concentration of CO_2 in air-filled soil pores was determined on a monthly basis using 80 gas wells made of PVC tubes (length × diameter: 12 × 2 cm). The aboveground end of the vertically inserted gas wells was sealed with a silicon septum while the belowground end remained open and a pair of opposite slits allowed CO_2 diffusion from soil between 3 and 11 cm depth. The gas wells were arranged in a grid of 3 m within the 550 m^2 FACE area while an extended grid of 6 m was established in the larger control area (Steinmann *et al.*, 2004). The soil air samples were analysed for their C isotope ratio using a mass spectrometer (Delta Plus XL; Thermo Finnigan, Bremen, Germany). For each sample, the CO_2 concentration was estimated from the calibration line derived from standard gases of known CO_2 concentrations (340 and 5015 ppm). The area below the voltage signal peak of the mass spectrometer for CO_2 (masses 44, 45, and 46) was integrated over time and corresponded to the CO_2 concentration of the soil air sample. Each series of soil air samples included a series of reference gas samples.

We used the Keeling plot approach (Keeling, 1958) for each sampling date and CO_2 treatment separately, to determine the $\delta^{13}C$ of soil CO_2, i.e. the intercept of the linear regression between the measured $\delta^{13}C$ values and the inverse soil air CO_2 concentration corresponded to the mean $\delta^{13}C$ of soil air and the standard error of the intercept was used as a measure of spread. Our data were corrected for isotope fractionation resulting from slower diffusion of the heavier $^{13}CO_2$ by subtracting 4.4‰ (Hesterberg & Siegenthaler, 1991).

Soil respiration

CO_2 release from the forest soil was recorded from April 2007, before the seasonal start of CO_2 enrichment, to November 2007 at weekly intervals during the growing season (weather permitting) and monthly after the growing season. Flux measurements were taken using two identical custom-made static chambers equipped 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 water vapour cross-sensitivity of the IRGA was corrected on-line by linking it with the relative humidity/temperature sensor. The polypropylene chamber housing (height × diameter: 20 × 20 cm) had a sealable vent on top (3 cm diameter) to prevent overpressurization during chamber placement over the soil (Davidson *et al.*, 2002). Inside the chamber, a small battery-driven computer fan, down-regulated to 3 V, provided gentle mixing of the air in the headspace, minimising mass flow of CO_2 from the soil into the chamber (diameter: 40 mm, Asia Vital Components Co., Ltd., Kaohsiung City, Taiwan; Pumpanen *et al.*, 2004). Closed cell neoprene gaskets on the chamber flange provided an airtight seal between chamber and soil rings. During measurements, the chambers were placed on polypropylene rings (5 cm in height and 20 cm in diameter) inserted 2 cm into the soil. A total of 44 soil collars were installed, 22 each under CO_2-enriched and control trees, respectively. However, due to soil disturbances caused by rodent activity the number of measurable soil collars varied from 19 to 22 for each treatment between sampling dates. We assigned one to three soil rings to one of each control or CO_2-enriched trees. Measurements of R_s under ambient and elevated CO_2 were taken simultaneously between 14:00 and 17:30 hours to coincide with maximum daytime rates of respiration. Litter inside the soil rings was left in place to provide near-natural conditions of CO_2 efflux. All seedlings that germinated in the collars were immediately removed throughout the growing season. For diel courses, R_s was recorded at five time intervals within 24 h, the mid-points of which were 08:00, 12:00, 15:00, 20:00 and 03:00 hours. Individual recordings of soil CO_2 efflux lasted 5 min with a 5 s reading interval. Since our measurement system operates on a nonflow-through nonsteady-state basis, the rate of R_s was calculated from the slope of a linear regression applied to the initial CO_2 increase in the chamber headspace. For the regression analyses, we ignored the first minute of the recordings to avoid slight flux disturbances following chamber placement (Davidson *et al.*, 2002; J. Pumpanen, personal communication).

Fine root respiration

Fine root respiration was measured using a portable CO_2/H_2O gas-exchange system equipped with a conifer cuvette (LI-6400, LI-6400-05; Licor). At the end of July 2007, we recorded R_s between 14:00 and 17:30 hours using a Licor system (LI-6400-09 soil respiration chamber linked to the LI-6400) at eight rings (10 cm in diameter) per CO_2 treatment. These measurements served as a reference to estimate the contribution of fine root respiration to total R_s. Subsequently, we took soil cores (depth: 10 cm, diameter: 5 cm) from the centre of each ring, separated roots from soil and rinsed the roots with distilled water in our field laboratory. Then, fine root samples were placed on a fine mesh and transferred into the conifer cuvette where root respiratory CO_2 release was determined at constant temperature of 13.5 °C (corresponding to the actual soil temperature), constant CO_2 concentration (1000 ppm), and 70%–80% relative humidity to resemble *in situ* conditions as close as possible (Subke *et al.*, 2006). All fine roots samples were measured within 2.5 h after excision. Afterwards, roots were dried for 48 h at 80 °C for dry mass determination. Excised herb and tree fine roots have been shown to maintain constant respiration rates for 4–6 h after collection (Burton & Pregitzer, 2002; Lipp & Andersen, 2003; Bahn *et al.*, 2006). With the help of a root reference collection established during a previous study at the same site (Bader *et al.*, 2009) we were able to distinguish between tree roots that made up $>90\%$ of the roots in the soil cores and nontree roots.

Soil microbial C and N

Soil samples for the determination of soil microbial biomass were derived from a parallel study at our site that considered only the three dominant and replicated tree species (*Fagus sylvatica*, *Quercus petraea*, *Carpinus betulus*). In July 2007, we collected three soil cores from each of the three individuals per tree species in ambient and elevated CO_2 ($n = 9$ per treatment). Soil cores were taken to a depth of 18 cm using a soil corer (5 cm diameter). Microbial biomass C and N (C_{mic}, N_{mic}) were estimated from these samples by the chloroform fumigation–extraction (CFE) method (Brookes, 1985; Vance *et al.*, 1987). From sieved (2 mm mesh) and root-free soil samples (~50% water holding capacity) one subsample (15–20 g dry mass equivalent) was fumigated with ethanol-free $CHCl_3$ for 3 days. Then the reagent was removed and the sample extracted with 0.5 M K_2SO_4 for 30 min using a table shaker (200 rpm). A second nonfumigated subsample was extracted in the same way concurrently to the start of fumigation. The extract from the soil was filtered (Whatman no. 1) and then frozen until quantification using a TOC analyser (TOC 100 equipped with a TNb-Module; Dimatec GmbH, Essen, Germany). Filtered K_2SO_4 solution served as a blank for the TOC analyser accounting for background C from chemicals and/or filter material. Soil microbial biomass C and N was calculated from the difference between organic C and N extracted from fumigated an nonfumigated soil samples (EC, EN)

$$C_{mic} = EC/k_{EC} = EC/0.45,$$
$$N_{mic} = EN/k_{EN} = EN/0.54,$$

where k_{EC} is the extraction yield for microbial C suggested by Wu *et al.* (1990) and the k_{EN} the extraction yield for microbial nitrogen given by Brookes (1985).

Statistical analysis

The SCC FACE experiment was a tree-oriented study, therefore individual trees (incl. roots and soil sphere) exposed to ambient and elevated CO_2 were our statistical replicates. In practice, one to five samples (gas wells, soil respiration rings, spot measurements of volumetric soil water content, excised root respiration, and soil samples in the case of microbial biomass) were assigned to individual trees.

We used two linear mixed-effects models with restricted maximum likelihood to assess the impact of elevated atmospheric CO_2 on soil air $\delta^{13}C$, soil air CO_2 concentration, soil CO_2 efflux, soil water content (model I), and soil microbial biomass (model II). The first model comprised the fixed factors 'CO_2', 'soil temperature', ('soil water content'), and 'sampling date', and the random factor 'tree'. The second model included 'CO_2' as single fixed factor and the random factor 'tree'. Heteroscedasticity of within-group errors was modelled using a constant variance function. The residuals and the random effects of the final models were tested for normality using quantile–quantile plots. In addition, we applied the Kolmogorov–Smirnov test by comparing the model residuals to a normally distributed random sample of the same size with identical mean and standard deviation. We used an autocorrelation function to check the model residuals for temporal autocorrelation and applied an autoregressive correlation structure of order 1 [AR(1)] to model dependence among observations. Whenever necessary, suitable transformations were derived using the Box–Cox method. Nonnormally distributed rates of R_s were $\log_{10}$-transformed and soil water content was power-transformed by -0.4. Rates of fine root respiration under ambient and elevated CO_2 could not be successfully transformed to normality and were compared using the Wilcoxon's rank-sum test.

The soil temperature response of R_s was estimated by nonlinear least squares regression using the Arrhenius function

$$R_s = R_{s10} \times e^{\left(\frac{E_0}{R(T+273.15)}\frac{T-10}{283.15}\right)}$$

and the Q_{10} function

$$R_s = R_{s10} \times Q_{10}^{\left(\frac{T-10}{10}\right)},$$

where R_s is soil respiration, R_{s10} is soil respiration at 10 °C soil temperature, Q_{10} is the temperature sensitivity of soil respiration, E_0 is the activation energy, R is the universal gas constant ($8.314\,J\,K^1\,mol^1$), and T is the soil temperature in °C (Fang & Moncrieff, 2001). Attempts to apply the modified Arrhenius function suggested by Lloyd & Taylor (1994) to our data were not successful because the estimation algorithm failed to achieve convergence indicating overparameterization for our data set. Therefore, we used the following equation to derive the Q_{10} value from the Arrhenius model

$$Q_{10} = \left(\frac{R_s2}{R_s1}\right)^{\left(\frac{10}{T2-T1}\right)},$$

where R_s2 and R_s1 are the soil respiration rates at the higher ($T2 = 15$ °C) and lower ($T1 = 5$ °C) soil temperature, respectively. Model selection was based on F-test comparisons between the model assuming common parameter estimates for both treatments and the model with separate parameter estimates for each of the treatments. We computed 95% bootstrap confidence intervals to obtain confidence limits for the derived Q_{10} values. Attempts to incorporate a soil moisture term into the Arrhenius model and the application of a multiplicative model considering soil water (e.g. Hui & Luo, 2004; Palmorth *et al.*, 2005) both failed because of insignificant parameter estimates.

However, the residuals of the Arrhenius model were related to VWC and could be modelled using a common Gauss function

$$R_{s\ \mathrm{residuals}} = a + e^{\frac{-(\mathrm{VWC}-b)^2}{2c^2}},$$

where a is the asymptote, VWC is the soil volumetric water content in vol.%, b is the VWC value under the peak of the curve, and c is a value controlling the spread of the curve.

Here, Q_{10} is a seasonal measure that gives the temperature sensitivity integrated over the whole measuring period (May–November), i.e., the rate of change in bulk soil respiration for a 10 K increase in soil temperature. Given the miscellaneous contributions of roots and heterotrophic organisms to R_s and other environmental factors that vary along with soil temperature throughout the growing season, seasonal Q_{10} does not correspond to the strict definition of Q_{10}. However, it is suitable for the analysis of the temperature sensitivity of bulk soil processes (Widén & Majdi, 2001). Rates of R_s were integrated over time to arrive at seasonal and annual estimates of soil C efflux. This was achieved by linear interpolation of R_s rates between sample dates (growing season only) and by modelling using the Arrhenius equation with the fitted parameter estimates for ambient and elevated CO_2 in combination with a continuous soil temperature record at 10 cm depth.

The free software R, version 2.9.0, was used for all statistical computations and graphics (R Development Core Team, 2009; www.r-project.org).

Results

Weather conditions during the study period

In 2007, the average air temperature at the study site was 10.3 °C. Weekly mean air temperature reached its maximum at the end of July (19.3 °C) and was lowest in early November (0.1 °C). During the measurement period, from April to November, precipitation was 517 mm, which was ca. 75% of the annual total of this year (708 mm; for daily precipitation data, see Fig. 1).

$\delta^{13}C$ of soil air

Throughout the entire sampling period (April–November 2007) soil air $\delta^{13}C$ was significantly lower under CO_2-enriched trees, on average 2.2 ± 0.2‰ (mean ± SE, Fig. 1a, Table 2). Using the isometer $\delta^{13}C$ as reference, this isotopic signature corresponds to a contribution of 39% labelled C to the respired CO_2 in soil air. Irrespec-

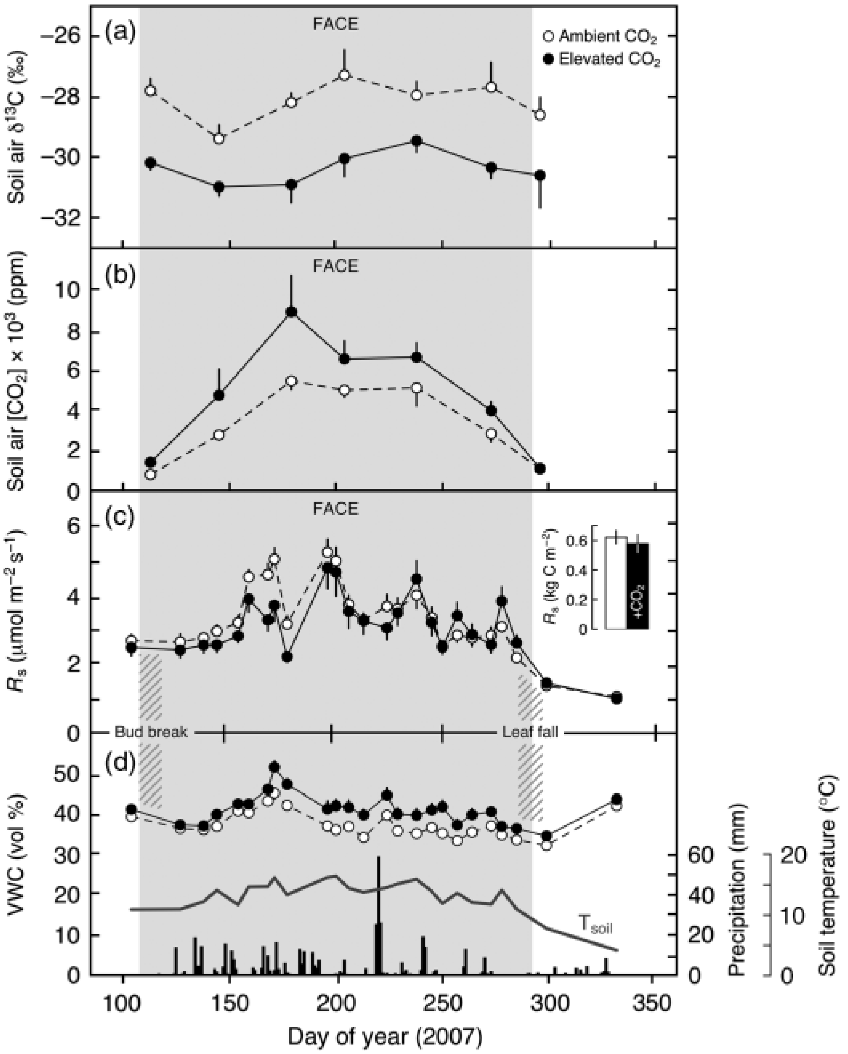


Fig. 1 Seasonal variation of (a) $\delta^{13}C$ in soil air at 3–11 cm depth, (b) soil air CO_2 concentration at 3–11 cm depth, (c) soil respiration (R_s), (d) soil temperature (T_{soil}) and volumetric soil water content (VWC) in the top 10 cm, and precipitation (vertical lines), recorded at the Swiss Canopy Crane (SCC) site under CO_2-enriched and control trees (ambient CO_2: $n = 17$, elevated CO_2: $n = 11$, means ± SE). The grey-shaded area indicates the free air CO_2 enrichment (FACE) period that corresponds to the 6-month growing season. The bar chart inset gives the R_s integrated over the growing season.

tive of treatment, there was significant seasonal variation in soil air $\delta^{13}C$, which also influenced the difference in the isotopic signature between treatments indicated by a significant CO_2 × month interaction (Table 2).

Soil air CO_2 concentration

At the time of leaf budbreak and after leaf fall, soil air CO_2 concentration was similarly low under all study trees (<1500 ppm). However, along with leaf maturation and thus, increasing canopy assimilation, respiratory CO_2 accumulated at significantly higher concentrations in soil air under CO_2-treated trees compared with soil air under controls (Fig. 1b, Table 2). There was particularly strong accumulation of respiratory CO_2 in the soil under CO_2-enriched trees at the end of June (elevated CO_2: 8900 ppm, ambient CO_2: 5400 ppm) when exceedingly high soil water contents coincided with a drop in soil temperature of 3 K (Fig. 1b). Averaged over the growing season the concentration of CO_2 in soil air was 52% higher under trees exposed to elevated CO_2.

Soil respiration

Instantaneous rates of R_s showed pronounced seasonal variation that correlated strongly with the seasonal course of soil temperature (Fig. 1c and d). VWC also

Table 2 Linear mixed-effects model results for soil air $\delta^{13}C$, soil air CO_2 concentration, rates of soil respiration, and VWC under control and CO_2-enriched trees

Factor	df	*F*	*P*
Soil air $\delta^{13}C$			
CO_2	1, 47	19.508	$<0.001^{***}$
Month	6, 249	53.263	$<0.001^{***}$
$CO_2 \times$ month	6, 249	3.703	0.002^{**}
Soil air CO_2 *concentration*			
CO_2	1, 47	5.420	0.024^{*}
Month	6, 249	171.675	$<0.001^{***}$
$CO_2 \times$ month	6, 249	2.085	0.056
Soil respiration			
CO_2	1, 26	0.499	0.486
T_s	1, 447	487.885	$<0.001^{***}$
VWC	1, 447	6.607	0.011^{*}
Doy	22, 447	6.569	$<0.001^{***}$
$CO_2 \times T_s$	1, 447	0.387	0.534
$CO_2 \times$ VWC	1, 447	21.417	$<0.001^{***}$
$CO_2 \times$ doy	22, 447	1.689	0.027^{*}
$T_s \times$ VWC	1, 447	0.857	0.355
$T_s \times$ doy	22, 447	0.755	0.782
VWC $\times$ doy	22, 447	2.019	0.004^{**}
$CO_2 \times T_s \times$ VWC	1, 447	16.085	$<0.001^{***}$
$CO_2 \times T_s \times$ doy	22, 447	1.594	0.044^{*}
$CO_2 \times$ VWC $\times$ doy	22, 447	1.784	0.016^{*}
VWC			
CO_2	1, 26	10.605	0.003^{**}
T_s	1, 517	5.673	0.018^{*}
Doy	22, 517	27.094	$<0.001^{***}$
$CO_2 \times T_s$	1, 517	2.232	0.136
$CO_2 \times$ doy	1, 517	1.519	0.062
$T_s \times$ doy	22, 517	1.666	0.030^{*}

doy, day of year; T_s, soil temperature; VWC, soil volumetric water content.

$^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

significantly influenced the release of CO_2 from the soil, but CO_2 enrichment as a main effect was not significant (Table 2). The response of R_s to canopy CO_2 enrichment was dependent on VWC and changed with progressing season as evidenced by significant two-way interactions (Table 2). The highly significant $CO_2 \times$ soil temperature $\times$ VWC interaction indicates that the effect of CO_2 enrichment on R_s was controlled by soil temperature and soil water content and the interaction of these soil factors. Before the seasonal start of CO_2 enrichment and during leaf expansion, rates of R_s were similar under all study trees ($\sim 2.5\,\mu mol\,m^{-2}\,s^{-1}$). In June, when VWC had reached its seasonal maximum, higher moisture levels in soil under CO_2-enriched trees (>45%) resulting from reduced sap flow, caused R_s to decline below rates measured under control trees (where VWC never reached 45%). The steep drop in R_s observed under all study trees in late June corresponded to a decline in soil temperature of almost 3 K coincident with the exceptionally high soil water content at that time (Fig. 1c and d). Maximal rates of R_s under both treatments reached about $5\,\mu mol\,m^{-2}\,s^{-1}$ and coincided with the soil temperature maximum of about 16 °C in July. During the second half of the summer, elevated CO_2 occasionally stimulated R_s, presumably driven by soil temperature peaks when VWC under the CO_2-exposed trees had declined to values $\leq 40\%$. After leaf fall, R_s was nearly identical under control and CO_2-enriched trees and decreased to rates of about $1\,\mu mol\,m^{-2}\,s^{-1}$ when soil temperature dropped below 5 °C in November.

Soil CO_2 efflux integrated over the 6-month growing season (by linear interpolation between sampling dates) was similar under treated and control trees (ambient $CO_2 = 0.62 \pm 0.05\,kg\,C\,m^{-2}$, elevated $CO_2 = 0.58 \pm 0.06\,kg\,C\,m^{-2}$, mean ± SE; Fig. 1c inset). We also used the Arrhenius function to model cumulative R_s over the growing season, which yielded estimates very close to the interpolated values (ambient $CO_2 = 0.60\,kg\,C\,m^{-2}$, 95% CI = 0.49–0.64, elevated $CO_2 = 0.56\,kg\,C\,m^{-2}$, 95% CI = 0.45–0.60). Yearly model estimates of R_s at the SCC site yielded $0.84\,kg\,C\,m^{-2}\,yr^{-1}$ (95% CI = 720–878) under control trees and $0.81\,kg\,C\,m^{-2}\,yr^{-1}$ (95% CI = 691–852) under CO_2-enriched trees.

The diel courses of R_s recorded in the early, mid, and late growing season showed similar rates between both CO_2 treatments. Moreover, day- and nighttime rates of R_s were mostly similar. In the early growing season before leaves had fully expanded and when soil temperature had just exceeded 10 °C, daily R_s averaged $2.49 \pm 0.05\,\mu mol\,m^{-2}\,s^{-1}$. In mid-July at peak season, when soil temperature rose to 16.4 °C, R_s had nearly doubled and reached $4.77 \pm 0.11\,\mu mol\,m^{-2}\,s^{-1}$. This was the only diel data set that showed differences in R_s on a daily basis with highest rates in the afternoon and lowest rates during the night (-17%, $F = 10.134$, $P < 0.01$). In August, the daily average decreased to $4.02 \pm 0.10\,\mu mol\,m^{-2}\,s^{-1}$ and further declined to $2.49 \pm 0.07\,\mu mol\,m^{-2}\,s^{-1}$, shortly before leaf fall when soil temperature was similar to that in spring. Since soil temperature under the forest canopy hardly varied in the course of a day the close coupling of R_s to soil temperature was not evident on a diel time scale (Fig. 3).

Soil temperature and soil water content

Soil temperature at 10 cm depth was nearly identical across treatments and increased from 10.9 °C in April to a maximum of 16.4 °C in mid-July, declining to 4.2 °C (minimum temperature of the measuring period) in November (Fig. 1d).

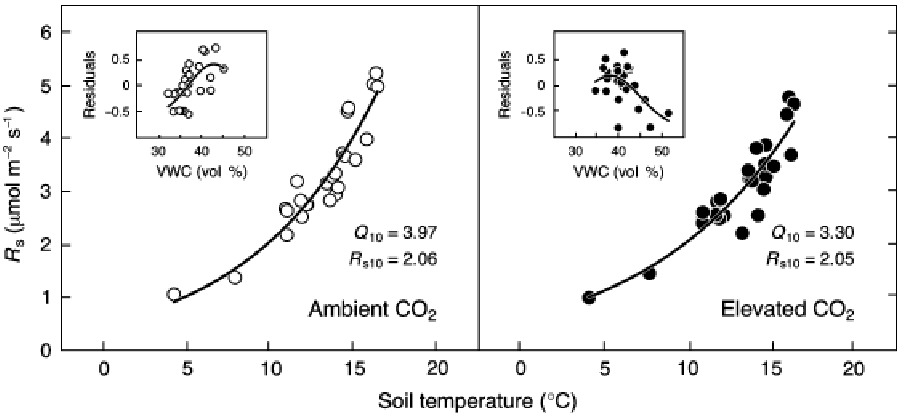


Fig. 2 The seasonal response of soil respiration to soil temperature at 10 cm depth under trees exposed to ambient and elevated CO_2. Both regression lines are highly significant ($P < 0.001$). Soil temperature explained 87% (ambient CO_2) and 83% (elevated CO_2) of the seasonal variation in soil respiration. The insets show the model residuals as a function of soil volumetric water content (VWC) in the top 10 cm.

Table 3 Nonlinear regression estimates of soil respiration rates at 10 °C (R_{s10}) and temperature sensitivity (Q_{10}) including bootstraped confidence intervals at the SCC FACE site

Model	Treatment (CO_2)	R_{s10}	95% CI of R_{s10}	Q_{10}	95% CI of Q_{10}	Explained variation (%)
Arrhenius function	Ambient	2.06	1.84–2.13	3.97	3.14–4.34	87
(All dates)	Elevated	2.05	1.80–2.13	3.30	2.62–3.58	83
Arrhenius function	Ambient	2.07	1.86–2.15	3.74	2.99–4.04	87
(Truncated data set)	Elevated	2.06	1.86–2.13	3.47	2.82–3.75	88
Q_{10} function	Ambient	2.06	1.86–2.14	3.86	3.08–4.17	88
(All dates)	Elevated	2.05	1.83–2.13	3.22	2.59–3.52	83
Q_{10} function	Ambient	2.08	1.87–2.14	3.65	2.90–3.95	88
(Truncated data set)	Elevated	2.07	1.85–2.14	3.39	2.74–3.63	88

SCC, Swiss Canopy Crane; FACE, free air CO_2 enrichment; VWC, soil volumetric water content; CI, confidence interval.
The analyses comprised the entire sampling period (all dates) and a truncated data set excluding the three samplings dates where VWC peaked in both treatments.

Soil temperature accounted for 87% and 83% of the seasonal variation observed in R_s under control and CO_2-enriched trees, respectively (Fig. 2). The temperature sensitivity of R_s for the measuring period (April–November) was significantly reduced under elevated ($Q_{10} = 3.30$, Table 2) compared with ambient CO_2 ($Q_{10} = 3.97$; F-test, $F = 3.4945$, $P = 0.039$, Table 3). However, after removal of three sampling dates in June when VWC peaked in both treatments (exceeding 45% under CO_2-enriched trees), the temperature sensitivity of R_s was similar in both treatments (F-test, $F = 0.6636$, $P = 0.521$, Table 3). VWC at 0–10 cm depth showed considerable seasonal variation but never dropped below 33%, regardless of the CO_2-treatment (Fig. 1d). Canopy CO_2 enrichment and soil temperature significantly affected VWC and there was evidence that the magnitude of both effects varied with the course of the season (see interactions Table 2). Before the start of CO_2 enrichment and during leaf development, VWC was similar under all study trees. When leaves had fully expanded and transpiration in the canopy approached maximum values, soil moisture savings resulting from reduced water consumption (Leuzinger & Körner, 2007) became rapidly apparent under CO_2-enriched trees. Higher VWC under elevated CO_2 (on average + 8.5%) persisted throughout the season and did not converge towards ambient VWC until leaf ageing commenced. The residuals from the temperature response models were highly correlated to VWC which accounted for 93% and 88% of the unexplained variation under ambient and elevated CO_2, respectively (inserts Fig. 2). Irrespective of treatment, the residual models suggest an optimal VWC of around 40% for R_s (corresponding to ~70% of the field capacity).

Table 4 Soil microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) in soil under three dominant European forest trees (*Fagus sylvatica*, *Quercus petraea*, *Carpinus betulus*) exposed to elevated or ambient CO_2 (means ± SE, $n = 9$, 1 n = averaged soil core triplicate sampled in July 2007)

	Ambient CO_2	Elevated CO_2	P
C_{mic} (μg C g^{-1} soil)	1566 ± 73	1813 ± 98	0.06*
N_{mic} (μg N g^{-1} soil)	279 ± 16	319 ± 23	0.17
C_{mic}/N_{mic}	5.7 ± 0.2	5.8 ± 0.2	0.69

*$P = 0.05$–0.1.

Fine root respiration

At peak season, respiration of excised fine roots measured by gas-exchange was 33% higher under elevated CO_2 ($31 \pm 5\,\mathrm{nmol\,g^{-1}\,s^{-1}}$, mean ± SE), compared with ambient CO_2 ($20 \pm 4\,\mathrm{nmol\,g^{-1}\,s^{-1}}$) but this difference was statistically insignificant (Wilcoxon's test; $W = 20$, $P = 0.235$, $n = 8$). We do not believe that disrupting the root–microbe–soil matrix allows reliable flux partitioning into autotrophic respiration (fine roots and rhizosphere) and heterotrophic respiration (bulk soil). However, when we ignore these artefacts and those due to root excision, extrapolation of the root respiratory fluxes suggests that fine roots from control trees contributed roughly 50% to total R_s, while the CO_2 release from fine roots of CO_2-enriched trees accounted for 83% of the respiration originating from the forest soil.

Soil microbial biomass

At peak season in year 7 of CO_2-enrichment, microbial C was ca. 14% and microbial N was nearly 13% higher in soil under CO_2-enriched trees compared with soil under trees growing in ambient CO_2 (Table 4).

Discussion

We aimed at quantifying the respiratory soil C release under 11 mature forest trees belonging to five broad-leaved species after 7 years of exposure to elevated atmospheric CO_2. Since photosynthesis remained enhanced under elevated CO_2 but growth rates across trees were largely unaffected, we expected an increased C flux to the soil. Strong CO_2 accumulation in the soil pore space confirmed this assumption and led us to hypothesize that the additional C channelled to the soil under the CO_2-enriched canopy was largely respired back to the atmosphere. Surprisingly, our chamber-based rates of soil CO_2 efflux did not provide evidence to support this hypothesis neither on a daily nor on a seasonal time scale.

Soil air $\delta^{13}C$

The use of fossil ^{13}C-depleted CO_2 for canopy enrichment allowed us to trace the C flux from the crowns to the rhizosphere of our ca. 100-year-old study trees. After the start of canopy CO_2 enrichment in autumn 2000, the isotopic signal rapidly appeared in soil air under CO_2-exposed trees and persisted throughout the study years (Steinmann *et al.*, 2004; Keel *et al.*, 2006). This soil air signal mirrors the contribution of the metabolization of freshly assimilated ^{13}C-depleted CO_2 and the respiratory mix of carbohydrates produced and stored in previous years. Our data from year 7 of the experiment suggest that respiratory CO_2 in soil under CO_2-enriched trees consisted of 39% labelled C, which is in line with the quasi-steady state of 35%–40% reached within the 1st year of enrichment (Steinmann *et al.*, 2004; Keel *et al.*, 2006), but lower than reported previously from other forests (55%–80%; Andrews *et al.*, 1999; Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003; Pregitzer *et al.*, 2006). This may partly result from differences between the investigated stands such as tree age, species identity/composition or climatic conditions affecting soil temperature, VWC, and plant metabolism that in turn affect soil air $\delta^{13}C$. Pulse-labelling experiments and the comparison of stable C isotope data from the early and later years of CO_2-enrichment suggest slowly diluting C pools in our trees which may also contribute to the smaller-than-expected fraction of isotopically labelled soil air (Keel *et al.*, 2006, 2007; Bader *et al.*, 2009). Obviously, new C is blended into existing C-pools before the mixture becomes metabolized. A further minor dilution of the isotopic signal comes from the release of unlabelled respiratory CO_2 by the roots of the understorey vegetation.

Soil air CO_2 concentration and soil CO_2 efflux

Similar to the previous years of the experiment, soil air CO_2 concentration during the growing season of year 7 was higher under CO_2-enriched trees (on average + 52%). However, this increase in soil air CO_2 concentration did not translate into a corresponding increase in soil CO_2 efflux under CO_2-enriched trees. In fact, R_s integrated over the entire growing season (by linear interpolation and modelling) yielded quite similar soil C emissions under ambient and elevated atmospheric CO_2 ($\sim 0.6\,\mathrm{kg\,C\,m^{-2}}$ over the 6-month growing season). Our modelled annual estimates of the cumulative soil C release ($\sim 0.8\,\mathrm{kg\,C\,m^{-2}\,yr^{-1}}$) are in line with the estimates from other FACE projects (except for the Duke

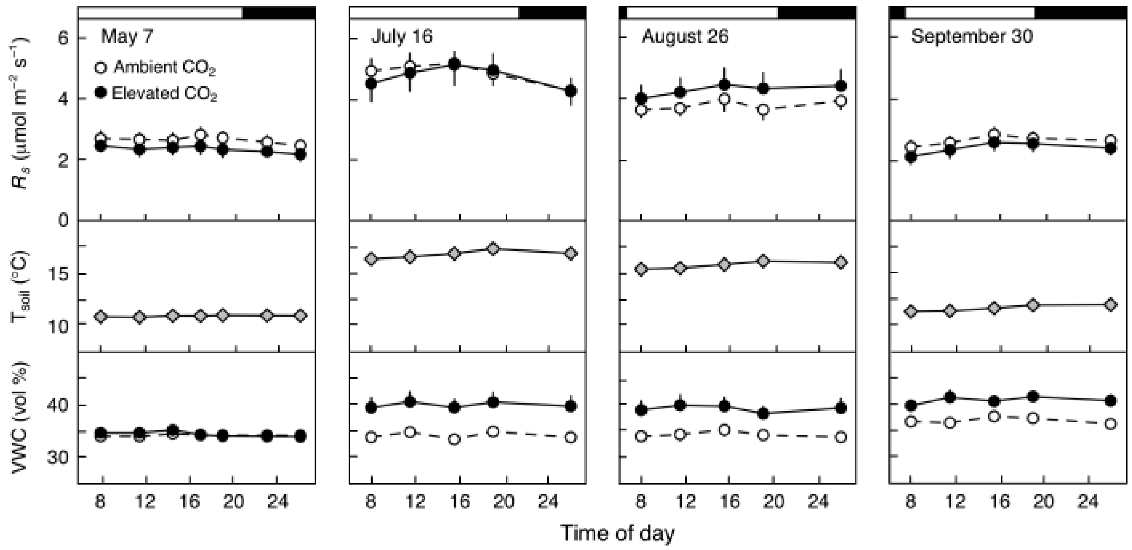


Fig. 3 Diel courses of soil respiration, soil temperature and soil water content (VWC) at 10 cm depth recorded at the Swiss Canopy Crane (SCC) site under CO_2-enriched and control trees in the early (May 18), mid (July 16), mid-late (August 26) and late (September 30) growing season (ambient CO_2: $n = 17$, elevated CO_2: $n = 11$, means ± SE). White and black bars on top of the panels denote day- and nighttime hours.

forest that had substantially higher yearly rates, Jackson *et al.*, 2009) and more recent values for temperate forests (Malhi *et al.*, 1999; Granier *et al.*, 2003; Wang *et al.*, 2006). However, our annual estimates are higher than previously reported values for temperate forests (Raich & Schlesinger, 1992; Raich & Potter, 1995).

For most of the 2007 growing season, the instantaneous rates of R_s were similar in both treatments but at times of high or continuous precipitation, rates under CO_2-enriched trees were suppressed relative to rates under control trees. Yet, when soil temperature peaks coincided with moderate VWC levels, soil CO_2 efflux under CO_2-enriched trees exceeded control rates. Given the generally high VWC in this forest, water savings resulting from reduced sapflow under elevated CO_2 (Cech *et al.*, 2003; Leuzinger & Körner, 2007) may rapidly diminish soil diffusivity and thus lead to periodic reductions of soil gas exchange compared with ambient CO_2 conditions. Our data imply that gas diffusion in this fine textured forest soil becomes impaired once VWC exceeds 45% (corresponding to $>80\%$ of the field capacity and $>60\%$ water-filled pores, Fig. 2 insets). Linn & Doran (1984) previously demonstrated that soil aeration rapidly declines once water-filled pore space exceeds 60%. However, changes in soil diffusivity may alter temporal patterns of soil respiration only at a subdaily timescale but cannot change the balance of soil CO_2 production and release in the long-term (Hui & Luo, 2004; Daly *et al.*, 2009). Higher CO_2 production belowground should therefore translate into enhanced CO_2 efflux from the soil surface, even at high VWC as has been shown for the Duke forest (Suwa *et al.*, 2004). However, in none of our four 24 h measurements taken from the early to the late growing season did we find any daytime-dependent stimulation of R_s under CO_2-enriched trees (Fig. 3). The absence of a CO_2-effect in the course of a day together with the mostly small diel variation in R_s strengthened our confidence in the estimates of cumulative R_s (derived from statistical modelling and linear interpolation of the weekly afternoon measurements).

Even though we found higher soil air CO_2 concentration, there was no evidence – neither during the growing season nor during the early dormant season – that R_s under CO_2-enriched trees could account for the dissipation of the extra C assimilated under elevated CO_2. Given the consistently higher VWC in soil under CO_2-enriched trees, losses of C through enhanced leaching of dissolved organic or inorganic C (DOC, DIC) provide one possible pathway for C dissipation. After 2 years of CO_2 enrichment at the SCC, DOC leaching rates from litter of the CO_2-exposed trees was increased by 16% compared with litter from control trees, supposedly due to enhanced concentrations of nonstructural carbohydrates and phenolics in litter (Körner *et al.*, 2005; Hagedorn & Machwitz, 2007). Moreover, the DOC leaching from CO_2-enriched litter was less biodegradable and could therefore enter the soil organic matter (SOM) pool via sorptive stabilization and thus increase soil C storage in the long-term. Preliminary data suggest that DOC leaching rates in the mineral soil of our stand remained unaffected by elevated CO_2 whereas annual C losses via leaching of DIC were 29% enhanced under CO_2-enriched trees (F. Hagedorn, unpublished data measured in 2004 and 2005). Enhanced C losses due to a 22% increase in DIC leaching resulting from a 14% increase in soil air CO_2 concentration have previously been reported from the Aspen-FACE site (Karberg *et al.*, 2005). Tracing the δ^{13}C-signature of the fumigation gas revealed that $\sim 90\%$ of the leaching DIC in the soil of these stands consisted of recently assimilated C. At the Duke FACE forest, strongly increased cation concentrations and alkalinity in deeper soil (200 cm) due to higher rates of mineral weathering also indicated increases in DIC under elevated CO_2 (Jackson *et al.*, 2009). In a late successional treeline ecotone in the Swiss Alps, CO_2 enrichment increased DOC in the organic layer and in soil solution (at 5 cm depth) by 17% and 20%, respectively (Hagedorn *et al.*, 2008). The CO_2-induced increases observed in DOC were in excess of the actual input of recently fixed C suggesting a priming effect that may even involve increasing losses of old C.

Soil temperature and soil water content

Seasonal estimates of Q_{10} must be regarded as a combined response to changes in soil temperature, VWC, plant metabolism and the dynamics of roots and soil microbes (Davidson *et al.*, 1998, 2006; Janssens & Pilegaard, 2003). At the SCC FACE site, elevated CO_2 significantly reduced the seasonal temperature sensitivity (Q_{10}) of R_s. However, when we excluded the three consecutive sampling dates where soil water content in both treatments reached its maximum during the growing season, Q_{10} estimates were similar under ambient and elevated CO_2. The latter is consistent with findings reported from four FACE sites of expanding and established forest stands (Duke-FACE, ORNL-FACE, POP-FACE, and ASPEN-FACE) where seasonal Q_{10} values remained unaffected by elevated CO_2 (King *et al.*, 2004). In our mature forest, canopy CO_2 enrichment reduced tree water consumption, which gave rise to higher soil water levels (2007: + 8.5%) persisting throughout the growing season (Cech *et al.*, 2003; Leuzinger & Körner, 2007; Bader *et al.*, 2009). The soil in the top 10 cm is finely textured (loamy clay) providing a pore system characterized by a high field capacity making these soils prone to water logging and by relatively low air capacity resulting in poor soil aeration. At times of high or continuous precipitation, these soil characteristics may cause soil pore diffusivity to decrease sooner under elevated CO_2 where VWC persistently exceeded that under control trees. Therefore, we conclude that the reduction of the seasonal temperature sensitivity of R_s (Q_{10}) under elevated CO_2 resulted from particularly high VWC levels ($>45\%$) periodically limiting soil pore diffusivity and thus soil gas exchange. Moreover, a shift in soil microbial community composition toward a greater importance of fungi, which show lower temperature sensitivity than bacteria might have contributed to the decline in Q_{10} under elevated CO_2 (Boone *et al.*, 1998; Epron *et al.*, 2001; Pendall *et al.*, 2004; Treseder, 2004).

Regardless of CO_2 enrichment, our seasonal temperature sensitivities agree very well with those reported for (mixed) hardwood stands in northeastern USA, Europe and China ($Q_{10} = 3.3$–5.6; Davidson *et al.*, 1998; Borken *et al.*, 2002; Janssens & Pilegaard, 2003; Wang *et al.*, 2006) but are higher than the Q_{10} of 3.1 reported for hardwood forests on a global scale (Kicklighter *et al.*, 1994). In a mixed beech forest in France where climate and species composition was similar to that of our site, a Q_{10} of 3.9 was estimated (Epron *et al.*, 1999).

Fine root respiration

Respiration of fine roots and the rhizosphere may constitute 20%–90% to total R_s (Bowden *et al.*, 1993; Hanson *et al.*, 2000; Sulzman *et al.*, 2005), and represents the most temperature-sensitive contribution to the CO_2 released from soils (Boone *et al.*, 1998; Epron *et al.*, 2001). The CO_2 release from tree fine roots at our site (20–31 nmol CO_2 g^{-1} s^{-1}) was in the upper range of reported values (George *et al.*, 2003). Although statistically not significant, 33% higher fine root respiration in CO_2-enriched trees probably reflects the rapid flow of extra C through these trees to the soil. A CO_2-driven increase in fine root respiration was reported from the Duke-FACE experimental forest where elevated CO_2 stimulated annual stand-level root respiration in *Pinus taeda* by 34% (Drake *et al.*, 2008).

Soil microbial biomass

The availability of labile soil C often limits microbial metabolism, therefore, increased C availability due to elevated atmospheric CO_2 is expected to stimulate microbial growth and activity in soils (Hu *et al.*, 1999). In accordance with this view and with our hypothesis we observed greater soil microbial biomass at peak season under CO_2-enriched trees (C_{mic}: + 14%). This increment suggests either (i) increased flow of labile C to the soil or (ii) a stimulating soil moisture effect, where the CO_2-induced soil water savings may support a larger microbial community, or (iii) a combination of both factors. Indeed, we assumed increased C-flux to the soil under our CO_2-enriched trees through enhanced root exudation or mycorrhization simply because neither biomass above- or below-ground nor litter inputs increased in response to CO_2 enrichment and stable C isotope data suggest a slow turnover of fine roots (Körner *et al.*, 2005 and C. Körner, newer unpublished data; Asshoff *et al.*, 2006; Bader *et al.*, 2009).

We also observed rapid transfer of labelled C to mycorrhizal fungi (Keel *et al.*, 2006), whose abundance has been shown to increase by 47% across all biomes studied under elevated CO_2, inevitably increasing the flux of labile C to the soil (Treseder, 2004). However, the CO_2 efflux from the forest floor did not mirror the difference between treatments in soil microbial biomass indicating that the related increase in microbial respiration was too small to enhance R_s under CO_2-treated trees. Small contributions of microbial respiration to total R_s have been reported previously for forest soils (Johnson *et al.*, 1994).

Most studies also show increases in soil microbial biomass under elevated CO_2, but no changes and declines had been reported as well (Sadowsky & Schortemeyer, 1997; Hu *et al.*, 1999; Zak *et al.*, 2000). At the Duke-FACE and ORNL-FACE studies and also at the scrub oak open top chamber (OTC) system in Florida, elevated CO_2 did not increase soil microbial biomass (Allen *et al.*, 2000; Schortemeyer *et al.*, 2000; Sinsabaugh *et al.*, 2003). However, the combined biomass of bacteria, actinomycetes, and fungi in the soil contributes merely 1%–3% to the total soil C and N (Wardle, 1992). Therefore, changes in microbial biomass alone are unlikely to alter soil C storage noticeably, but the flow of substrates through microbial biomass is key to C sequestration and mineralization processes in soils (Zak *et al.*, 2000).

Conclusions

Our data imply that the extra C assimilated under elevated CO_2 in this stand is not rapidly respired back to the atmosphere via belowground metabolism. The present study illustrates how canopy CO_2 enrichment may stimulate or impede soil respiration in mature forest stands depending on the interactive effects of soil temperature and VWC, thus highlighting the significance of indirect CO_2 effects via soil moisture (cf. Morgan *et al.*, 2004). In finely textured forest soils with inherently high water availability, additional water savings resulting from reduced tree water consumption under elevated CO_2 (Leuzinger & Körner, 2007) may lead to excessive VWC, periodically limiting soil gas-exchange and thus soil respiration under CO_2-enriched trees. Although diminished soil diffusivity may not disrupt the long-term balance between soil CO_2 production and the CO_2 efflux at the soil surface, more frequent anaerobic soil conditions may stimulate trace gas emissions other than CO_2 such as N_2O and CH_4. More importantly, higher VWC may stimulate leaching of nutrients and particularly DOC and DIC. Higher leaching rates and diminished biodegradability of DOC from CO_2-enriched leaf litter indeed suggest that some of the extra C could enter the SOM pool by sorptive stabilization. A larger portion of the extra C may also leave the system through enhanced DIC leaching in the mineral soil as indicated by preliminary data suggesting increased soil acidity and mineral weathering in a CO_2-rich future.

Acknowledgements

We are grateful to Rolf Siegwolf and Matthias Saurer (Paul Scherrer Institute, Switzerland) for stable isotope analysis. Further, we thank Erwin Amstutz for on-site support, Debbie Coldwell and several helpers for field assistance, Michael Bahn and Jukka Pumpanen for methodological discussions and we greatly appreciate the statistical advice of Pascal Niklaus and Andy Hector and the constructive comments on earlier versions of the manuscript by Erika Hiltbrunner. The SCC FACE study was supported by the Swiss National Science Foundation (grant 3100AO-111914/1).

References

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. *Ecological Applications*, **10**, 437–448.

Amundson R (2001) The carbon budget in soils. *Annual Review of Earth and Planetary Sciences*, **29**, 535–562.

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 Science Society of America Journal*, **63**, 1429–1435.

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

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

Bahn M, Knapp M, Garajova Z, Pfahringer N, Cernusca A (2006) Root respiration in temperate mountain grasslands differing in land use. *Global Change Biology*, **12**, 995–1006.

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 S, Nordgren A, Lofvenius MO, Hogberg MN, Mellander PE, 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 and Environment*, **26**, 1287–1296.

Boone RD, Nadelhoffer KJ, Canary JD, Kaye JP (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, **396**, 570–572.

Borken W, Xu YJ, Davidson EA, Beese A (2002) Site and temporal variation of soil respiration in European beech, Norway spruce, and Scots pine forests. *Global Change Biology*, **8**, 1205–1216.

Bowden RD, Nadelhoffer KJ, Boone RD, Melillo JM, Garrison JB (1993) Contributions of aboveground litter, belowground litter and root respiration to total soil respiration in a temperate mixed hardwood forest. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **23**, 1402–1407.

Brookes PC (1985) The microbial biomass in soil. In: *Ecological Interactions in Soil. Special Publication of the British Ecological Society* (ed. Fitter AH), pp 123–125. Blackwell Science Publishing, Oxford.

Burton AJ, Pregitzer KS (2002) Measurement carbon dioxide concentration does not affect root respiration of nine tree species in the field. *Tree Physiology*, **22**, 67–72.

Cech PG, Pepin S, Körner C (2003) Elevated CO_2 reduces sap flux in mature deciduous forest trees. *Oecologia*, **137**, 258–268.

Daly E, Palmroth S, Stoy P *et al.* (2009) The effects of elevated atmospheric CO_2 and nitrogen amendments on subsurface CO_2 production and concentration dynamics in a maturing pine forest. *Biogeochemistry*, **94**, 271–287.

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. *Global Change Biology*, **4**, 217–227.

Davidson EA, Janssens IA, Luo YQ (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. *Global Change Biology*, **12**, 154–164.

Davidson EA, Savage K, Verchot LV, Navarro R (2002) Minimizing artifacts and biases in chamber-based measurements of soil respiration. *Agricultural and Forest Meteorology*, **113**, 21–37.

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.

Drake JE, Stoy PC, Jackson RB, Delucia EH (2008) Fine-root respiration in a loblolly pine (*Pinus taeda* L.) forest exposed to elevated CO_2 and N fertilization. *Plant, Cell and Environment*, **31**, 1663–1672.

Epron D, Farque L, Lucot E, Badot PM (1999) Soil CO_2 efflux in a beech forest: dependence on soil temperature and soil water content. *Annals of Forest Science*, **56**, 221–226.

Epron D, Le Dantec V, Dufrene E, Granier A (2001) Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest. *Tree Physiology*, **21**, 145–152.

Fang C, Moncrieff JB (2001) The dependence of soil CO_2 efflux on temperature. *Soil Biology & Biochemistry*, **33**, 155–165.

George K, Norby RJ, Hamilton JG, Delucia EH (2003) Fine-root respiration in a loblolly pine and sweetgum forest growing in elevated CO_2. *New Phytologist*, **160**, 511–522.

Goulden ML, Munger JW, Fan SM, Daube BC, Wofsy SC (1996) Exchange of carbon dioxide by a deciduous forest: response to interannual climate variability. *Science*, **271**, 1576–1578.

Granier A, Aubinet M, Epron D *et al.* (2003) Deciduous forests: carbon and water fluxes, balances and ecophysiological determinants. In: *Fluxes of Carbon Water and Energy of European Forests* (ed. Valentini R), pp 55–70. Springer, Berlin.

Hagedorn F, Machwitz M (2007) Controls on dissolved organic matter leaching from forest litter grown under elevated atmospheric CO_2. *Soil Biology & Biochemistry*, **39**, 1759–1769.

Hagedorn F, Van Hees PAW, Handa IT, Hättenschwiler S (2008) Elevated atmospheric CO_2 fuels leaching of old dissolved organic matter at the alpine treeline. *Global Biogeochemical Cycles*, **22**, GB2004.

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115–146.

Hättenschwiler S, Körner C (2000) Tree seedling responses to in situ CO_2-enrichment differ among species and depend on understorey light availability. *Global Change Biology*, **6**, 213–226.

Hesterberg R, Siegenthaler U (1991) Production and stable isotopic composition of CO_2 in a soil near Bern, Switzerland. *Tellus Series B-Chemical and Physical Meteorology*, **43**, 197–205.

Högberg P, Nordgren A, Buchmann N *et al.* (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789–792.

Hu SJ, Firestone MK, Chapin FS (1999) Soil microbial feedbacks to atmospheric CO_2 enrichment. *Trends in Ecology & Evolution*, **14**, 433–437.

Hui DF, Luo YQ (2004) Evaluation of soil CO_2 production and transport in Duke Forest using a process-based modeling approach. *Global Biogeochemical Cycles*, **18**, GB4029.

Hungate BA, Reichstein M, Dijkstra P *et al.* (2002) Evapotranspiration and soil water content in a scrub-oak woodland under carbon dioxide enrichment. *Global Change Biology*, **8**, 289–298.

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.

Janssens IA, Dore S, Epron D *et al.* (2003) Climatic influences on seasonal and spatial differences in soil CO_2 efflux. In: *Fluxes of Carbon Water and Energy of European Forests* (ed. Valentini R.), pp 235–253. Springer, Berlin.

Janssens IA, Lankreijer H, Matteucci G *et al.* (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, **7**, 269–278.

Janssens IA, Pilegaard K (2003) Large seasonal changes in Q_{10} of soil respiration in a beech forest. *Global Change Biology*, **9**, 911–918.

Johnson D, Geisinger D, Walker R, Newman J, Vose J, Elliot K, Ball T (1994) Soil pCO_2, soil respiration, and root activity in CO_2-fumigated and nitrogen-fertilized *Ponderosa pine*. *Plant and Soil*, **165**, 129–138.

Karberg NJ, Pregitzer KS, King JS, Friend AL, Wood JR (2005) Soil carbon dioxide partial pressure and dissolved inorganic carbonate chemistry under elevated carbon dioxide and ozone. *Oecologia*, **142**, 296–306.

Keel SG, Siegwolf RTW, Jaggi 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.

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.

Keeling CD (1958) The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. *Geochimica et Cosmochimica Acta*, **13**, 322–334.

Kicklighter DW, Melillo JM, Peterjohn WT, Rastetter EB, Mcguire AD, Steudler PA, Aber JD (1994) Aspect of spatial and temporal aggregation in estimating regional carbon-dioxide fluxes from temperate forest soils. *Journal of Geophysical Research-Atmospheres*, **99**, 1303–1315.

Kimball BA, Kobayashi K, Bindi M (2002) Responses of agricultural crops to free-air CO_2 enrichment. *Advances in Agronomy*, **77**, 293–368.

King JS, Hanson PJ, Bernhardt E, Deangelis 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 (2006) Plant CO_2 responses: an issue of definition, time and resource supply. *New Phytologist*, **172**, 393–411.

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

Körner C, Asshoff R, Bignucolo O *et al.* (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. *Science*, **309**, 1360–1362.

Lavigne MB, Ryan MG, Anderson DE *et al.* (1997) Comparing nocturnal eddy covariance measurements to estimates of ecosystem respiration made by scaling chamber measurements at six coniferous boreal sites. *Journal of Geophysical Research-Atmospheres*, **102**, 28977–28985.

Law BE, Baldocchi DD, Anthoni PM (1999) Below-canopy and soil CO_2 fluxes in a ponderosa pine forest. *Agricultural and Forest Meteorology*, **94**, 171–188.

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

Linn DM, Doran JW (1984) Effect of water-filled pore-space on carbon-dioxide and nitrous-oxide production in tilled and nontilled soils. *Soil Science Society of America Journal*, **48**, 1267–1272.

Lipp CC, Andersen CP (2003) Role of carbohydrate supply in white and brown root respiration of ponderosa pine. *New Phytologist*, **160**, 523–531.

Liu LL, King JS, Booker FL, Giardina CP, Allen HL, Hu SJ (2009) Enhanced litter input rather than changes in litter chemistry drive soil carbon and nitrogen cycles under elevated CO_2: a microcosm study. *Global Change Biology*, **15**, 441–453.

Lloyd J, Taylor JA (1994) On the temperature-dependence of soil respiration. *Functional Ecology*, **8**, 315–323.

Luo YQ, Jackson RB, Field CB, Mooney HA (1996) Elevated CO_2 increases belowground respiration in California grasslands. *Oecologia*, **108**, 130–137.

Malhi Y, Baldocchi DD, Jarvis PG (1999) The carbon balance of tropical, temperate and boreal forests. *Plant, Cell and Environment*, **22**, 715–740.

Morgan JA, Pataki DE, Korner C *et al.* (2004) Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. *Oecologia*, **140**, 11–25.

Palmorth S, Maier CA, McCarthy HR *et al.* (2005) Contrasting responses to drought of forest floor CO_2 efflux in a Loblolly pine plantation and a nearby Oak–Hickory forest. *Global Change Biology*, **11**, 421–434.

Pendall E, Bridgham S, Hanson PJ *et al.* (2004) Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. *New Phytologist*, **162**, 311–322.

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.

Pregitzer K, Loya W, Kubiske M, Zak D (2006) Soil respiration in northern forests exposed to elevated atmospheric carbon dioxide and ozone. *Oecologia*, **148**, 503–516.

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 Phytologist*, **180**, 153–161.

Pumpanen J, Kolari P, Ilvesniemi H *et al.* (2004) Comparison of different chamber techniques for measuring soil CO_2 efflux. *Agricultural and Forest Meteorology*, **123**, 159–176.

R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Raich JW, Nadelhoffer KJ (1989) Belowground carbon allocation in forest ecosystems – global trends. *Ecology*, **70**, 1346–1354.

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

Raich JW, Potter CS, Bhagawati D (2002) Interannual variability in global soil respiration, 1980–94. *Global Change Biology*, **8**, 800–812.

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

Sadowsky MJ, Schortemeyer M (1997) Soil microbial responses to increased concentrations of atmospheric CO_2. *Global Change Biology*, **3**, 217–224.

Schlesinger WH, Andrews JA (2000) Soil respiration and the global carbon cycle. *Biogeochemistry*, **48**, 7–20.

Schortemeyer M, Dijkstra P, Johnson DW, Drake BG (2000) Effects of elevated atmospheric CO_2 concentration on C and N pools and rhizosphere processes in a Florida scrub oak community. *Global Change Biology*, **6**, 383–391.

Sinsabaugh RL, Saiya-Corka K, Long T, Osgood MP, Neher DA, Zak DR, Norby RJ (2003) Soil microbial activity in a *Liquidambar* plantation unresponsive to CO_2-driven increases in primary production. *Applied Soil Ecology*, **24**, 263–271.

Skopp J, Jawson MD, Doran JW (1990) Steady-state aerobic microbial activity as a function of soil-water content. *Soil Science Society of America Journal*, **54**, 1619–1625.

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

Subke JA, Inglima I, Cotrufo MF (2006) Trends and methodological impacts in soil CO_2 efflux partitioning: a metaanalytical review. *Global Change Biology*, **12**, 921–943.

Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. *Biogeochemistry*, **73**, 231–256.

Suwa M, Katul GG, Oren R, Andrews J, Pippen J, Mace A, Schlesinger WH (2004) Impact of elevated atmospheric CO_2 on forest floor respiration in a temperate pine forest. *Global Biogeochemical Cycles*, **18**, GB2013.

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist*, **164**, 347–355.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry*, **19**, 703–707.

Vincent G, Shahriari AR, Lucot E, Badot PM, Epron D (2006) Spatial and seasonal variations in soil respiration in a temperate deciduous forest with fluctuating water table. *Soil Biology & Biochemistry*, **38**, 2527–2535.

Wang CK, Yang JY, Zhang QZ (2006) Soil respiration in six temperate forests in China. *Global Change Biology*, **12**, 2103–2114.

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological Reviews of the Cambridge Philosophical Society*, **67**, 321–358.

Widén B, Majdi H (2001) Soil CO_2 efflux and root respiration at three sites in a mixed pine and spruce forest: seasonal and diurnal variation. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **31**, 786–796.

Wofsy SC, Goulden ML, Munger JW *et al.* (1993) Net exchange of CO_2 in a midlatitude forest. *Science*, **260**, 1314–1317.

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation extraction – an automated procedure. *Soil Biology & Biochemistry*, **22**, 1167–1169.

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 Phytologist*, **147**, 201–222.

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

Chapter 4

Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment

Planta
DOI 10.1007/s00425-010-1240-8

ORIGINAL ARTICLE

Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment

Martin Karl-Friedrich Bader · Rolf Siegwolf · Christian Körner

Received: 12 June 2010 / Accepted: 22 July 2010

Abstract Carbon uptake by forests constitutes half of the planet's terrestrial net primary production; therefore, photosynthetic responses of trees to rising atmospheric CO_2 are critical to understanding the future global carbon cycle. At the Swiss Canopy Crane, we investigated gas exchange characteristics and leaf traits in five deciduous tree species during their eighth growing season under free air carbon dioxide enrichment in a 35-m tall, ca. 100-year-old mixed forest. Net photosynthesis of upper-canopy foliage was 48% (July) and 42% (September) higher in CO_2-enriched trees and showed no sign of down-regulation. Elevated CO_2 had no effect on carboxylation efficiency (V_{cmax}) or maximal electron transport (J_{max}) driving ribulose-1, 5-bisphosphate (RuBP) regeneration. CO_2 enrichment improved nitrogen use efficiency, but did not affect leaf nitrogen (N) concentration, leaf thickness or specific leaf area except for one species. Non-structural carbohydrates accumulated more strongly in leaves grown under elevated CO_2 (largely driven by *Quercus*). Because leaf area index did not change, the CO_2-driven stimulation of photosynthesis in these trees may persist in the upper canopy under future atmospheric CO_2 concentrations without reductions in photosynthetic capacity. However, given the lack of growth stimulation, the fate of the additionally assimilated carbon remains uncertain.

Keywords Elevated CO_2 · Global change · Photosynthetic acclimation · Swiss Canopy Crane

Abbreviations

A_{growth}	Light-saturated net photosynthesis measured at growth CO_2 concentration (ambient CO_2: A^a_{growth}; elevated CO_2: A^e_{growth})
A_{550}	Light-saturated net photosynthesis measured at 550 ppm leaf chamber CO_2 concentration
A_{380}	Light-saturated net photosynthesis measured at 380 ppm leaf chamber CO_2 concentration
C	Carbon
E	$A^e_{growth}/A^a_{growth}$
E'	A_{550}/A_{380}
FACE	Free air carbon dioxide enrichment
J_{max}	Maximal photosynthetic electron transport rate (a proxy for ribulose-1,5-bisphosphate regeneration)
LAI	Leaf area index
N	Nitrogen
PPFD	Photosynthetic photon flux density
SLA	Specific leaf area
NSC	Non-structural carbohydrates
SCC	Swiss canopy crane
SE	Standard error of the mean
V_{cmax}	Maximal carboxylation rate of Rubisco
ALVPD	Air-to-leaf vapour pressure deficit

Introduction

Every year, the burning of fossil fuels and dramatic changes in land use feed vast amounts of CO_2 to the

---

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

R. Siegwolf
Paul Scherrer Institute, 5323 Villigen PSI, Switzerland

atmosphere (Le Quéré et al. 2009). The uptake of CO_2 from the atmosphere through photosynthesis and its recycling through respiratory processes represent the largest fluxes in the global carbon (C) cycle (Schimel 1995; Sabine et al. 2004). If the C assimilated by plants is not completely recycled and some remains stored in the biosphere for prolonged periods, this could mitigate atmospheric CO_2 enrichment. The knowledge of the long-term response of photosynthesis to elevated atmospheric CO_2 is key to understanding such future ecosystem responses. Under the current atmospheric CO_2 concentration that exceeds the pre-industrial level by nearly 40%, photosynthesis in C_3-plants is still CO_2 limited (Farquhar et al. 1980; Tans 2008). Hence, photosynthetic rates increase in response to elevated CO_2, because the increased substrate availability stimulates Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) carboxylation whilst competitively inhibiting the oxygenation process (Drake et al. 1997; Ainsworth and Rogers 2007). In trees that harbour ~90% of the terrestrial biomass carbon (Roy et al. 2001) and account for 50% of the terrestrial net primary production (Bonan 2008), elevated CO_2 has been reported to increase leaf photosynthesis by 30–60%, regardless of tree age (Gunderson and Wullschleger 1994; Curtis and Wang 1998; Saxe et al. 1998; Medlyn et al. 1999; Norby et al. 1999; Nowak et al. 2004; Ainsworth and Long 2005). The magnitude of this stimulation is species dependent and modulated by environmental factors such as light, temperature, soil water and nutrient supply (Curtis and Wang 1998; Nowak et al. 2004).

Sustained photosynthetic stimulation is one of the prerequisites for growth stimulation under elevated CO_2. However, there is no direct translation of CO_2 uptake per unit leaf area into plant growth (Körner 2006). In fact, long-term exposure to elevated CO_2 may affect morphological, biochemical and physiological plant properties that feed back to both photosynthesis and net carbon incorporation into the plant body (Gunderson and Wullschleger 1994; Egli et al. 2001). Photosynthetic acclimation to elevated CO_2 commonly occurs at the biochemical level through decreases in Rubisco carboxylation (V_{cmax}) resulting from reductions in Rubisco concentration and, less importantly, through declines in the maximal electron transport rate (J_{max}; Drake et al. 1997; Moore et al. 1999; Stitt and Krapp 1999; Ellsworth et al. 2004; Ainsworth and Rogers 2007). Imbalances in sink–source relations due to reduced or insufficient sink capacity often result from nutrient limitations (e.g. growth at low fertility sites or progressive nitrogen limitation) and lead to accumulation of photosynthates in leaves (Körner and Miglietta 1994). This sugar signal triggers a response mechanism that targets the small subunit of Rubisco and eventually leads to selective down-regulation of Rubisco (Rogers and Ellsworth 2002; Long et al. 2004; Ainsworth and Rogers 2007). Non-selective down-regulation on the other hand emerges from a loss of total leaf N or the dilution of leaf N by non-structural carbohydrates (NSC), which indirectly affect Rubisco concentration (Ellsworth et al. 2004). Early observations of photosynthetic down-regulation could be largely attributed to experimental constraints such as pot size (restricted rooting volume; Drake et al. 1997). However, also in field experiments, where the spatial constraints on the root system had been overcome, down-regulation of photosynthesis occurred in CO_2-enriched trees, resulting mainly from reduced carboxylation capacity due to leaf N dilution (on mass and area basis) by NSC accumulation, i.e. from a non-selective, indirect effect on Rubisco (Medlyn et al. 1999; Ellsworth et al. 2004). When photosynthetic down-regulation occurs, the stimulative effect of elevated CO_2 is diminished but not completely eliminated, and photosynthesis is still enhanced under elevated CO_2 even after several years of enrichment (Saxe et al. 1998; Crous et al. 2008). In a meta-analysis comprising CO_2 fertilisation experiments other than free air carbon dioxide enrichment (FACE), photosynthesis of (mostly young) European forest trees declined over time by 10–20% under elevated CO_2, but was still stimulated by 51% relative to control trees (350 vs. 700 ppm CO_2; Medlyn et al. 1999). In FACE experiments, down-regulation of photosynthetic capacity was observed in the aggrading Aspen-FACE stands; however, this was a transitory effect that disappeared after steady-state LAI had been reached (Ellsworth et al. 2004; Uddling et al. 2009). A strong sugar-mediated, selective down-regulation of Rubisco occurred in old needles of mature *Pinus taeda* growing in an N-limited steady-state system at the Duke-FACE facility (Rogers and Ellsworth 2002; Crous et al. 2008).

Despite the bulk of literature on the effects of elevated CO_2 on photosynthesis in trees, very few studies have addressed the responses of mature dominant trees that have reached steady-state canopy development (constant leaf area index). The FACE experiment at the Swiss Canopy Crane (SCC) is the only study worldwide where several species of hardwood trees growing in a near-natural, mature mixed forest were exposed to elevated atmospheric CO_2. We evaluated the long-term CO_2 response of photosynthesis and associated leaf traits in five tree species during the 8th year of canopy CO_2 enrichment. Our objective was to assess the magnitude of photosynthetic enhancement and potential down-regulation under elevated CO_2. Gas exchange, biochemical and morphological leaf parameters were measured in sunlit foliage during the mid and late growing season to account for seasonal sink–source dynamics. Given the lack of persistent growth stimulation above and below ground in the CO_2-enriched trees of this stand (Körner et al. 2005; Asshoff et al. 2006;

Bader et al. 2009), we anticipated that reduced sink capacity would feed back to photosynthesis, thereby partially offsetting the CO_2-induced stimulation of leaf-level C uptake. Such a decline in photosynthetic enhancement would involve: (1) a reduction of the maximal rate of leaf photosynthesis; (2) a decline in V_{cmax} and/or J_{max}; (3) increased foliar non-structural carbohydrate (NSC) concentration; (4) diminished leaf nitrogen concentration (mass based); and (5) reduced specific leaf area (SLA).

Materials and methods

Study site

The Swiss Canopy Crane (SCC) facility is located in a species-rich forest 15 km south of Basel, Switzerland (47°28′N, 7°30′E, 550 m a.s.l.). The ca. 100-year-old stand grows on a gentle NNW-exposed slope and reaches canopy heights from 30 to 35 m. The leaf area index (LAI) is around 5, tree density is 415 trees ha^{-1} (breast height diameter $\geq$0.1 m) and stem basal area amounts to 46 m^2 ha^{-1}. The forest is dominated by *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl. and *Carpinus betulus* L., accompanied by less abundant tree species such as *Tilia platyphyllos* Scop., *Acer campestre* L., *Prunus avium* L. and four species of conifers (*Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.). The soil type is a Rendzic Leptosol (WRB) (Rendzina, FAO; Lithic Rendoll, USDA) with an accessible profile depth of maximal 25 cm followed by rocky subsoil merging into the calcareous bedrock at depths of 40–90 cm. The soil texture was classified as loamy clay with a pH of 5.8 in the top 10 cm of the profile.

The climate in this temperate region is distinguished by mild winters and moderately warm summers with mean air temperatures in January and July of 2 and 19°C, respectively. Long-term mean annual precipitation in the study region is 990 mm. Approximately two-thirds of the yearly precipitation falls during the 6-month growing season (Pepin and Körner 2002).

Free air CO_2-enrichment system

Future CO_2 levels in the tree canopies were established by applying a novel free air CO_2-enrichment (FACE) technique called web-FACE (Pepin and Körner 2002). Briefly, pure CO_2 was pulse released through a fine web of perforated tubes plaited into tree crowns with the help of a construction crane. Canopy CO_2 supply was governed via computer-controlled magnetic valves to maintain the target CO_2 concentration of 550 ppm as accurately as possible. CO_2 concentration in the canopy served as the main control signal and was monitored by an air sampling system consisting of multiple suction heads per tree feeding canopy air through sampling lines into infrared gas analysers. CO_2 release was confined to daylight hours (photosynthetic photon flux density, PPFD > 100 µmol m^{-2} s^{-1}) of the growing season and was disengaged from the time of leaf shedding to bud break (end of October to mid-April). Twelve deciduous trees growing in the 60 m operating range of the crane were selected for CO_2 enrichment (three *Fagus sylvatica*, three *Quercus petraea*, three *Carpinus betulus*, one *Tilia platyphyllos*, one *Acer campestre,* one *Prunus avium*) and received elevated atmospheric CO_2 since autumn 2000. In 2006, the individual *Prunus avium* tree suffered from storm damage and was therefore excluded from the CO_2 enrichment. An adequate number of control trees were accessible in the remaining crane area at sufficient distance to the CO_2-enriched zone.

Leaf gas exchange measurements

In early July and mid-September of 2008, instantaneous rates of CO_2/H_2O gas exchange were measured with two identical portable photosynthesis systems (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) on 25 trees of five species. Light-saturated net photosynthetic rates (PPFD = 1,200 µmol m^{-2} s^{-1}, LI-COR 6400-02 LED light unit) were determined between 8:30 and 12:30 h at ambient and elevated CO_2 concentrations (i.e. at 380 and 550 ppm) at 25°C leaf temperature and air-to-leaf vapour pressure deficit (ALVPD) of 1.18 ± 0.01 and 1.47 ± 0.02 kPa (means ± SE) in June and September, respectively. Measurements were taken on eight mature leaves per tree from different sunlit branches. Leaves that initially showed low stomatal conductance <70 mmol m^{-2} s^{-1} were replaced by leaves exceeding this threshold. By means of the adjustable leaf chamber CO_2 supply, gas exchange was first measured at 380 ppm CO_2 and subsequently, on the same leaf, at 550 ppm CO_2, designated hereafter as A_{380} and A_{550}, respectively. The light-saturated net photosynthesis measured at growth CO_2 concentration is termed A_{growth}. Recordings were taken as soon as the net rate of photosynthesis and stomatal conductance (g_s) remained constant. Individual measurements did not exceed 5 min. The instantaneous photosynthetic enhancement ratio (E') was calculated as the leaf-intrinsic ratio of A_{550}/A_{380} for any tree in ambient and elevated CO_2. The enhancement ratio that compares A_{growth} of CO_2-enriched trees (A^{e}_{growth}) with A_{growth} of control trees growing under ambient conditions (A^{a}_{growth}) is denoted by E. We calculated E using weighted species means to account for the varying number of trees available in each species (less weight assigned to *Acer* and *Tilia* that occurred with only one tree individual in each CO_2 treatment). Both photosynthetic enhancement ratios, E' and E, were expressed as percentages (A_{550}/A_{380} or $A^{\mathrm{e}}_{\mathrm{growth}}/A^{\mathrm{a}}_{\mathrm{growth}} - 1 \times 100\%$). In addition, A/C_{i} curves of the replicated species (*F. sylvatica*, *Q. petraea*, *C. betulus*) were recorded on two to four leaves per tree. Gas exchange rates were first recorded at a leaf chamber CO_2 concentration (C_{a}) of 400 ppm CO_2, before C_{a} was stepwise reduced to 300, 200, 100 and 40 ppm, subsequently C_{a} was returned to 400 ppm (to check if the initial rate could be restored) and then increased to 600, 800, 1,000, 1,500 and 2,000 ppm. Individual response curves were completed within 25–35 min. A/C_{i} curves were analysed using a Farquhar-type photosynthesis model (Long and Bernacchi 2003). Nonlinear least squares regression was applied to estimate V_{cmax}, the maximal carboxylation rate of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), J_{max}, the maximal rate of electron transport, and photosynthetic limitation due to triose-phosphate utilisation (TPU-limitation).

Leaf properties

At peak season (July 2008), we also measured leaf thickness (SM 112, Teclock, Nagano, Japan, precision 0.01 mm) and foliar chlorophyll content non-destructively (CCM-200, Opti-Sciences, Tyngsboro, MA, USA) on 30 leaves per tree canopy. A different set of ten leaf samples per tree was collected with a leaf puncher for determination of SLA, leaf N and NSC concentrations. Immediately after sampling, leaf discs (1.2 cm^2) were dried at 80° C for at least 48 h and then weighed for biomass quantification. Then, samples were ground and all samples from one tree were pooled for determination of leaf N concentration (mg N g^{-1} dry mass) using a CHN-analyzer (Vario EL *III*, Elementar Analysensysteme GmbH, Hanau, Germany). Non-structural carbohydrates (NSC = starch, sucrose, glucose and fructose) were analysed applying an enzymatic starch digestion followed by a spectrophotometric glucose test after invertase and isomerase addition (Körner and Miglietta 1994).

Statistical analysis

Gas exchange parameters were analysed using a linear mixed-effects model. We fitted the model using restricted maximum likelihood (REML) and included the fixed factors ‘species’, ‘FACE-treatment’ and ‘leaf chamber CO_2’. ‘Leaf chamber CO_2’ was also nested within the random factor ‘tree individual’. The photosynthetic enhancement ratios E and E' were analysed in three-way repeated measures ANOVAs with the fixed factors ‘FACE-treatment’, ‘species’ and ‘date’. CO_2 effects on leaf properties were analysed with two-way ANOVAs with the fixed factors ‘species’ and ‘FACE-treatment’. All statistical analyses and graphics were performed using R version 2.9.0 (http://www.r-project.org).

Results

Leaf gas exchange

In the early and late growing season during year 8 of CO_2 enrichment, the instantaneous enhancement of light-saturated net photosynthesis by elevated CO_2 ($E' = A_{550}/A_{380}$) was similar in trees growing under ambient and elevated

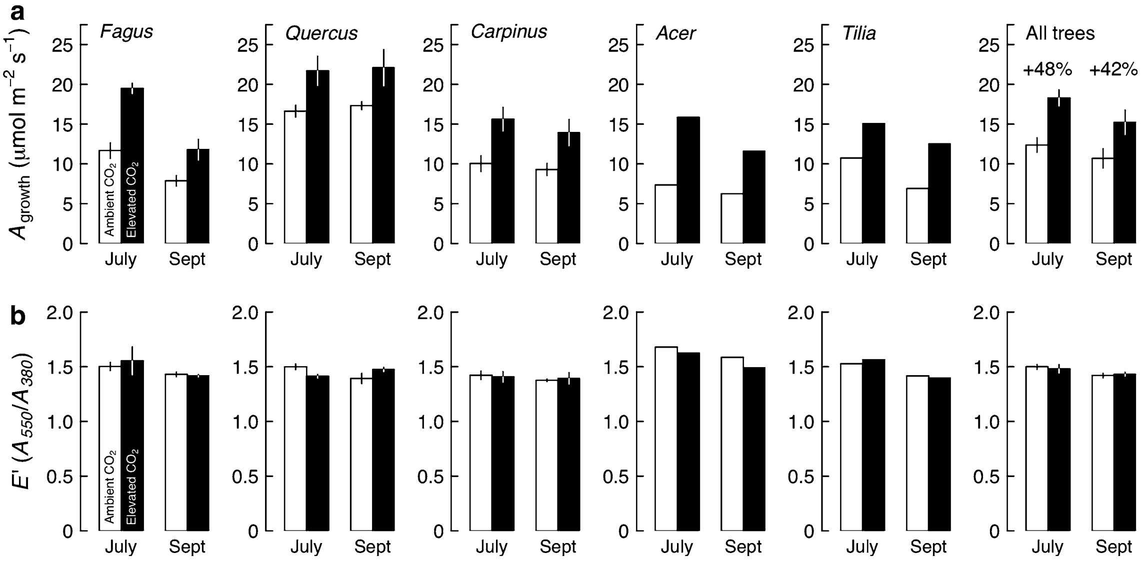


Fig. 1 CO_2-induced enhancement of light-saturated leaf photosynthesis of five deciduous tree species measured in the mid and late growing season of 2008 at the SCC FACE site, Switzerland. Trees were growing in a near-natural stand under ambient (*white*) or elevated CO_2 (*black*). **a** Light-saturated net photosynthesis measured at growth CO_2 concentration, A_{growth}. **b** Instantaneous leaf-intrinsic photosynthetic enhancement, $E' = A_{550}/A_{380}$. Means ± SE, $n = 11$ (elevated CO_2), $n = 14$ (ambient CO_2)

Table 1 Results of a three-way ANOVA on the effects of species identity, FACE and sampling date (peak and late growing season) on the instantaneous leaf-intrinsic photosynthetic enhancement (E') involved with a switch in leaf chamber CO_2 from 380 to 550 ppm

Factor	*Df*	*F*	*P*
Species	4, 15	4.380	<0.010**
FACE	1, 15	0.005	0.947
Date	1, 15	8.030	<0.010**
Species × FACE	4, 15	0.177	0.948
Species × date	4, 15	0.535	0.710
FACE × date	1, 15	0.603	0.444
Species × FACE × date	4, 15	1.032	0.407

Df degrees of freedom (numerator, denominator); *F F*-value

** $P < 0.01$

Table 2 Linear mixed-effects model results on the effects of species identity, FACE and leaf chamber CO_2 on photosynthetic capacity of five deciduous tree species growing under ambient and elevated CO_2 (FACE = CO_2 effect, CO_2-LC = leaf chamber CO_2 concentration)

Factor	*Df*	*F*	*P*
Species	4, 15	17.838	<0.001***
Date	1, 781	190.947	<0.001***
FACE	1, 15	0.008	0.929
CO_2-LC	1, 19	1045.290	<0.001***
Species × FACE	4, 15	0.833	0.525
Species × date	4, 781	44.540	<0.001***
Species × CO_2-LC	4, 19	1.281	0.312
FACE × CO_2-LC	1, 19	0.047	0.830
FACE × date	1, 781	0.398	0.528
Date × CO_2-LC	1, 781	5.421	0.020*
Species × FACE × date	4, 781	3.865	0.004**

Df degrees of freedom (numerator, denominator); *F F*-value

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

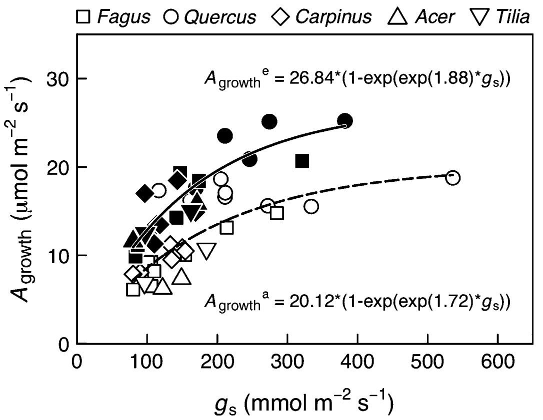


Fig. 2 Net photosynthesis under growth CO_2 concentration (A_{growth}) as a function of stomatal conductance (g_s) in ambient (*white*) and CO_2-enriched foliage (*black*) of five mature deciduous tree species, measured in the peak and late growing season of 2008. Means ± SE, $n = 1$–5 per species and treatment

CO_2 (Fig. 1; Table 1). Averaged across all trees, the short-term switch in CO_2 supply to the leaf chamber from 380 to 550 ppm led to a photosynthetic enhancement of 49% in early July and declined significantly to 42% in September (Fig. 1; Table 2). As a consequence of this strong CO_2 stimulation, the rate of light-saturated net photosynthesis measured at growth CO_2 concentration (A_{growth}) was significantly higher in CO_2-enriched trees compared to ambient controls ($E = A^{e}_{growth}/A^{a}_{growth}$) reaching 48% in July and 42% in September (Fig. 1). There was significant variation amongst the study species with *Quercus petraea* showing the highest rates of net photosynthesis under both treatments and at both measured leaf chamber CO_2 levels, whilst the lowest rates were seen in *Acer campestre* (Table 2). The significance of the species × FACE × date interaction (indicating species-specific down-regulation over the growing season, Table 2) was caused by the single *Tilia* tree and when this unreplicated species was disregarded in the model the interaction term lost its significance. From the early to the late growing season, the average A_{growth} declined significantly by 15–17% under ambient (12.4 vs. 10.7 µmol m^{-2} s^{-1}) and elevated CO_2 (18.3 vs. 15.2 µmol m^{-2} s^{-1}, three-way ANOVA, $P < 0.001$). Stomatal conductance declined towards the end of the growing season by 41 and 33% in ambient and elevated CO_2 (three-way ANOVA, $P < 0.001$). Comparing foliage grown and measured under ambient CO_2 with foliage grown and measured under elevated CO_2 yielded similar stomatal conductance (g_s), but 6 and 9% lower transpiration rates in July and September, respectively. However, these differences in transpiration were statistically not significant (three-way repeated measures ANOVA, $P > 0.2$). Rates of A_{growth} and g_s were tightly coupled, and A^{e}_{growth} was always higher than A^{a}_{growth} at a common g_s (Fig. 2). Since leaf N in CO_2-enriched trees was not reduced (see later), photosynthetic net carbon uptake per unit leaf nitrogen (nitrogen-use efficiency, PNUE) was significantly higher compared to control trees (Table 4).

The carboxylation efficiency of Rubisco (V_{cmax}) and the maximal rate of electron transport leading to RubP regeneration (J_{max}) showed significant inter-specific variation, but CO_2 enrichment as main effect had no significant influence. The significant species × FACE × date interaction for V_{cmax} resulted mainly from increases in V_{cmax} in *Quercus* control trees towards the end of the growing season, rather than from a late-seasonal down-regulation in CO_2-enriched trees. In a number of leaves, A/C_i curves also showed that photosynthesis became limited by triose-phosphate utilisation (5.3–12.6 µmol m^{-2} s^{-1}) when C_i exceeded ~750 ppm.

Across species and treatments, V_{cmax} and J_{max} had significantly declined by 11 and 8% over the growing season

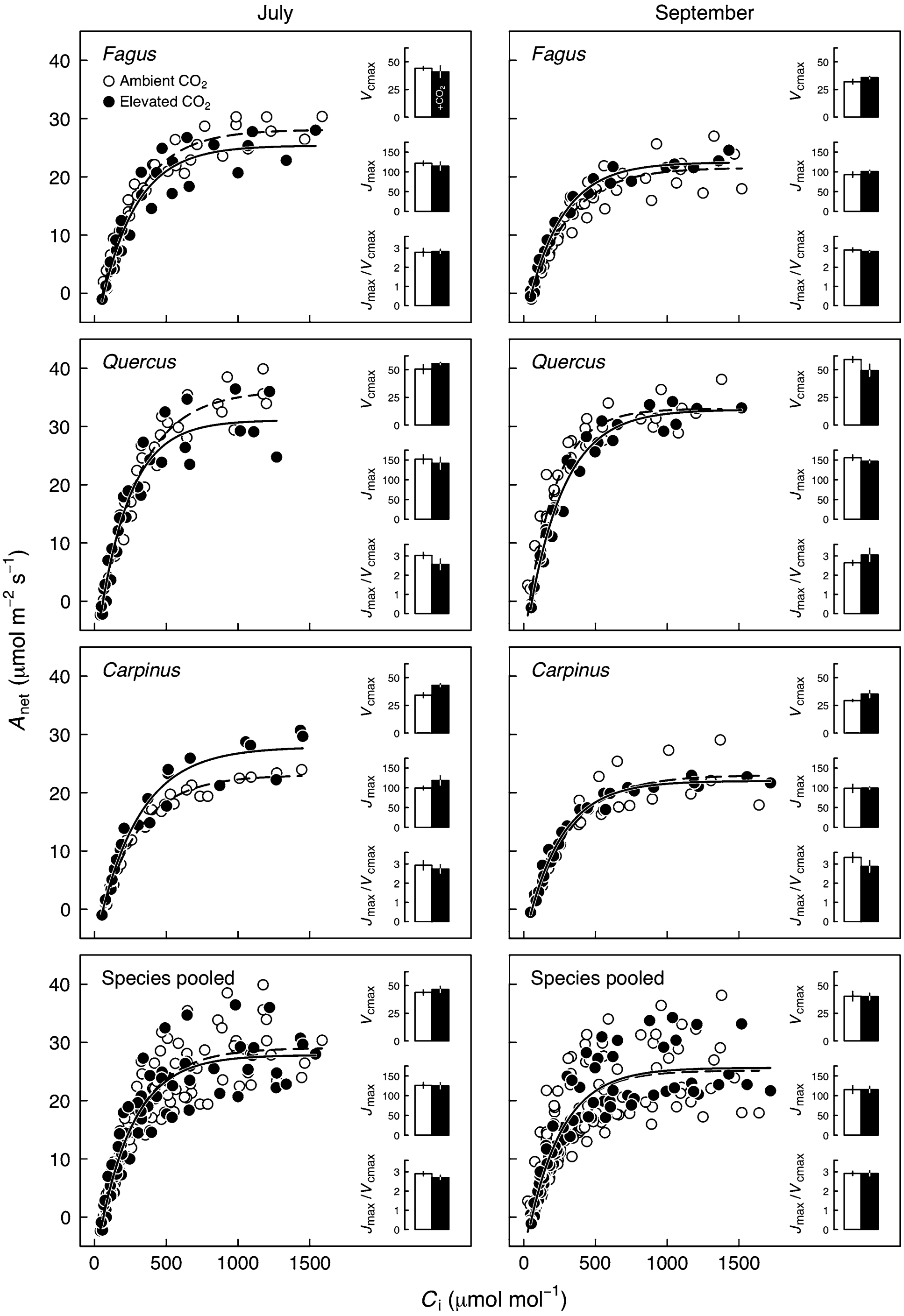


Fig. 3 The seasonal response of light-saturated net photosynthesis (A_{net}) to intercellular CO_2 concentration (C_i) in upper-canopy leaves of mature individuals of the three dominant deciduous tree species growing under ambient (*white*, *dashed lines*) and elevated CO_2 (*black*, *solid lines*) in a near-natural stand at the SCC FACE site, Switzerland. The bar chart inserts give the maximum rate of Rubisco carboxylation (V_{cmax}), the maximum electron transport rate (J_{max}) and the J_{max}/V_{cmax} ratio derived from a Farquhar-type photosynthesis model. The response curves shown in the graph were fitted using nonlinear least squares regression based on the equation used by Herrick and Thomas (2001): $A = A_{max}[1 - (1 - \alpha/A_{max})(1 - C_i/\Gamma)]$, where $A_{max} = A_{net}$ at CO_2 saturation, $\alpha = y$-intercept and $\Gamma = CO_2$ compensation point. Means ± SE, $n = 3$–5 per species and treatment

(Fig. 3; Table 3). We observed a common positive linear relationship between J_{max} and V_{cmax}, which was similar in leaves grown under ambient and elevated CO_2 and did not change with progressing growing season (F test, $F = 0.232$, $P = 0.963$, Fig. 4). Neither V_{cmax} nor J_{max} was significantly correlated with leaf N on an area basis (N m^{-2}, data not shown).

Leaf traits

In year 8 of the FACE study, all measured leaf traits showed significant inter-specific variation, but CO_2 enrichment as main effect had no significant influence (Table 4). However, across all trees, CO_2 exposure tended to increase leaf non-structural carbohydrates (NSC,

Table 3 Results of a three-way ANOVA on the effects of species identity, FACE and sampling date (peak and late growing season) on the maximum rate of Rubisco carboxylation (V_{cmax}), maximum electron transport rate (J_{max}) and the ratio J_{max}/V_{cmax} in the three replicated tree species (*Fagus sylvatica*, *Quercus petraea*, *Carpinus betulus*)

Factor	*Df*	*F*	*P*
V_{cmax}			
Species	2, 16	45.976	<0.001***
FACE	1, 16	0.712	0.405
Date	1, 15	7.889	0.009**
Species × FACE	2, 16	2.792	0.078
Species × date	2, 15	4.712	0.016*
FACE × date	1, 15	1.012	0.322
Species × FACE × date	2, 15	3.606	0.039*
J_{max}			
Species	2, 16	37.534	<0.001***
FACE	1, 16	0.003	0.954
Date	1, 15	4.570	0.041*
Species × FACE	2, 16	1.272	0.295
Species × date	2, 15	2.888	0.071
FACE × date	1, 15	0.003	0.961
Species × FACE × date	2, 15	0.977	0.388
J_{max}/V_{cmax}			
Species	2, 16	0.606	0.552
FACE	1, 16	0.948	0.338
Date	1, 15	0.788	0.382
Species × FACE	2, 16	0.693	0.508
Species × date	2, 15	0.464	0.633
FACE × date	1, 15	0.527	0.474
Species × FACE × date	2, 15	2.294	0.118

Df degrees of freedom (numerator, denominator); *F* *F*-value

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

+15%), which was largely driven by *Quercus* that showed 36% increase in leaf NSC under elevated CO_2 (Table 4). There was also a trend towards a decline in specific leaf area (SLA) under elevated CO_2, which disappeared when the two unreplicated species were excluded from the statistical analysis or when SLA was expressed on an NSC-free basis (Table 4). We found species-dependent CO_2 effects on leaf chlorophyll content and leaf N (significant species × treatment interaction). CO_2-enriched *Quercus* trees showed 20% less foliar chlorophyll than conspecific control trees, whilst *Fagus* leaves exhibited ca. 30% higher chlorophyll contents under elevated CO_2. The significant species × treatment interaction with leaf N (on an area basis) resulted solely from the decline seen in *Acer* (−28%) and *Tilia* (−14%). When these two unreplicated species were excluded from the analysis, the interaction term lost significance (Table 4).

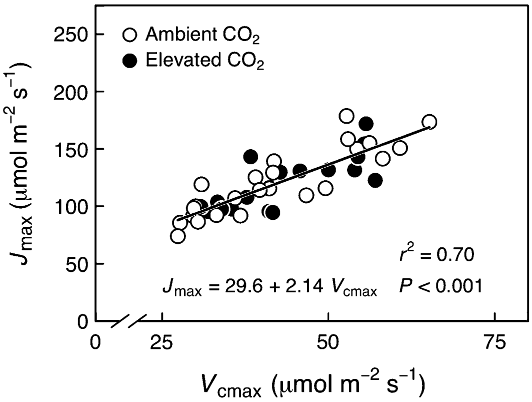


Fig. 4 Correlation between the maximum rate of electron transport (J_{max}) driving RubP regeneration and the maximum rate of Rubisco carboxylation (V_{cmax}) of mature leaves in the outer canopy of the three dominant deciduous tree species at the SCC site [*Fagus sylvatica*, *Quercus petraea*, and *Carpinus betulus*, means ± SE, $n = 9$ (elevated CO_2), $n = 12$ (ambient CO_2)]. Treatment- and date-wise regression was statistically not different from the regression analysis applied to the combined data from ambient and elevated CO_2 in July and September; therefore the pooled data set is shown

Discussion

Leaf gas exchange

The Swiss Canopy Crane gave us the unique possibility to reveal the long-term effects of CO_2 enrichment on photosynthesis in mature, 30–35 m tall deciduous forest trees. In the canopy of the mixed forest stand at the SCC site, net photosynthetic rates of sunlit foliage from mature trees that had been grown under future atmospheric CO_2 for 8 years were consistently higher compared with rates in leaves of control trees ($E = 48\%$ in July and 42% in September, Fig. 1). In the same stand, Zotz et al. (2005) found 36 and 49% photosynthetic enhancement (E) in the mid and late growing season, respectively, after 3 years of CO_2 enrichment (E given here is corrected for the loss of one CO_2-exposed tree in 2006 and was recalculated with the original data using weighted species means). Thus, the magnitude of photosynthetic enhancement at the SCC forest did not change over the study years and agrees well with the numbers reported from other multi-year FACE experiments performed with trees (Herrick and Thomas 2001, *Liquidambar styraciflua* in the understory at Duke-FACE: +63%; Sholtis et al. 2004, *L. styraciflua* at ORNL-FACE: +44%; Liberloo et al. 2007, three *Populus* species at POP-FACE: +49%; Ainsworth and Rogers 2007, review: +46% in trees, Crous et al. 2008, *Pinus taeda* at Duke-FACE: +68% in current year needles, +40% in 1-year-old needles). At the Aspen-FACE site, *Populus tremuloides* clones initially showed somewhat lower stimulation especially under $CO_2 + O_3$ conditions

Table 4 Traits of fully expanded foliage of five forest tree species (*Fagus sylvatica, Quercus petraea, Carpinus betulus, Acer campestre, Tilia platyphyllos*)

Species	*Fagus*		*Quercus*		*Carpinus*		*Acer*		*Tilia*		*P*	FACE	Species × FACE
Treatment	a	e	a	e	a	e	a	e	a	e	Species		
NSC	14.2 ± 0.8	15.7 ± 1.2	12.9 ± 1.5	17.5 ± 0.4	17.8 ± 1.0	17.3 ± 1.1	12.7	8.7	10.2	11.7	<0.01**	0.10	0.13
(% d.m.)											(<0.01**)	(0.11)	(0.10)
Chlorophyll	27.8 ± 2.7	35.9 ± 3.3	33.1 ± 2.4	26.4 ± 1.8	20.3 ± 1.5	22.6 ± 1.3	30.9	28.1	27.5	15.4	<0.05*	0.92	<0.05*
(rel. units)											(<0.01**)	(0.38)	(<0.05*)
Leaf N	2.5 ± 0.1	2.5 ± 0.0	2.8 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.5	1.8	3.7	3.2	<0.001***	0.27	<0.05*
(% d.m.)											(0.08)	(0.80)	(0.49)
NSC-free leaf N	2.9 ± 0.2	3.0 ± 0.1	3.2 ± 0.1	3.2 ± 0.2	2.9 ± 0.1	3.0 ± 0.1	2.9	2.0	4.1	3.6	<0.001***	0.65	0.06
(% d.m.)											(0.19)	(0.38)	(0.90)
Leaf N	2.0 ± 0.1	1.8 ± 0.2	2.2 ± 0.1	2.2 ± 0.2	2.3 ± 0.0	2.2 ± 0.1	2.2	1.5	3.5	3.2	<0.001***	<0.05*	0.08
(g m^{-2})											(<0.01**)	(0.22)	(0.84)
SLA	10.2 ± 0.5	9.7 ± 0.2	8.8 ± 0.3	8.3 ± 0.1	13.3 ± 0.2	13.9 ± 1.3	11.5	8.2	14.3	14.8	<0.001***	0.09	0.10
(m^2 kg^{-1})											(<0.001***)	(0.76)	(0.75)
NSC-free SLA	11.9 ± 0.7	11.5 ± 0.4	10.1 ± 0.2	10.1 ± 0.3	16.2 ± 0.0	16.8 ± 1.5	13.2	9.0	16.0	16.8	<0.001***	0.37	0.08
(m^2 kg^{-1})											(<0.001***)	(0.90)	(0.96)
Leaf thickness	389 ± 14	384 ± 12	337 ± 21	326 ± 5	395 ± 16	403 ± 6	272	302	410	431	<0.001***	0.75	0.71
(μm)											(<0.001***)	(0.82)	(0.71)
PNUE	86.4 ± 7.1	152.5 ± 12.1	110.6 ± 6.7	140.6 ± 4.0	61.6 ± 6.2	99.3 ± 6.6	47.1	146.0	43.3	66.5	<0.001***	<0.001***	<0.05*
(μmol mol N^{-1} s^{-1})											(<0.001***)	(<0.001***)	(0.09)
Trees (*n*)	5	3	4	3	3	3	1	1	1	1			

Numbers are means ± SE or represent single trees, a = ambient CO_2, e = elevated CO_2. The terminal columns represent the results of two-way ANOVAs for species and treatment effects and the interaction term. For each parameter, the upper row contains the statistical results for all species and the lower row the results for the three replicated species only (see last row for number of replications)

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

(Noormets et al. 2001). However, when LAI had reached steady-state, photosynthetic capacity in CO_2-enriched *Populus* clones and *Betula papyfera* trees was increased by 76 and 115%, respectively, and the addition of O_3 (elevated $CO_2 + O_3$ treatment) did not significantly reduce the CO_2-driven stimulation in photosynthesis (Uddling et al. 2009).

Although previous measurements revealed reduced sapflow in our CO_2-enriched trees (Cech et al. 2003; Leuzinger and Körner 2007), we did not measure reduced g_s under elevated CO_2. However, the relationship between A_{growth} and g_s suggests improved water-use-efficiency (Fig. 2). A similar relationship between A_{growth} and g_s was reported for *Liquidambar styraciflua* trees at the ORNL-FACE site, which, in contrast to our trees, showed a significant 24% decline in g_s under elevated CO_2 (Gunderson et al. 2002).

In their meta-analysis of photosynthetic responses to elevated CO_2 in FACE experiments, Ainsworth and Rogers (2007) reported moderate but significant decreases in V_{cmax} (−6%) in trees growing under high CO_2. At our site, trees receiving CO_2 enrichment did not show reduced V_{cmax} not even towards the end of the growing season when down-regulation due to declining sink strength is most commonly observed. Downward adjustment of J_{max} in response to elevated CO_2 has less often been reported (Long et al. 2004; Ainsworth and Long 2005) and was not apparent in our CO_2-enriched trees.

The lack of photosynthetic down-regulation is consistent with the findings for mature and understory sweetgum trees growing at the ORNL- and Duke-FACE site, respectively, and three poplar species growing at short-rotation coppice at the POP-FACE site as well as for aspen and birch at the ASPEN-FACE stands (Herrick and Thomas 2001; Sholtis et al. 2004; Liberloo et al. 2007; Uddling et al. 2009).

Down-regulation of photosynthesis in response to CO_2 enrichment is often associated with a decline in leaf N (Stitt 1991; Medlyn et al. 1999) and thus occurs more often under limited soil N availability (Oren et al. 2001). Although the steady-state pine forest at the DUKE-FACE facility is such an N-limited system, long-term exposure to elevated CO_2 did not affect needle N irrespective of needle age. Nonetheless, V_{cmax} and J_{max} declined significantly and reduced photosynthetic enhancement by 37% in 1-year-old needles of CO_2-enriched *Pinus taeda* trees (Rogers and Ellsworth 2002; Crous et al. 2008). These declines resulted from NSC accumulation that caused strong selective down-regulation of Rubisco (Rogers and Ellsworth 2002). In current-year needles that showed no down-regulation, the photosynthetic enhancement by elevated CO_2 was therefore substantially higher compared to 1-year-old needles (68 vs. 40%; Crous et al. 2008). Interestingly, N-fertilisation in the last FACE year restored photosynthetic capacity in 1-year-old needles suggesting that CO_2 enrichment reduced the allocation of N to Rubisco and RuBP regeneration and to proteins associated with electron transport in 1-year-old needles. The authors concluded that curtailing the N supply to photosynthesis could make more N available for new foliage growth at low fertility sites (Crous et al. 2008). However, even in well-fertilised sour orange trees, photosynthesis was gradually down-regulated during 14 years of growth in elevated CO_2 (Adam et al. 2004).

The SCC stand grows in an area with ample precipitation and high wet nitrogen deposition (20–25 kg N ha^{-1} a^{-1}) and is thus believed to be free of N-limitation. Consequently, we assumed strong initial growth stimulation by elevated CO_2 that would only start to acclimatise a few years after the FACE initiation (Körner 2006). However, apart from transient growth stimulation in *Fagus* during the early years of the experiment, we did not observe any consistent, significant increases in growth or biomass or the acceleration of turnover processes above or below ground under elevated CO_2 (Körner et al. 2005; Asshoff et al. 2006; Keel et al. 2006; Bader et al. 2009). Although stable isotope data and soil air CO_2 concentration both indicated an increased flux of C into the soil (Keel et al. 2006), we did not detect a corresponding signal in soil respiration that could account for the fate of the extra C assimilated under elevated CO_2 (Bader and Körner 2010). The lack of strong sink capacity for assimilates led us to assume considerable down-regulation of photosynthesis in trees receiving CO_2 enrichment. Instead, we found persistent stimulation of photosynthesis implying a lack of closure in the C budget, clearly pointing to so far unaccounted C fluxes in this CO_2 enrichment experiment. Preliminary findings indicate that parts of the extra C assimilated under elevated CO_2 may leave the system through enhanced leaching of dissolved organic and inorganic C (<20%, F. Hagedorn, personnel communication).

Leaf traits

Similar to previous years, the overall trend towards increased leaf NSC under elevated CO_2 resulted mainly from the strong buildup seen in *Quercus* leaves (Table 4; Körner et al. 2005). As discussed earlier, NSC accumulation in leaves may lead to photosynthetic down-regulation, but photosynthesis (A_{growth} and E') in *Quercus* foliage remained unaffected by the CO_2-induced increase in NSC. Similarly strong accumulation of leaf NSC (+37%) was observed in *Liquidambar styraciflua* (sweetgum) leaves at the ORNL-FACE site and there the sugar signal also failed to down-regulate photosynthesis (Sholtis et al. 2004). Consistent with the lack of photosynthetic down-regulation, leaf N assessed at peak season was not affected by CO_2 enrichment, except for the single *Acer* tree that showed a large decrease in leaf N. This contrasts with the findings from the early years of the experiment that showed an overall reduction of 10% in leaf N driven by a pronounced decline in *Carpinus* and the dilution by NSC (Körner et al. 2005). A 10% reduction in leaf N (mass based) was also reported for sweetgum trees at the ORNL stands (Sholtis et al. 2004), whilst needle N in *Pinus taeda* growing on low fertile soil at the Duke-FACE forest and leaf N in deciduous trees at the POP- and Aspen-FACE stands remained unaffected by CO_2 exposure (Liberloo et al. 2007; Crous et al. 2008; Uddling et al. 2009).

The changes in area-based chlorophyll content observed in *Fagus* and *Quercus* were not linked to changes in SLA and had no effect on V_{cmax} or J_{max}. Commonly, foliar chlorophyll content is little affected under FACE conditions (Long et al. 2004; Ainsworth and Long 2005), and declines on a mass basis observed in sweetgum trees (ORNL-FACE) were fully explained by reductions in SLA (ca. −10%, Sholtis et al. 2004). During the first 4 years of the SCC experiment, elevated CO_2 diminished SLA by 5–8% in all species but *Fagus* (Körner et al. 2005), whereas in year 8 only *Acer* showed lower SLA under elevated CO_2. Also, *Pinus taeda* growing at the Duke forest showed no SLA response to elevated CO_2 (Rogers and Ellsworth 2002), but SLA declined up to 24% in the upper canopy of a poplar coppice exposed to elevated CO_2 (POP-FACE, Liberloo et al. 2007). In general, leaf traits of our study trees were surprisingly little affected by CO_2 enrichment.

Even if the CO_2-induced decline in SLA observed during the early years of the experiment disappeared over time, it did not preclude changes in leaf production. However, at our site, annual leaf litter production and thus LAI remained unchanged under elevated CO_2 (Körner et al. 2005 and later unpublished data), which was consistent with earlier studies reporting the lack of an LAI response to CO_2 enrichment in closed canopy stands (Hättenschwiler et al. 1997; Gielen et al. 2003; Norby et al. 2003). Steady-state LAI had, however, increased in pure aspen and mixed aspen birch stands growing under elevated CO_2 at the Aspen-FACE site (Uddling et al. 2008).

Conclusions

Photosynthetic enhancement (42–48%) in mature trees of five broad-leaved species was sustained without reductions over 8 years of canopy CO_2 enrichment. Provided that future climatic trends will not strongly affect photosynthesis directly and nutrient availability will remain sufficient, these findings suggest that the enhancement of photosynthesis may persist in these mature deciduous trees under high future atmospheric CO_2 concentrations. The fate of the additional C assimilated by CO_2-exposed trees growing in this closed canopy forest remains uncertain. Above- and below-ground growth responses to elevated CO_2 were inconsistent suggesting that the extra C was not used to build up significantly more biomass in these old trees.

Acknowledgments We thank Erwin Amstutz for crane operations and for his efforts in data collection. We are grateful to the Paul Scherrer Institute (Switzerland) for the provision of equipment. Further, we thank Olivier Bignucolo for various laboratory analyses of leaf parameters. The SCC FACE study was supported by the Swiss National Science Foundation (grant 3100AO-111914/1).

References

Adam NR, Wall GW, Kimball BA, Idso SB, Webber AN (2004) Photosynthetic down-regulation over long-term CO_2 enrichment in leaves of sour orange (*Citrus aurantium*) trees. New Phytol 163:341–347

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 Phytol 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 Environ 30:258–270

Asshoff R, Zotz G, Körner C (2006) Growth and phenology of mature temperate forest trees in elevated CO_2. Glob Change Biol 12:848–861

Bader M, Körner C (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Glob Change Biol. doi:10.1111/j.1365-2486.2010.02159.x (in press)

Bader M, 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

Bonan GB (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320:1444–1449

Cech PG, Pepin S, Körner C (2003) Elevated CO_2 reduces sap flux in mature deciduous forest trees. Oecologia 137:258–268

Crous KY, Walters MB, Ellsworth DS (2008) Elevated CO_2 concentration affects leaf photosynthesis–nitrogen relationships in *Pinus taeda* over nine years in FACE. Tree Physiol 28:607–614

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

Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? Annu Rev Plant Physiol Plant Mol Biol 48:609–639

Egli P, Maurer S, Spinnler D, Landolt W, Gunthardt-Georg MS, Körner C (2001) Downward adjustment of carbon fluxes at the biochemical, leaf, and ecosystem scale in beech-spruce model communities exposed to long-term atmospheric CO_2 enrichment. Oikos 92:279–290

Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Glob Change Biol 10:2121–2138

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90

Gielen B, Liberloo M, Bogaert J, Calfapietra C, De Angelis P, Miglietta F, Scarascia-Mugnozza G, Ceulemans R (2003) Three years of free-air CO_2 enrichment (POPFACE) only slightly affect profiles of light and leaf characteristics in closed canopies of *Populus*. Glob Change Biol 9:1022–1037

Gunderson CA, Wullschleger SD (1994) Photosynthetic acclimation in trees to rising atmospheric CO_2—a broader perspective. Photosynth Res 39:369–388

Gunderson CA, Sholtis JD, Wullschleger SD, Tissue DT, Hanson PJ, Norby RJ (2002) Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. Plant Cell Environ 25:379–393

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

Herrick JD, Thomas RB (2001) No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO_2 enrichment at the Duke forest FACE experiment. Plant Cell Environ 24:53–64

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

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

Körner C, Miglietta F (1994) Long-term effects of naturally elevated CO_2 on mediterranean grassland and forest trees. Oecologia 99:343–351

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

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 2:421–436

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

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

Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J Exp Bot 54:2393–2401

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol 55:591–628

Medlyn BE, Badeck FW, De Pury DGG, Barton CVM, Broadmeadow M, Ceulemans R, De Angelis P, Forstreuter M, Jach ME, Kellomaki S, Laitat E, Marek M, Philippot S, Rey A, Strassemeyer J, Laitinen K, Liozon R, Portier B, Roberntz P, Wang K, Jarvis PG (1999) Effects of elevated CO_2 on photosynthesis in European forest species: a meta-analysis of model parameters. Plant Cell Environ 22:1475–1495

Moore BD, Cheng SH, Sims D, Seemann JR (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. Plant Cell Environ 22:567–582

Noormets A, McDonald EP, Dickson RE, Kruger EL, Sober A, Isebrands JG, Karnosky DF (2001) The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. Trees Struct Funct 15:262–270

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, Sholtis JD, Gunderson CA, Jawdy SS (2003) Leaf dynamics of a deciduous forest canopy: no response to elevated CO_2. Oecologia 136:574–584

Nowak RS, Ellsworth DS, Smith SD (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

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

Rogers A, Ellsworth DS (2002) Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO_2 (FACE). Plant Cell Environ 25:851–858

Roy J, Saugier B, Mooney HA (2001) Terrestrial global productivity. Academic Press, San Diego

Sabine CL, Heimann M, Artaxo P, Bakker DCE, Chen C-TA, Field CB, Gruber N, Le Quéré C, Prinn RG, Richey JE, Lankao PR, Sathaye JA, Valentini R (2004) Current status and past trends of the global carbon cycle. In: Field CB, Raupach MR (eds) Global carbon cycle—integrating humans climate and the natural world. Island Press, Washington, DC, pp 17–44

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139:395–436

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. Glob Change Biol 1:77–91

Sholtis JD, Gunderson CA, Norby RJ, Tissue DT (2004) Persistent stimulation of photosynthesis by elevated CO_2 in a sweetgum (*Liquidambar styraciflua*) forest stand. New Phytol 162:343–354

Stitt M (1991) Rising CO_2 level and their potential significance for cabon flow in photosynthetic cells. Plant Cell Environ 14:741–762

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ 22:583–621

Tans P (2008) Trends in atmospheric carbon dioxide. Web page NOAA/ESRL http://www.esrl.noaa.gov/gmd/ccgg/trends/. Accessed 27 July 2009

Uddling J, Teclaw RM, Kubiske ME, Pregitzer KS, Ellsworth DS (2008) Sap flux in pure aspen and mixed aspen—birch forests exposed to elevated concentrations of carbon dioxide and ozone. Tree Physiol 28:1231–1243

Uddling J, Teclaw RM, Pregitzer KS, Ellsworth DS (2009) Leaf and canopy conductance in aspen and aspen—birch forests under free-air enrichment of carbon dioxide and ozone. Tree Physiol 29:1367–1380

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

Chapter 5

Water relations and photosynthetic performance in *Larix sibirica* growing in the forest-steppe ecotone of northern Mongolia

Tree Physiology 29, 99–110
doi:10.1093/treephys/tpn008

Water relations and photosynthetic performance in *Larix sibirica* growing in the forest-steppe ecotone of northern Mongolia

CHOIMAA DULAMSUREN,[1,2,3] MARKUS HAUCK,[1] MARTIN BADER,[4]
DALAIKHUU OSOKHJARGAL,[5] SHAGJJAV OYUNGEREL,[5]
SURAN NYAMBAYAR,[5] MICHAEL RUNGE[1] and CHRISTOPH LEUSCHNER[1]

[1] *Department of Plant Ecology, Albrecht von Haller Institute of Plant Sciences, University of Göttingen, D-37073 Göttingen, Germany*

[2] *Corresponding author (dchoima@gwdg.de)*

[3] *Center of Nature Conservation, University of Göttingen, Von-Siebold-Straße 2, D-37075 Göttingen, Germany*

[4] *Botanical Institute, University of Basel, CH-4056 Basel, Switzerland*

[5] *Faculty of Biology, National University of Mongolia, Ikh Surguuliin Gudamj 1, 210646 Ulaanbaatar, Mongolia*

Received July 30, 2008; accepted September 5, 2008; published online December 3, 2008

Summary Shoot water relations were studied in Siberian larch (*Larix sibirica* Ledeb.) trees growing at the borderline between taiga and steppe in northern Mongolia. *Larix sibirica* is the main tree species in these forests covering 80% of Mongolia's forested area. Minimum shoot water potentials (Ψ_m) close to the point of zero turgor (Ψ_0) repeatedly recorded throughout the growing season suggest that the water relations in *L. sibirica* were often critical. The Ψ_m varied in close relation to the atmospheric vapor pressure deficit, whereas Ψ_0 was correlated with monthly precipitation. Young larch trees growing at the forest line to the steppe were more susceptible to drought than mature trees at the same sites. Furthermore, isolated trees growing on the steppe exhibited lower Ψ_m and recovered to a lower degree from drought overnight than the trees at the forest line. Indications of drought stress in *L. sibirica* were obtained in two study areas in Mongolia's forest-steppe ecotone: one in the mountain taiga of the western Khentey in northernmost Mongolia, the other in the forest-steppe at the southern distribution limit of *L. sibirica* on Mt. Bogd Uul, southern Khentey. *Larix sibirica* growing in riverine taiga with contact to the groundwater table was better water-supplied than the larch trees growing at the forest line to the steppe. Larch trees from the interior of light taiga forests on north-facing slopes, however, exhibited more critical water relations than the trees at the forest line. Frequent drought stress in mature trees and even more in young larch trees at the forest-steppe borderline suggests that *L. sibirica* does not have the potential to encroach on the steppe under the present climate, except in a sequence of exceptionally moist and cool years. A regression of the present borderline between forest and steppe is likely to occur, as average temperatures are increasing everywhere and precipitation is decreasing regionally in Mongolia's taiga forest region. Higher stomatal conductance concomitant to lower Ψ_m in trees of northern-slope forests compared to trees from the forest line to the steppe may be the result of a recent increase in drought intensity that affects better drought-adapted trees at the forest edge less than the trees in the forest interior. We conclude that drought is a key factor explaining the forest-steppe borderline in northern Mongolia. The proportion of forests within the present vegetation pattern of forests on north-facing slopes and the grasslands on south-facing slopes in Mongolia's forest-steppe ecotone is not likely to increase under the present climate, but may decrease with increasing aridity due to global warming.

Keywords: chlorophyll fluorescence, CO_2/H_2O gas exchange, drought stress, shoot water potential, $\delta^{13}C$.

Introduction

Northern Eurasia is covered by the huge belt of boreal coniferous forests. In Eurasia's oceanic parts, the boreal forest gives way to broadleaved forests in the south, whereas steppe grasslands are located south of it in continental Eurasia, i.e., eastern Siberia, Kazakhstan and Mongolia (Lavrenko and Karamysheva 1993). While the transition from the boreal forest to the temperate deciduous forests in oceanic areas with high precipitation is caused by the inferiority of conifers to the competition of broadleaved trees with increasing length of the growing season, drought limits the boreal forest at its southern distribution limit in the center of the Eurasian landmasses (Walter and Breckle 1994). The ecotone between the northern coniferous forests and the steppe is an aspect-dependent vegetation pattern of forests on north-facing slopes and grasslands on south-facing slopes (Wallis de Vries et al. 1996, Dulamsuren et al. 2005*a*, Chytrý et al. 2008). The southern distribution limit of the individual tree species occurring in this ecotone and, with it, the entire forest-steppe border migrates depending on the variations of precipitation and temperature (Dinesman et al. 1989, Gunin et al. 1999, Miehe et al. 2007). Also, anthropo-zoogenic influences affect the position of the forest-steppe borderline (Hilbig 1995, Rösch et al. 2005, Sankey et al. 2006).

The temperature increase during the past decades at the forest-steppe transition in southern Siberia, Kazakhstan and Mongolia is far beyond the global average (Batima et al. 2005, Henderson et al. 2006), whereas precipitation trends differ between regions (Aizen et al. 2001, Giese and Moßig 2004). In parts of northern Mongolia, precipitation has significantly decreased in the late 20th century (Batima et al. 2005), and a further decline is predicted for the 21st century (Sato et al. 2007). Since forests already today occur at their drought limit in such areas with decreasing precipitation and increasing temperature, it is reasonable to assume that the increasing aridity will in future reduce the area of forests in the present forest-steppe mosaics.

Ecophysiological studies examining the current extent of drought stress in trees at forest-steppe ecotones are rare. This is also true for Mongolia, which harbors one of the world's largest and the least disturbed forest-steppe transition zones (Vostokova and Gunin 2005). In the forest-steppe of north-eastern Mongolia, Li et al. (2005*a*) had investigated the carbon balance in a stand of Siberian larch (*Larix sibirica* Ledeb.), which is Mongolia's most common tree species (Gunin et al. 1999), in comparison to the nearby steppe grassland (Li et al. 2005*b*). Li et al. (2006, 2007*a*) applied a stable isotope approach for identifying water sources used by *L. sibirica* in Mongolia's forest-steppe ecotone. Direct study, as to how *L. sibirica* or any other tree species occurring in Mongolia's forest-steppe ecotone respond to the seasonal shortages of water, is limited to a single investigation of $\delta^{13}C$ signatures in a *L. sibirica* stand in northeastern Mongolia (Li et al. 2007*b*). Therefore, we investigated the seasonal and diurnal dynamics of shoot water relations of *L. sibirica* trees growing at the forest-steppe borderline of northern Mongolia and supplemented these analyses with punctual measurements of CO_2/H_2O gas exchange and chlorophyll fluorescence characteristics. The aim was to assess the potential of the larch trees to encroach onto grasslands in front of the present forest lines under the current climate and to withstand increasing aridity in the future.

Special emphasis was given to the hypotheses that (1) trees at the forest line to the steppe suffer from drought stress during summer and (2) young larch trees at the forest edge are more susceptible to drought stress than mature trees. Other research questions addressed included a comparison of the water relations in larch trees growing at the forest line to the steppe and in the interior of the taiga forest occupying the north-facing slopes. Water relation in *L. sibirica* growing in a riverine forest was also punctually studied. While most parts of the study were conducted in the mountain taiga of the western Khentey Mountains in northernmost Mongolia, some comparative measurements of the shoot water potential were also carried out in the forest-steppe of the southern Khentey Mountains immediately at the southern distribution limit of the Eurosiberian taiga to the open steppe.

Materials and methods

Study sites

Investigations were conducted in two regions of northern Mongolia. The first study area, where most measurements were carried out, was located near Khonin Nuga Research Station in the western Khentey Mountains (49°04′48″ N and 107°17′15″ E), 130 km north of the Mongolian capital Ulan Bator. The second study area was selected in the southern Khentey at Mt. Bogd Uul, 12 km southeast of Ulan Bator. The study areas differ in their latitudinal position and vegetation. Khonin Nuga is located in the northernmost part of north-eastern Mongolia close to the Russian border, whereas the Bogd Uul mountain range forms the southernmost distribution limit of forests in north-eastern Mongolia. Khonin Nuga belongs to the mountain taiga, where steppe occurs as insular outposts on south-facing slopes surrounded by woodlands on north-facing slopes and in valleys (Dulamsuren et al. 2005*a*). Mt. Bogd Uul is located in the forest-steppe belt, which is principally dominated by grasslands with islands of light taiga forest limited to north-facing slopes (Hilbig et al. 2004).

Long-term climate data from the vicinities of the study areas are available from the weather station Eroo, ca. 70 km northwest Khonin Nuga Research Station, and from the airport in Ulan Bator, ca. 25 km west northwest of the sample plots on Mt. Bogd Uul (Table 1). Microclimate measurements from nine weather stations near Khonin Nuga Research Stations from spring 2005 to fall 2007 suggest similar temperatures as recorded at the weather station Eroo (Dulamsuren and Hauck 2009). However, precipitation is too variable in Mongolia to deduce long-term annual means from the weather data recorded from 2005 to 2007. The forest vegetation at Khonin Nuga suggests that the annual precipitation is higher than that at the weather stations Eroo and Ulan Bator, as the latter are located in the forest-steppe which is strongly dominated by the drought-tolerant *L. sibirica*, whereas the forests of Khonin Nuga, located in the mountain taiga, are more diverse and include moisture-demanding tree species, like *Abies sibirica* Ledeb. and *Sorbus sibirica* Hedl. (Dulamsuren et al. 2005*a*). The maximum precipitation is received in summer.

Table 1. Climate data from the weather stations Eroo (ca. 70 km northwest of Khonin Nuga) and Ulan Bator (ca. 25 km west northwest of Tor Khurkh, Mt. Bogd Uul). Arithmetic mean ± SE; ranges of annual mean temperature or annual precipitation in brackets.

	Eroo (ca. 900 m)	Ulan Bator (ca. 1365 m)
Study period	1961–2004	1950–2006
Annual mean temperature (°C)	−2.1 ± 0.2	−1.8 ± 0.2
Mean January temperature (°C)	−27.4 ± 0.5 (−4.0 to 3.2)	−23.9 ± 0.4 (−31.8 to −18.3)
Mean July temperature (°C)	18.7 ± 1.3 (16.4 to 22.3)	17.2 ± 0.2 (14.3 to 21.9)
Precipitation (mm)	277 ± 11 (157 to 459)	259 ± 10 (62 to 404)
January precipitation (mm)	3 ± 1 (0 to 19)	2 ± 0 (0 to 8)
July precipitation (mm)	78 ± 5 (30 to 160)	71 ± 5 (7 to 197)

Studies in the western Khentey were carried out on Mt. Bayantogol (1020–1180 m, 49°5′ N and 107°17′ E) north of Khonin Nuga Research Station. The south-facing slope of Mt. Bayantogol rises directly from the northern shore of the River Eroo and has a size of ca. 1.5 km × 250 m (parallel × perpendicular to the contour lines). This south-facing slope is covered with different communities of meadow steppe and mountain steppe as well as small savanna-like woodlands of *Ulmus pumila* L. (Dulamsuren et al. 2005*a*, 2005*b*). On its north-facing slope, Mt. Bayantogol is covered with a light taiga forest of *Betula platyphylla* Sukaczev and *L. sibirica* with a canopy cover between 20% and 40%. The borderline between the forest and the grasslands at the mountain ridge is almost exclusively built by *L. sibirica*.

Shoot water potential measurements on Mt. Bogd Uul were conducted in the north-eastern part of the mountain system that is northwest of the Tor Khurkh Valley on a sample plot of 1950 m altitude (Bayan Tokhom; 47°43′ N and 107°5′ E). Meadow steppe with single larch trees growing in strips of ca. 50 m from the forest line occurred on southeast-facing slopes. The light taiga forests of Mt. Bogd Uul solely consist of *L. sibirica*. The forested slope studied was exposed to the east. The bases of the studied slopes are high valleys within the Bogd Uul mountain system.

Measurements of the shoot water potential

The plant water status was assessed by conducting diurnal and seasonal measurements of the shoot water potential (Ψ) on rainless days with clear sky using a Model 1000 Pressure Chamber Instrument (PMS Instrument Company, Albany, Oregon) applying the method of Scholander et al. (1964). Measurements included the determination of the predawn water potential (Ψ_p), which represents the daily maximum of Ψ, and of the midday water potential (Ψ_m), which equals the daily minimum of Ψ, in the field. These measurements were conducted with freshly cut twigs of 10 cm length from sun-exposed branches at a height of 2 m. Five replicates were studied per tree at a specific point in time. In addition, pressure–volume curves were recorded from fully water-saturated twigs, sampled the day ($\leq$ 12 h) before the measurement to determine the point of zero turgor (Ψ_0) (Roberts et al. 1980). The Ψ_0 is the maximum value of Ψ, from which on irreversible damage due to cytorrhysis is possible. However, the cells can withstand values of Ψ below Ψ_0 if the stability of the cell wall allows negative turgor values (Rhizopoulou 1997). Twigs used for the determination of Ψ_0 were re-cut underwater and saturated with water in a glass wrapped in plastic foil for 12 h. The Ψ_0 is less affected by the length of the saturation phase before the measurements (Parker and Pallardy 1987). The Ψ_0 was deduced from plots of $-1/\Psi$ versus the relative water content (RWC) of the sample. The RWC was calculated following RWC (%) = [(fresh mass − dry mass)/(turgid mass − dry mass)] × 100. Three twigs per tree were sampled as pseudoreplicates for the determination of Ψ_0.

Water potential measurements on Mt. Bayantogol included monthly measurements of Ψ_p, Ψ_m and Ψ_0 throughout two subsequent growing seasons from May to September 2005 and June to September 2006. The Ψ_p and Ψ_m were determined in five mature trees (ca. 60–80-years old, 26 ± 5 cm in diameter at breast height) and three young trees (stem diameter 3.5 ± 0.3 cm, stem height between 60 and 100 cm). The 60–80-year-old trees represented the most common age class in the forest (Dulamsuren and Hauck unpublished). The five mature larch trees stood 15, 35, 45 or 50 m in front of or directly at the forest line. The three young trees grew 25, 35 or 40 m in front of the forest line on the montane meadow steppe. The Ψ_0 was determined each in one mature and young tree growing on the meadow steppe at a distance of 15–30 m from the forest line.

In addition to the monthly measurements of Ψ_p and Ψ_m, the diurnal variation of Ψ was measured on selected days in the middle of the growing season. These measurements included mature and young trees at the forest edge as well as a comparison between mature larch trees growing on the steppe and in the neighboring floodplain of a small creek. They were carried out at Mt. Baziin Am at 1050 m (49°2′ N and 107°15′ E), as the valley bottom below Mt. Bayantogol is deforested. During the measurements involving the floodplain, an ML-2x Theta soil moisture sensor (Umweltanalytische Produkte, Ibbenbüren, Germany) was used together with an HH2 portable logger to measure the volumetric soil water content in the upper 6 cm of the soil covering a soil volume of 75 cm^3. Furthermore, Ψ_m was determined in each of the five mature larch trees growing at the forest line or in the forest interior (120–140 m behind the forest line) on Mt. Bayantogol in August 2006.

In the Bogd Uul mountain range, Ψ was measured in the two warmest months of the growing season in July and August 2007. Diurnal courses, including Ψ_p and Ψ_m, were recorded from five trees growing in the steppe up to 50 m in front of the forest line and five trees growing in the forest up to 50 m behind the forest line. The Ψ_0 was determined in the laboratory for four to five trees per month on the steppe and in the forest.

Determination of $\delta^{13}C$ signatures

The δ^{13}C signature of needles of mature trees of *L. sibirica* was determined in samples from the western Khentey Mountains. Samples were taken from each of the five trees growing in the meadow steppe, 15–45 m in front of the forest line, or growing in the forest interior, 120–140 m behind the forest line. Five additional samples were taken from trees growing at the lower part of a north-facing slope in the valley of the River Eroo, opposite Mt. Bayantogol (970 m; 49°5′ N and 107°18′ E). These trees were sampled as an additional reference, as the site is only a little above the level of the floodplain, which was thought to be better water-supplied than that in the stand on Mt. Bayantogol. Sun-exposed needles were collected at 2 m height above the ground from the southward side of the trees in August 2006. The samples were dried at 105 °C for 24 h, ground to a fine powder, and weighed out in tin capsules. The analyses were conducted with a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA), which was combined with an NA 1500 C/N Elementar Analyzer (Carlo Erba Strumentazione, Milan, Italy) and Conflo III interface (Thermo Fisher Scientific). Acetanilide was used as an internal standard. Using this internal standard, the δ^{13}C signature was related to the Peedee belemnite limestone standard using $\delta^{13}C\ (‰) = ([R_{sample}/R_{standard}] - 1) \times 1000$, with $R = {}^{12}C/{}^{13}C$. The enrichment of ^{13}C indicating drought stress results in high (less negative) values of δ^{13}C.

Gas exchange measurements

Diurnal variation of CO_2/H_2O gas exchange of *L. sibirica* shoots was studied on Mt. Bayantogol in August 2006. Net photosynthesis, transpiration and stomatal conductance were measured under ambient conditions with an LCi Ultra Compact Photosynthesis System equipped with a standard conifer cuvette (ADC Bioscientific, Herts, UK). Gas exchange was measured in each three mature larch trees growing on the meadow steppe (15, 35 or 45 m) in front of the forest line, and in the forest interior, 120–140 m behind the forest line. Measurements were performed on 2-m high, sun-exposed branches. The measurements could not start earlier than 10:00 A.M., as needles were covered with a water film from nocturnal fog before.

Measurement and calculation of chlorophyll fluorescence parameters

The effective quantum yield (Φ_2) of photochemical energy conversion in photosystem II (PSII) was measured with a Mini-PAM chlorophyll fluorometer (Walz Mess- und Regeltechnik, Effeltrich, Germany). Diurnal variation of Φ_2 in the field was recorded on Mt. Bayantogol in August 2006 from five mature trees at the forest-steppe growing 15–45 m in front of or directly at the forest line, five young trees growing 25–40 m in front of the forest line and five mature trees growing in the interior of the forest 120–140 m behind the forest line. Five pseudoreplicate measurements were made per tree. In addition to Φ_2, the photosynthetically active radiation (PAR) and the leaf temperature were measured with sensors integrated in the leaf clip holder of the Mini-PAM. The PAR data were used to calculate the electron transport rate (ETR) using $\mathrm{ETR} = \Phi_2 \times \mathrm{PAR} \times 0.42$. The non-photochemical quenching (NPQ) was calculated following the equation $\mathrm{NPQ} = (F_M - F_M')/F_M'$ with F_M being the absolute maximum fluorescence in the dark-adapted state (measured at predawn) and F_M' being the absolute maximum fluorescence in the light-adapted state (Roháček 2002).

Weather conditions during the study period

Data from an HOBO weather station (Onset, Bourne, MA) located in the meadow steppe along the forest edge on Mt. Bayantogol at an elevation of 1060 m are available for the growing seasons covered by the present study (Dulamsuren and Hauck 2009). Direct weather information from Mt. Bogd Uul is lacking, but the data from Mt. Bayantogol can be used to assess the general trends for interannual variation during the study years. The weather station was equipped with an H21 data logger and a set of HOBO Smart Sensors including instruments for measuring air temperature and relative air humidity at 150 cm above soil level (temperature/RH sensor S-THA), soil temperature at 1 and 15 cm depth (8 Bit temperature sensor S-TMA), volumetric soil water content at 10 cm depth (soil moisture sensor S-SMA) and PAR in horizontal position at 2 m above the ground (PAR sensor S-LIA). Precipitation at 1 m (rain gauge sensor S-RGA) was sampled at another HOBO weather station 3 km southwest of Mt. Bayantogol in the floodplain of the River Eroo at 910 m altitude. Data were recorded every 10 min and are the averages of 10 consecutive measurements. Snow was sampled in five 10-l buckets near the weather station in the floodplain meadow.

To characterize the weather conditions in the study periods, weather data of July 2005 and July 2006 are compiled in Table 2. In the western Khentey, the summer was warmer and drier in the first study year (2005) than in the second one (2006). The weather conditions during our measurements in the southern Khentey in summer 2007 were characterized by a dry July (36 mm of precipitation at Ulan Bator) and a humid August (72 mm).

Table 2. July weather data for Mt. Bayantogol, western Khentey for 2005–2007. Arithmetic mean ± SE; absolute maxima or minima in brackets.

	2005	2006	2007
Air temperature, mean (°C)	18.4 ± 0.4	17.0 ± 0.5	18.7 ± 0.7
Air temperature, max. (°C)	27.5 ± 0.7 (37.4)	24.0 ± 0.7 (31.1)	27.8 ± 1.1 (37.9)
Soil temperature, 1 cm, mean (°C)	24.4 ± 0.4	21.8 ± 0.5	23.1 ± 0.6
Soil temperature, 1 cm, max. (°C)	38.1 ± 0.8 (45.4)	31.9 ± 1.1 (44.9)	35.0 ± 1.4 (49.6)
Soil temperature, 15 cm, mean (°C)	21.4 ± 0.2	19.7 ± 0.2	20.7 ± 0.3
Soil temperature, 15 cm, max. (°C)	23.5 ± 0.2 (25.2)	21.9 ± 0.3 (24.0)	22.9 ± 0.3 (26.0)
Relative humidity, mean (%)	69.7 ± 1.8	81.1 ± 1.7	69.8 ± 2.6
Relative humidity, min. (%)	35.2 ± 2.1 (15.8)	49.1 ± 2.5 (25.8)	35.9 ± 3.2 (8.8)
Soil water content (% vol.)	6.2 ± 0.3	6.9 ± 0.2	7.1 ± 0.5
Precipitation, sum (mm)	30	76	–
Precipitation, frequency (days)	10	15	–
PAR, sum (mol m^{-2})	2.26	1.98	2.21

Statistics

Arithmetic mean ± SE is given throughout the paper. Data were tested for normal distribution with the Shapiro–Wilk test. Pairwise comparisons of means were made with Student's *t* test. For repeated pairwise comparisons in the same data set (Ψ_m versus Ψ_0), the *t* test was Bonferroni-corrected. Duncan's multiple range test was used for multiple comparisons of the $\delta^{13}C$ data. Statistical analyses were computed with SAS Version 6.04 software (SAS Institute Inc., Cary, NC).

Results

Mountain taiga of the western Khentey

Both in mature and in young larch trees growing at the forest line to the steppe, Ψ_m decreased throughout the growing season (Figure 1A, B). The Ψ_p decreased from June to August and recovered in September 2005, but did not decline before September in the cooler and moister year 2006. In general, field values of Ψ varied with the atmospheric vapor pressure deficit (VPD) (Figure 1C). Except for the early growing season, Ψ_0 was correlated with the amount of precipitation in the current month in mature trees (Figure 1D). Linear regression of Ψ_0 versus monthly precipitation for the months June to September in 2005 and 2006 yielded a significant relationship with $r = 0.81$ ($P \leq 0.001$). The Ψ_0 of the young trees, however, did not recover after drought in July 2005 with increasing precipitation in August and September 2005 (Figure 1D). Therefore, linear regression of Ψ_0 in the young trees on monthly precipitation did not yield a significant relationship ($r = 0.43$, $P = 0.14$). The adjustment of Ψ_0 to water availability was sufficient in mature trees throughout the growing season in the moist year 2006, but not in 2005 when Ψ_0 was not significantly different from Ψ_m in June, August and September (Figure 1A). In the young trees growing at the forest-steppe borderline, Ψ_m dropped to the level of Ψ_0 throughout the dry growing season 2005 and in midsummer of the wetter year 2006 (Figure 1B).

Drought stress in trees forming the frontier to the steppe increased along with an increasing distance from the closed forest, though this relationship was only statistically significant in the drier growing season 2005 (Figure 2). In July 2005, Ψ_m in the tree advancing farthest into the steppe (at 50 m distance from the forest line) differed by -1 MPa from Ψ_m in a larch tree growing immediately at the forest line (Figure 2C). Furthermore, nocturnal recovery of water relations is increasingly hampered along with the increasing distance from the forest edge, as indicated by the Ψ_p values (Figure 2A). In July 2006, when twice as much precipitation was received as in the year before (Table 2), an insignificant trend for lower Ψ_m with increasing distance from the forest line occurred (Figure 2D), whereas Ψ_p was nearly independent of the position of the tree along the forest-steppe gradient (Figure 2D). As the young trees were more susceptible to drought than the mature ones (Figure 1B), the regression lines in Figure 2 refer only to the mature trees. The results in Figure 2 agree with the measurements of the diurnal variation of Ψ showing more strongly decreasing values of Ψ during daytime in mature and young *L. sibirica* growing in the meadow steppe, 30–45 m in front of the forest line, than in mature larch growing at the forest edge, only up to 15 m in front of the closed forest (Figure 3A).

Depending on the water supply of the forest stand, the shoot water status of larch trees growing in the interior of the light taiga forest was either better or worse than that of the trees growing on the steppe in front of the forest line. A comparison with *L. sibirica* growing in the floodplain of a small creek, where the roots most likely reached the groundwater table, showed higher values of Ψ_m in the forest than on the steppe (Figure 3B, C). Hourly measurements of soil moisture synchronously with the water potential measurements shown in Figure 3C exhibited soil water contents that were 10 times higher in the riverine forest than at the forest line to the steppe (24 ± 1% vol. versus 2.3 ± 0.2% vol.). Larch trees from the north-facing

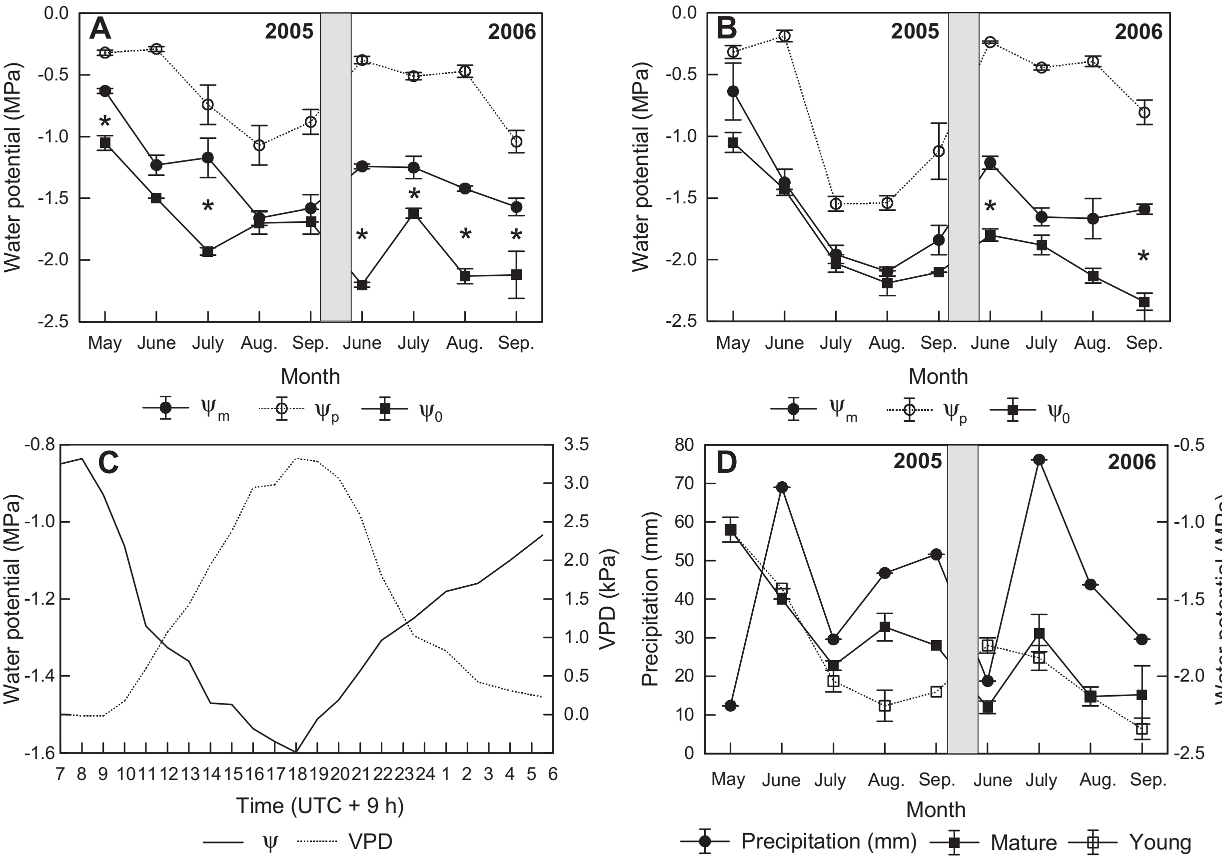


Figure 1. Seasonal and diurnal variation of the shoot water potential in *L. sibirica* growing at the forest line to the steppe at Mt. Bayantogol, western Khentey. (A, B) Minimum (Ψ_m) and predawn (Ψ_p) shoot water potentials as well as points of zero turgor Ψ_0 in (A) mature ($N = 5$) and (B) young trees ($N = 3$) in the growing seasons 2005 and 2006. Asterisks indicate significant difference between Ψ_m and Ψ_0 ($P \leq 0.05$, Bonferroni-corrected t test). (C) Diurnal variation of the shoot water potential in a mature tree and VPD on August 12, 2005. (D) Variation of the point of zero turgor (Ψ_0) with monthly precipitation in mature and young *L. sibirica*.

slope of Mt. Bayantogol had significantly lower values of Ψ_m (-1.75 ± 0.07 MPa) than trees at the neighboring forest edge (-1.27 ± 0.09 MPa; $N = 5$, t test, $P \leq 0.01$). Stomatal conductance (Figure 4A) and transpiration (not shown) concurrently measured with the shoot water potential were higher in the forest interior on the north-facing slopes than at the forest line to the steppe. Despite the lower Ψ_m and the higher stomatal conductance in the larch trees of the forest interior, the rate of net photosynthesis under ambient conditions was not different between trees from the forest interior and the forest line (Figure 4B). The $\delta^{13}C$ signatures were not significantly different (Duncan's multiple range test, $P \leq 0.05$) between larch trees from the forest edge ($-29.08 \pm 1.25‰$ at Mt. Bayantogol) and the interior of forests from north-facing slopes ($-27.35 \pm 0.48‰$ at Mt. Bayantogol and $-26.56 \pm 0.79‰$ in the Eroo Valley), though there was an insignificant trend for higher $\delta^{13}C$ values in the latter.

Chlorophyll fluorescence measured simultaneously with the photosynthetic gas exchange showed that the rapid and steep rise in PAR at the forest edge induced strong light quenching (NPQ) in the PSII antennae and thus caused Φ_2 to deteriorate dramatically (Figure 5). Under high-light conditions prevailing most of the day, Φ_2 of forest edge trees remained down-regulated at low values around 0.3 resulting in a tendentiously lower ETR compared to the trees from the forest interior that received less illumination and thus had to implement appreciably less NPQ (Figure 5). No difference was observed in the chlorophyll fluorescence characteristics between mature and young trees growing at the forest edge (Figure 5). When comparing fluorescence with gas exchange data, it is remarkable that between 10:00 and 17:00 h the rather stable ETR contrasts with the steadily declining CO_2 assimilation rate (Figures 4C and 5D).

Forest-steppe of the southern Khentey

Diurnal courses of Ψ on Mt. Bogd Uul showed no significant difference between larch trees growing in the light taiga forest and in the grasslands at the forest edge toward the steppe (not shown). The Ψ_m was always close to Ψ_0

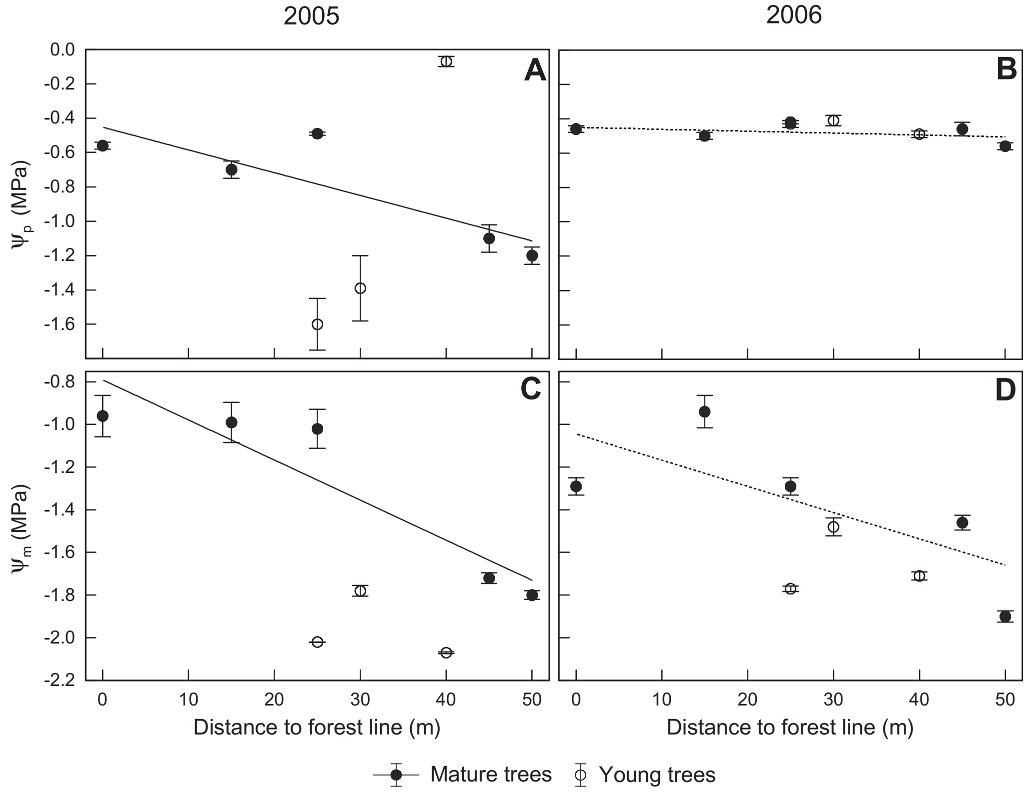


Figure 2. July values of predawn (Ψ_p) and minimum (Ψ_m) leaf water potentials in *L. sibirica* growing in the meadow steppe at different distances from the forest line on Mt. Bayantogol, western Khentey. (A) Ψ_p, 2005; (B) Ψ_p, 2006; (C) Ψ_m, 2005; (D) Ψ_m, 2006. Linear regression lines were calculated for mature trees only; dotted lines refer to non-significant relationships: (A) $r = -0.86$, $P = 0.03$; (B) $r = -0.43$, $P = 0.24$; (C) $r = -0.92$, $P = 0.01$; (D) $r = -0.73$, $P = 0.08$. Different scales for Ψ_p and Ψ_m on the ordinates should be noted.

(Table 3). In July, Ψ_m was even below Ψ_0; in August, when water potentials were generally higher, Ψ_0 was significantly above Ψ_m (Table 3).

Discussion

Our studies of the water relations in *L. sibirica* showed that the trees at the forest-steppe borderline in northern Mongolia frequently suffer from drought stress. The water supply of trees growing on mountain ridges at the forest line to the steppe strongly depends on the current precipitation, as the roots are not in contact with the groundwater table (Li et al. 2007*a*). The results of Li et al. (2007*a*) match with the correlation of Ψ_0 with the monthly precipitation observed in our study (Figure 1D). Velisevich and Kozlov (2006) found a dependence of wood formation in *L. sibirica* on the precipitation received in the early growing season. Permafrost is a source of water for trees growing in closed forests on north-facing slopes in northern Mongolia and southern Siberia, but not for trees on the south-facing slopes covered by grasslands (Sugimoto et al. 2002, Böhner and Lehmkuhl 2005, Etzelmüller et al. 2006).

Daily values of Ψ_m in the range of Ψ_0 or even $< \Psi_0$ were frequently encountered during dry weather (Figure 1A, B; Table 3). Such low in situ values of Ψ close to Ψ_0 show that the water supply of *L. sibirica* at the forest line to the steppe is often critical. The values of Ψ_m which are significantly below Ψ_0 suggest the occurrence of negative turgor values in *L. sibirica*, which are regularly found in plants with rigid cell walls (Kreeb 1961, Rhizopoulou 1997). The repeated occurrence of Ψ_m values around Ψ_0 implies that the larch trees growing in the forest-steppe transition are likely to suffer from extended drought periods where needle growth must be inhibited and the risk of xylem cavitation may be high. However, no vulnerability curves of shoot embolism exist for the larch trees studied. The North American *Larix occidentalis* Nutt. is known to be resistant to drought stress-induced cavitation (Piñol and Sala 2000). Similar results were obtained in *Larix laricina* (Du Roi) K.Koch, for which Wang (2005) showed only a slight decrease of specific hydraulic conductivity during the growing season in British Columbia.

The tense water relations inferred from the proximity of Ψ_m and Ψ_0 suggest that *L. sibirica* is currently unable to encroach from the forest onto the steppe, except in a

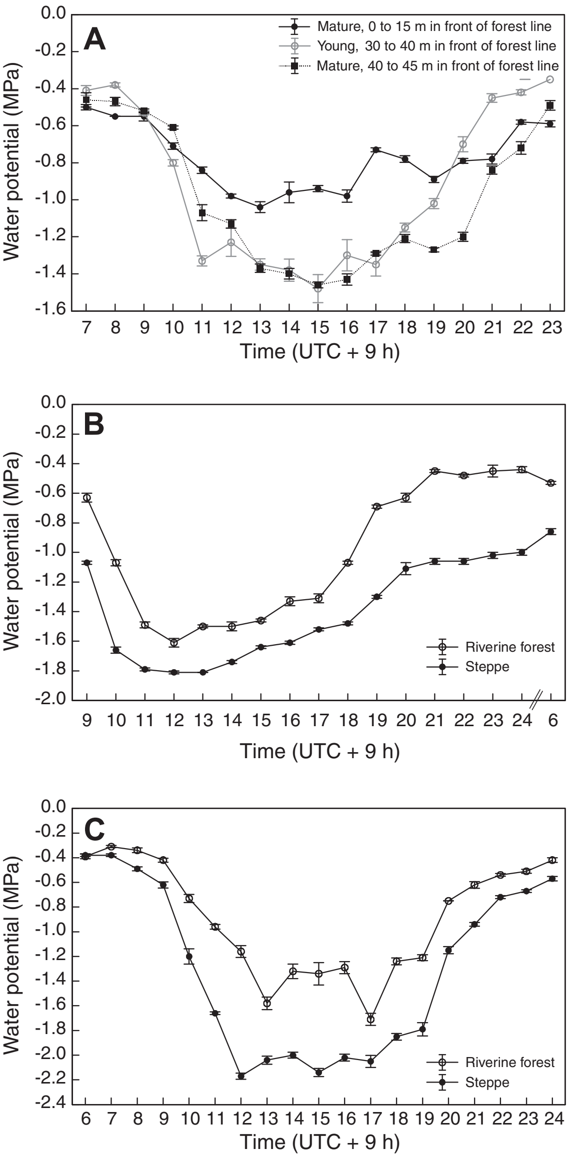


Figure 3. Diurnal variation of the shoot water potential in *L. sibirica* in the western Khentey. (A) Mature trees on the meadow steppe 0–15 m ($N = 3$) or 40–45 m ($N = 2$) in front of the forest line and young trees 30–40 m ($N = 3$) in front of the forest line on July 31, 2006. (B, C) Mature trees ($N = 5$) on the meadow steppe and in riverine forest on August 13, 2005 (B) and August 4, 2006 (C).

sequence of several particularly moist and cool years. This conclusion is supported by the fact that Ψ_m equaled Ψ_0 even more often in saplings than in mature trees (Figure 1B). While mature larch trees can efficiently regulate the concentration of solutes in the vacuole and, with it, the osmotic potential (Badalotti et al. 2000) along with the availability of water (Figure 1D), young trees on the steppe did not respond to increasing precipitation after drought (Figure 1D). Seedlings planted in two subsequent years on steppe slopes of the western Khentey near Khonin

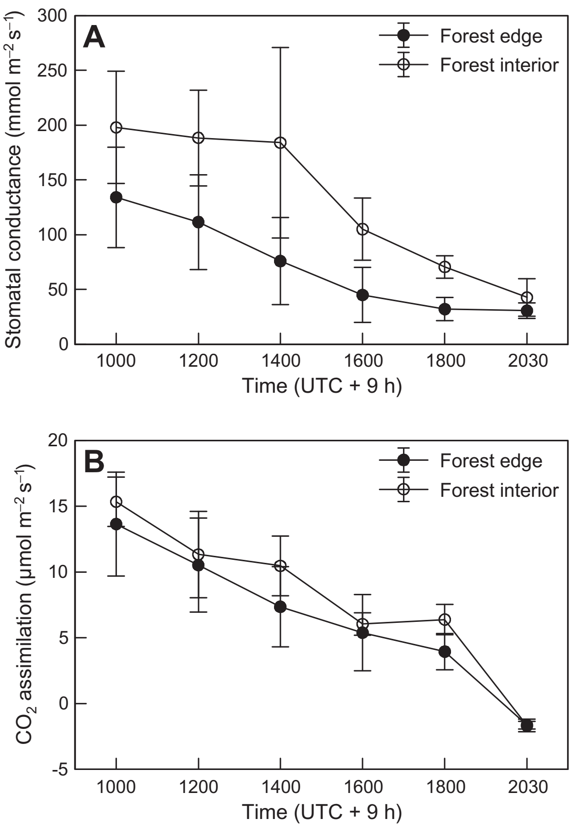


Figure 4. Diurnal variation of (A) stomatal conductance and (B) net CO_2 assimilation rate ($N = 3$) in *L. sibirica* growing at the forest line to the steppe and in the forest interior at Mt. Bayantogol, western Khentey on August 23, 2006.

Nuga corroborated that drought exacerbates the establishment of *L. sibirica* (Dulamsuren et al. 2008, Hauck et al. 2008). In combination with insect and small mammal herbivory, water shortage completely destroyed all planted seedlings within one growing season. Sowing experiments showed that low soil moisture, high soil temperatures and granivory make the emergence of *L. sibirica* from seeds in the steppes bordering on the north-facing taiga forests an improbable event (Dulamsuren et al. 2008).

The present results combined with the results of Dulamsuren et al. (2008) and Hauck et al. (2008) suggest that a spread of *L. sibirica* on the sun-exposed steppe slopes is currently unlikely, irrespective of the origin of the present vegetation pattern with forests on north-facing slopes and grasslands on south-facing slopes. Increasing temperatures concomitant to decreasing precipitation in parts of the Mongolian forest-steppe ecotone as predicted in scenarios (Sato and Kimura 2006, Sato et al. 2007) further reduce the probability of a future encroachment of *L. sibirica* onto the steppe. Rather, the current drought stress indicates that the steppe might expand to the disadvantage of forests with increasing aridity in future. Hereby, our study area differs from southern Siberia, where increased precipitation

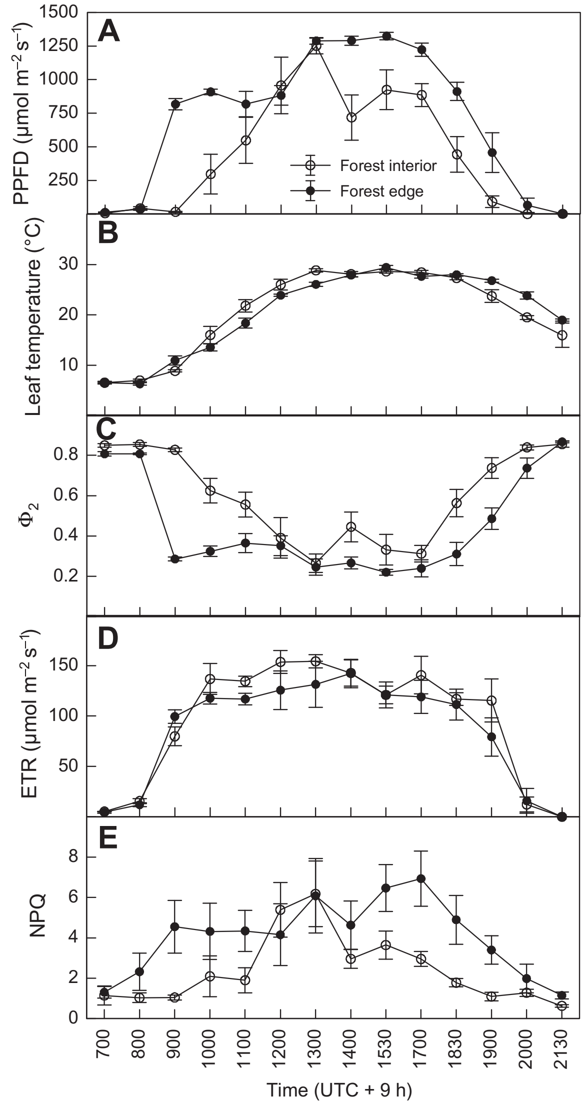


Figure 5. Diurnal variation of chlorophyll fluorescence and climatic parameters in *L. sibirica* growing in the forest interior and at the forest line to the steppe ($N = 5$) on August 23, 2006. (A) Photosynthetically active photon flux density (PPFD), (B) leaf temperature, (C) Φ_2, (D) ETR, (E) NPQ.

concomitant to increased temperature leads to improved growth conditions for forest trees (Lapenis et al. 2005, Tchebakova et al. 2005). It also differs from alpine forest lines in Mongolia, where tree growth is promoted by increased temperatures (Jacoby et al. 1996). Shifts of forest-steppe borderlines even on large spatial scales along with the changes in climate happened repeatedly in Mongolia throughout the Holocene (Gunin et al. 1999, Miehe et al. 2007). Spreads of *L. sibirica* into the steppe during the 20th century were correlated both to favorable moist and cool climate and to low grazing pressure by livestock (Treter 2000, Sankey et al. 2006).

Lower Ψ_m and Ψ_p values in larch trees growing as single trees in front of the forest line than in *L. sibirica* from the forest line itself (Figures 2 and 3A) reflect the warmer and drier microclimate on the open steppe slopes than at the forest edge. The shoot water status of larch trees in the forest strongly depends on the site. The *L. sibirica* growing in riverine forest exhibited higher Ψ_m than that at the forest edge (Figure 3B), as the water supply in floodplains is ensured by both groundwater and the current precipitation (Li et al. 2007*a*). In contrast to our expectations, larch trees growing on north-facing slopes had lower values of Ψ_m than the trees at the forest edge. The trend for higher $\delta^{13}C$ values in larch trees from the forest interior than from the forest line matches with these Ψ_m values (Peuke et al. 2006), though the difference in the $\delta^{13}C$ values was not large enough to be statistically significant. The more critical water relations in trees from the forest interior could theoretically be due to a higher competition for water as a result of the higher stand density in the forest interior than at the forest edge. However, higher stomatal conductance in the forest interior than at the forest line (Figure 4A) suggest that the larch trees on the northern slopes are less adapted to drought than the trees at the forest line. Conifers adapt to microclimate already during seed formation (Greenwood and Hutchinson 1996, Rehfeldt et al. 1999). Further, the strong selection pressure on the germinating seedlings at the forest edge is likely to promote those genotypes that are most efficiently adapted to drought. The higher drought stress observed in larch trees on north-facing slopes than at the forest edge to the steppe in the present study might be the result of a recent increase in aridity due to the global late 20th century warming.

Table 3. Points of zero turgor (Ψ_0) as well as minimum (Ψ_m) and maximum (Ψ_p) shoot water potentials (Ψ_m) in *L. sibirica* growing at the forest edge on the steppe or in the interior of light taiga forests at Mt. Bogd Uul, southern Khentey. Asterisks indicate significant differences between Ψ_0 and Ψ_m (Bonferroni-corrected *t* test, $P \leq 0.05$, d.f. = 5–6).

MPa	Month	Forest edge	Forest interior
Ψ_p	July	−0.99 ± 0.03	−0.98 ± 0.03
	August	−0.52 ± 0.02	−0.55 ± 0.02
Ψ_m	July	−2.29 ± 0.03	−2.26 ± 0.01
	August	−1.53 ± 0.03	−1.52 ± 0.04
Ψ_0	July	−1.90 ± 0.13*	−2.09 ± 0.04*
	August	−1.87 ± 0.06*	−1.88 ± 0.07*

Low $\delta^{13}C$ values in *L. sibirica* can also be caused by herbivores, as Li et al. (2007*b*) found lower values in newly emerged needles after an herbivore attack than in needles formed during foliation at the beginning of the growing season. The larch forest of the present study was subject to heavy infestation by gypsy moth (*Lymantria dispar* L.) in 2005 and to a minor invasion in 2006. Moreover, gypsy moth caused significantly more damage at the forest line to the steppe than in the forest interior (Hauck et al. 2008). However, the difference found for Ψ_m and stomatal conductance between the forest line and the forest interior in our study indicates that water supply, rather than the gypsy moth attack, was the main cause of the trend for more negative $\delta^{13}C$ values at the forest edge than in the forest interior.

Our data from the mountain taiga in the western Khentey and from the forest-steppe belt in the southern Khentey show that *L. sibirica* often suffers from drought stress both at its southernmost distribution limit (Table 3) and in the less arid mountain sites of northernmost Mongolia (Figure 1A, B). Whether trees in larch forests occurring as outposts within grasslands at the southern distribution limit of *L. sibirica* (Hilbig et al. 2004) are subject to more severe drought stress than trees growing in the mountain taiga, where steppe only occurs as islands inside the forest (Dulamsuren et al. 2005*a*, 2005*b*), cannot be reliably inferred from our data. Though trees growing both in the forest and on the steppe reached Ψ_m values significantly $< \Psi_0$ in the southern, but not in the western Khentey, any comparison between different regions would require simultaneous long-term measurements of Ψ because of the considerable interannual variation of precipitation and temperature in the highly continental Mongolia (Nandintsetseg et al. 2007).

The studied trees of *L. sibirica* were well able to deal with the high-light conditions in the forest-steppe ecotone. The capacity to dissipate surplus light energy as heat in the PSII antennae (NPQ) together with the energy quenching through alternative electron sinks was sufficient even in the larch trees growing under the high-light conditions of the forest edge to prevent photoinhibition or photodestructive damage (Figure 5E). This was obvious from the rapid and complete recovery of the PSII efficiency (Φ_2) to values > 0.8 in all trees after sunset (Figure 5C). However, the strong mismatch between stable ETR (Figure 5D) and steadily declining CO_2 uptake (Figure 5C) suggests intense dissipation of excess electrons through either photorespiration or the water–water cycle or both in addition to NPQ (Niyogi 2000, Ort and Baker 2002). The photoprotective role of the photorespiratory pathway was extensively demonstrated on transgenic tobacco plants with increased and reduced capacity for photorespiration as well as on wild-type soybean plants (Kozaki and Takeba 1996, Jiang et al. 2006), whereas Asada (1999, 2000) clearly showed the close association of the water–water cycle with light protection.

Though our data on the present water relations in *L. sibirica* suggest that the species does not have the potential to colonize new sites in Mongolia's forest-steppe ecotone, which are presently covered with grasslands, our results do not allow us to conclude as to whether the present vegetation pattern with forests on north-facing slopes and steppes on south-facing slopes is natural or was originally created by anthropogenic activities. Studies on the vegetation history of Mongolia suggest that the significance of anthropo-zoogenic factors for the formation of the present landscape differs between regions. Pollen analyses of our particular study area at Khonin Nuga in the western Khentey showed that the present vegetation pattern existed throughout the late Holocene, and this pattern probably reflects the spatial variation of microclimate (Schlütz et al. 2008). In Khonin Nuga, this conclusion is supported by convincing evidence that the area is avoided by pastoral nomads (Schlütz et al. 2008). In other more densely populated areas of Mongolia, which are more attractive for livestock breeding than Khonin Nuga, human impact is thought to have modified the natural, climate-dependent pattern of forests and grasslands to the disadvantage of the former (Hilbig 1995, Rösch et al. 2005, Miehe et al. 2007).

Conclusions

The results of the present study show that the trees of *L. sibirica* growing at the forest line to the steppe at the southern distribution limit of the northern Eurasian coniferous forest belt regularly suffer from drought stress. Comparison of Ψ_m and Ψ_0 values revealed that the water relations in *L. sibirica* were often critical, though the study years were no particular drought years. This suggests that *L. sibirica* is currently unable to encroach on the steppe at the studied forest-steppe ecotones, but that the drought years could readily cause a retreat of the forest line. The low potential of *L. sibirica* to colonize areas currently covered with grasslands is supported by the observation that young individuals were particularly susceptible to drought stress (Figure 1B). Strongly increasing temperatures in the forested areas of Mongolia in recent times and regionally decreasing precipitation (Batima et al. 2005, Sato and Kimura 2006, Sato et al. 2007) suggest that the potential of *L. sibirica* to encroach onto the steppe in the forest-steppe ecotone of Mongolia will deteriorate in future. Lower shoot water potentials and higher stomatal conductance in trees from the interior of larch forests on north-facing slopes than in trees growing at the forest line to the steppe could be due to a recent increase in aridity during the lifetime of the trees and thus forewarn of future declines of *L. sibirica* at these sites. A retreat of *L. sibirica* from the forest-steppe ecotones would equal a decline of forests per se, as *L. sibirica* covers around 80% of Mongolia's forested area (Savin et al. 1978, Gunin et al. 1999).

Acknowledgments

This study was supported by grants of the German Science Foundation (Deutsche Forschungsgemeinschaft) to Ch. Dulamsuren (Du 1145/1–1, 1–2). The authors are thankful to Prof. Dr. Michael Mühlenberg (Center of Nature Conservation, University of Göttingen) for making the facilities of Khonin Nuga Research Station (a biological field station jointly run by the University of Göttingen and the National University of Mongolia, Ulan Bator) available to the authors. Dr. Doris Möller, Denise Schwerdtner (University of Göttingen), Dr. S. Byambasuren (Ulan Bator, †) and T. Sain-Erdene (National University of Mongolia, Ulan Bator) helped with the measurements of the shoot water potentials.

Prof. Dr. Milan Chytrý (Masaryk University, Brno, Czech Republic) and an unknown reviewer are thanked for their helpful comments.

References

Aizen, E.M., V.B. Aizen, J.M. Melack, T. Nakamura and T. Otha. 2001. Precipitation and atmospheric circulation patterns at mid-latitudes of Asia. Int. J. Climatol. 21: 535–556.

Asada, K. 1999. The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:601–639.

Asada, K. 2000. The water–water cycle as alternative photon and electron sink. Philos. Trans. R. Soc. Lon. B Biol. Sci. 355:1419–1430.

Badalotti, A., T. Anfodillo and J. Grace. 2000. Evidence of osmoregulation in *Larix decidua* at alpine forest line and comparative responses to water availability of two co-occurring evergreen species. Ann. For. Sci. 57:632–633.

Batima, P., L. Natsagdorj, P. Gombluudev and B. Erdenetsetseg. 2005. Observed climate change in Mongolia. Assess. Impacts Adapt. Climate Change Work. Pap. 12:1–26.

Böhner, J. and F. Lehmkuhl. 2005. Environmental change modelling for Central and High Asia: Pleistocene, present and future scenarios. Boreas 34:220–231.

Chytrý, M., J. Danihelka, S. Kubešová et al. 2008. Diversity of forest vegetation across a strong gradient of climatic continentality: western Sayan Mountains, southern Siberia. Plant Ecol. 196:61–83.

Dinesman, L.G., A.V. Knyazev and N.K. Kiseleva. 1989. History of the steppe ecosystems of the Mongolian People's Republic. Nauka, Moscow, 215 p (in Russian).

Dulamsuren, Ch. and M. Hauck. 2009. Spatial and seasonal variation of climate on steppe slopes of the northern Mongolian mountain taiga. Grassl. Sci. (in press).

Dulamsuren, Ch., M. Hauck and M. Mühlenberg. 2005*a*. Vegetation at the taiga forest-steppe borderline in the western Khentej Mountains, northern Mongolia. Ann. Bot. Fenn. 42:411–426.

Dulamsuren, Ch., M. Hauck and M. Mühlenberg. 2005*b*. Ground vegetation in the Mongolian taiga forest-steppe ecotone does not offer evidence for the human origin of grasslands. Appl. Veg. Sci. 8:149–154.

Dulamsuren, Ch., M. Hauck and M. Mühlenberg. 2008. Insect and small mammal herbivores limit tree establishment in northern Mongolian steppe. Plant Ecol. 195:143–156.

Etzelmüller, B., E.S.F. Heggem, N. Sharkhuu, R. Frauenfelder, A. Kääb and C. Goulden. 2006. Mountain permafrost distribution modelling using a multi-criteria approach in the Hövsgöl area, northern Mongolia. Permafrost Periglac. Process 17:91–104.

Giese, E. and I. Moßig. 2004. Klimawandel in Zentralasien. Schriftenr. Zentr Int. Entw. Umweltforsch. 17:1–70.

Greenwood, M.S. and K.W. Hutchinson. 1996. Genetic aftereffects of increased temperature in *Larix*. *In* Proceedings 1995 Meeting of the Northern Global Change Program, 14–16 March 1995. Eds. J. Hom, R. Birdsey and K. O'Brian. USDA. Forest Service, Pittsburgh, PA, pp 56–62.

Gunin, P.D., E.A. Vostokova, N.I. Dorofeyuk, P.E. Tarasov and C.C. Black. 1999. Vegetation dynamics of Mongolia. Kluwer, Dordrecht, 233 p.

Hauck, M., Ch. Dulamsuren and C. Heimes. 2008. Effects of insect herbivory on the performance of *Larix sibirica* in a forest-steppe ecotone. Environ. Exp. Bot. 62:351–356.

Henderson, K., A. Laube, H.W. Gäggeler, S. Olivier, T. Papina and M. Schwikowski. 2006. Temporal variations of accumulation and temperature during the past two centuries from Belukha ice core, Siberian Altai. J. Geophys. Res. 111:D03104, doi:10.1029/2005JD005819.

Hilbig, W. 1995. The vegetation of Mongolia. SPB, Amsterdam, 258 p.

Hilbig, W., E.J. Jäger and H.D. Knapp. 2004. Die Vegetation des Bogd-uul bei Ulaanbaatar (Mongolei) – Standortsbindung und pflanzengeographische Stellung. Feddes Repert. 115:265–342.

Jacoby, G.C., R.D. D'Arrigo and T. Davaajamts. 1996. Mongolian tree-rings and 20th-century warming. Science 273:771–773.

Jiang, C.-D., H.-Y. Gao, Q. Zou, G.-M. Jiang and L.-H. Li. 2006. Leaf orientation, photorespiration and xanthophyll cycle protect young soybean leaves against high irradiance in the field. Environ. Exp. Bot. 55:87–96.

Kozaki, A. and G. Takeba. 1996. Photorespiration protects C_3 plants from photooxidation. Nature 384:57–560.

Kreeb, K. 1961. Zur Frage des negative Turgors bei mediterranen Hartlaubpflanzen unter natürlichen Bedingungen. Planta 56:479–489.

Lapenis, A., A. Shvidenkow, D. Shepaschenkoz, S. Nilsson and A. Aiyyer. 2005. Acclimation of Russian forests to recent changes in climate. Glob. Change Biol. 11:2090–2102.

Lavrenko, E.M. and Z.V. Karamysheva. 1993. Steppes of the former Soviet Union and Mongolia. *In* Natural Grasslands. Eastern Hemisphere and Résumé. Ed. R.T. Coupland. Elsevier, Amsterdam, pp 3–59.

Li, S.-G., J. Asanuma, A. Kotani, W. Eugster, G. Davaa, D. Oyunbaatar and M. Sugita. 2005*a*. Year-round measurements of net ecosystem CO_2 flux over a montane larch forest in Mongolia. J. Geophys. Res. 110:D09303, doi:10.1029/2004JD005453.

Li, S.-G., J. Asanuma, A. Kotani, W. Eugster, G. Davaa, D. Oyunbaatar and M. Sugita. 2005*b*. Net ecosystem carbon dioxide exchange over grazed steppe in central Mongolia. Glob. Change Biol. 11:1941–1955.

Li, S.-G., M. Tsujimura, A. Sugimoto, L. Sasaki, T. Yamanaka, G. Davaa, D. Oyunbaatar and M. Sugita. 2006. Seasonal variation in oxygen isotope composition of waters for a montane larch forest in Mongolia. Trees 20:122–130.

Li, S.-G., H. Romero-Saltos, M. Tsujimura, A. Sugimoto, L. Sasaki, G. Davaa and D. Oyunbaatar. 2007*a*. Plant water sources in the cold semiarid ecosystem of the upper Kherlen river catchment in Mongolia: a stable isotope approach. J. Hydrol. 333:109–117.

Li, S.-G., M. Tsujimura, A. Sugimoto, G. Davaa, D. Oyunbaatar and M. Sugita. 2007*b*. Temporal variation of $\delta^{13}C$ of larch leaves from a montane boreal forest in Mongolia. Trees 21:479–490.

Miehe, G., F. Schlütz, S. Miehe, L. Opgenoorth, J. Cermak, R. Samiya, E.J. Jäger and K. Wesche. 2007. Mountain forest islands and Holocene environmental changes in Central Asia: a case study from the southern Gobi Altay, Mongolia. Palaeogeogr. Palaeoclimatol. Palaeoecol. 250:150–166.

Nandintsetseg, B., J.S. Greene and C.E. Goulden. 2007. Trends in extreme daily precipitation and temperature near Lake Hövsgöl, Mongolia. Int. J. Climatol. 27:341–347.

Niyogi, K.K. 2000. Safety valves for photosynthesis. Curr. Opin. Plant Biol. 3:455–460.

Ort, D.R. and N.R. Baker. 2002. A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Curr. Opin. Plant Biol. 5:193–198.

Parker, W.C. and S.G. Pallardy. 1987. The influence of resaturation method and tissue type on pressure–volume analysis of *Quercus alba* L. seedlings. J. Exp. Bot. 28:535–549.

Peuke, A.D., A. Gessler and H. Rennenberg. 2006. The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. Plant Cell Environ. 29:823–835.

Piñol, J. and A. Sala. 2000. Ecological implications of xylem cavitation for several Pinaceae in the Pacific Northern USA. Funct. Ecol. 14:538–545.

Rehfeldt, G.E., C.C. Ying, D.L. Spittlehouse and D.A. Hamilton. 1999. Genetic responses to climate in *Pinus contorta*: niche breadth, climate change, and reforestation. Ecol. Monogr. 69:375–407.

Rhizopoulou, S. 1997. Is negative turgor fallacious? Physiol. Plant. 99:505–510.

Roberts, S.W., B.R. Strain and K.R. Knoerr. 1980. Seasonal patterns of leaf water relations in four co-occurring forest tree species: parameters from pressure–volume curves. Oecologia 46:330–337.

Roháček, K. 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. Photosynthetica 40:13–29.

Rösch, M., E. Fischer and T. Märkle. 2005. Human diet and land use in the time of the Khans – archaeobotanical research in the capital of the Mongolian Empire, Qara Qorum, Mongolia. Veg. Hist. Archaeobot. 14:485–492.

Sankey, T.T., C. Montagne, L. Graumlich, R. Lawrence and J. Nielsen. 2006. Lower forest-grassland ecotones and 20th century livestock herbivory effects in northern Mongolia. For. Ecol. Manag. 233:36–44.

Sato, T. and F. Kimura. 2006. Regional climate simulations to diagnose environmental changes in Mongolia. Bull. Terr. Environ. Res. Cent. Univ. Tsukuba 7:59–69.

Sato, T., F. Kimura and A. Kitoh. 2007. Projection of global warming onto regional precipitation over Mongolia using a regional climate model. J. Hydrol. 333:144–154.

Savin, E.N., A.V. Ogorodnikov, I.V. Semechkin, Ch. Dugarzhov and I.A. Korotkov. 1978. Forests of the Mongolian People's Republic. Nauka, Moscow, 127 p (in Russian).

Schlütz, F., Ch. Dulamsuren, M. Wieckowska, M. Mühlenberg and M. Hauck. 2008. Late Holocene vegetation history suggests natural origin of steppes in the northern Mongolian mountain taiga. Palaeogeogr. Palaeoclimatol. Palaeoecol. 261:203–217.

Scholander, P.F., H.T. Hammel, E.A. Hemmingsen and E.D. Bradstreet. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. Natl. Acad. Sci. USA 52:119–125.

Sugimoto, A., N. Yanagisawa, D. Naito, N. Fujita and T.C. Maximov. 2002. Importance of permafrost as a source of water for plants in east Siberian taiga. Ecol. Res. 17: 493–503.

Tchebakova, N.M., G.E. Rehfeldt and E.I. Parfenova. 2005. Impacts of climate change on the distribution of *larix* spp. and *Pinus sylvestris* and their climatypes in Siberia. Mitigat. Adapt. Strat. Glob. Change 11:861–882.

Treter, U. 2000. Recent extension and regeneration of the larch forest in the mountain forest steppe of north-west Mongolia. Marburger Geogr. Schr. 135:156–170.

Velisevich, S.N. and D.S. Kozlov. 2006. Effects of temperature and precipitation on radial growth of Siberian larch in ecotopes with optimal, insufficient, and excessive soil moistening. Russ. J. Ecol. 37:241–246.

Vostokova, E.A. and P.D. Gunin Eds. 2005. Ecosystems of Mongolia, Atlas. Russian Academy of Sciences, Moscow, 48 p.

Wallis de Vries, M.F., N. Manizabar and S. Dügerlham. 1996. The vegetation of the forest-steppe region of Hustain Nuruu, Mongolia. Vegetatio 122:111–127.

Walter, H. and S.-W. Breckle. 1994. Ökologie der Erde. Band 3. Spezielle Ökologie der gemäßigten und arktischen Zonen Euro-Nordasiens: Zonobiom VI–IX. 2nd Edn. Fischer, Stuttgart, 726 p.

Wang, J.R. 2005. Spring and summer hydraulic conductivity of 14 woody species of the sub-boreal forest in British Columbia. Can. J. For. Res. 35:2727–2733.

Chapter 6

Performance of Siberian elm (*Ulmus pumila*) on steppe slopes of the northern Mongolian mountain taiga: Drought stress and herbivory in mature trees

Environmental and Experimental Botany 66 (2009) 18–24

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Performance of Siberian elm (*Ulmus pumila*) on steppe slopes of the northern Mongolian mountain taiga: Drought stress and herbivory in mature trees

Choimaa Dulamsuren[a,b], Markus Hauck[a,*], Suran Nyambayar[c], Martin Bader[d], Dalaikhuu Osokhjargal[c], Shagjjav Oyungerel[c], Christoph Leuschner[a]

[a] *Department Plant Ecology, Albrecht von Haller Institute of Plant Sciences, University of Göttingen, D-37073 Göttingen, Germany*
[b] *Center of Nature Conservation, University of Göttingen, Von-Siebold-Straße 2, D-37075 Göttingen, Germany*
[c] *Faculty of Biology, National University of Mongolia, Ikh Surguuliin Gudamj 1, 210646 Ulaanbaatar, Mongolia*
[d] *Botanical Institute, University of Basel, CH-4056 Basel, Switzerland*

ARTICLE INFO

Article history:
Received 16 May 2008
Received in revised form 16 December 2008
Accepted 28 December 2008

Keywords:
Ulmus pumila
Steppe
Shoot water potential
Age structure
Fertility
Herbivory

ABSTRACT

Vegetation, soil conditions, age structure, water relations, light response of the chlorophyll fluorescence yield, and herbivore damage were studied in native savanna-like stands of Siberian elm (*Ulmus pumila*) on south-facing slopes in the mountain taiga of the western Khentey Mountains in northern Mongolia. These stands limited to the middle and lower parts of the slopes consist of single trees or small groups of trees of up to 5 m height. Parts of the tree crowns are characteristically dead. The stands have a marked shrub layer of, e.g., small individuals of *U. pumila* as well as *Spiraea aquilegifolia*, *Padus asiatica*, and *Ribes diacantha*, and a diverse, but patchy ground vegetation. *U. pumila* is limited to loose, deep, stony soils on the slopes, which preferably occur near rock outcrops, mostly in contact to mountain steppe. Fine-grained, non-skeletal soils are avoided by *U. pumila*, but inhabited by meadow steppe. The preference for stony soils is attributable to the high water requirements of *U. pumila*, as rock cracks are known to form a reservoir for rain and dew water. The high demand for water is a partly a consequence of a low VPD sensitivity of the stomata regulation known for *U. pumila*. High transpiration rates may help *U. pumila* to avoid elevated leaf temperatures under high solar irradiation on the exposed steppe slopes. Increasing non-photochemical quenching at a $\text{PPFD} > 2000\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ gives additional evidence of the good adaptation of *U. pumila* to sunlit environments. Minimum shoot water potentials far above the point of zero turgor throughout the growing season suggest sufficient water supply in summer in years with average rainfall. Literature data indicate that *U. pumila* can also suffer lethal damage on the rocky slopes during exceptionally dry summers. Insect herbivory was of subordinate significance for the elm trees. Therefore, desiccation is thought to be the main cause of the frequent occurrence of dead crown parts and the early death of the elm trees on the sun-exposed slopes. Mean age of the sample trees was as low as 17 years. At this age, the trees are not fertile, but proliferate vegetatively. The low lifespan of *U. pumila* on the sun-exposed slopes suggests that these slopes are a suboptimal habitat for this tree species. The dependence on loose, stony soils alone explains the irregular distribution of *U. pumila* on the sun-exposed slopes and rules out the formation of closed elm forest at these sites.

1. Introduction

Ulmus pumila L. is regularly found in savanna-like stands on sun-exposed slopes of northern Mongolia, which are otherwise covered with steppe grasslands (Hilbig, 1995; Dulamsuren et al., 2005a,b). In contrast to valleys of permanently or periodically flooded rivers, where *U. pumila* trees can reach a height of up to 20 m and an age of more than 200 years (Blažková, 1985; Lindeman et al., 1994), this species is usually only 1–5 m high on sun-exposed slopes (Lindeman et al., 1994) suggesting a low age of these trees. Significant parts of the tree crowns on the sun-exposed slopes are often dead (Lindeman, 1981). In addition to the formation of the savanna-like tree layer, the often dense shrub layer of these stands is partly formed by *U. pumila* (Hilbig, 1995; Dulamsuren et al., 2005b).

On the sun-exposed slopes, the elm trees never form dense forests, but are scattered as single trees or small groups over the slopes, which are otherwise covered with different communities of steppe grasslands (Dulamsuren et al., 2005a,b). Whether this savanna-like distribution is the result of a natural growth limitation due to unfavorable microclimate or edaphic factors, or attack by indigenous herbivores (Lindeman, 1981; Blažková, 1985) or

---

* Corresponding author. Tel.: +49 551 39 5721; fax: +49 551 39 5701.
E-mail address: mhauck@gwdg.de (M. Hauck).

0098-8472/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2008.12.020

whether it is primarily caused by livestock grazing, logging and anthropogenic fire (Hilbig, 1995) is disputed. The present paper sums up the results of a case study in the western Khentey Mountains, northern Mongolia, which was carried out to assess the potential of natural site factors to limit the abundance of *U. pumila* on the sun-exposed slopes. The study area near Khonin Nuga Research Station in the western Khentey is well suited for the investigation, as it has been traditionally avoided by pastoral nomads and as the continuous existence of steppes and *U. pumila* stands on the south-facing slopes throughout the late Holocene has been demonstrated by pollen analysis in this region (Schlütz et al., 2008).

Our investigations included the analysis of the vegetation of the *U. pumila* stands and the description of the supporting soils, measurements of the shoot water potential, the recording of light-response curves of the chlorophyll fluorescence yield at photosystem II (PS II), the assessment of the susceptibility to insect herbivory, and the determination of the age structure of mature *U. pumila* stands. The study aim was to test the hypotheses that (1) the elm trees are young despite their often partly dead crowns, (2) the elm trees suffer from drought stress in dry seasons, (3) the elm trees do not suffer from photoinhibition during periods of high solar irradiation on the south-facing steppe slopes, and (4) the elm trees are subject to substantial insect herbivory.

2. Materials and methods

2.1. Study sites

Elm stands at two south-facing slopes were studied near Khonin Nuga Research Station (49°04′48″ N, 107°17′15″ E) in the western Khentey Mountains, northern Mongolia, Mandal county (Mandal somon), 130 km north of Ulan Bator. Site A was located at Mt. Baziin Am at 1050–1180 m (49°2′ N, 107°15′ E) and site B at Mt. Bayantogol (1020–1120 m, 49°5′ N, 107°17′ E) (Dulamsuren et al., 2008). While site A is on the northern shore of the River Eroo, the river floating at the foot of the slope, site B faces the valley of a small tributary creek. The slopes are steeply inclined (up to 60°) and have a size of c. 1.5 km × 250 m (parallel to × perpendicular to the contour lines) at site A and of c. 2 km × 350 m at site B. A survey of the climate, geology, vegetation, and present land use intensity of the study area is given in Dulamsuren et al. (2005a) and Dulamsuren and Hauck (2008). Information on the vegetation history and the past land use is compiled in Schlütz et al. (2008).

2.2. Vegetation and soil description

The vegetation of 24 plots of a size of 15 m × 15 m was quantitatively analyzed in savanna-like *U. pumila* stands at sites A and B by estimating the cover of all vascular plant species in percent. Sample plots were non-randomly selected in the irregularly distributed *U. pumila* stands and checked for homogeneity of the physiognomy and ground vegetation. Thirteen relevés were recorded from the lower slopes and eleven from the middle slopes of the two sites. *U. pumila* was generally absent from the upper slopes. Non-random selection of sample plots for vegetation analysis was justified, as this analysis aimed at providing information on the vegetation characteristics of the *U. pumila* stands, but was not used for multivariate analysis to detect correlations with potential site factors or to describe plant communities. Woody plants of more than 2 m in height were classified as trees and below that height as shrubs. Nomenclature of plant species follows Gubanov (1996). In every plot, a soil profile was dug and the structure and depth of soil horizons were recorded. Additional 24 soil profiles were dug in the treeless meadow steppe of the open slopes to compare its soil structure with that of sites inhabited by *U. pumila*. Percent values in the soil descriptions (Table 3) are consistently expressed by volume.

2.3. Microclimate

Data from a HOBO weather station (Onset, Bourne, MA, USA) were used for the interpretation of shoot water potential measurements at site A. The weather station, located in the open meadow steppe, was equipped with a H21 data logger and a set of HOBO Smart Sensors including instruments for measuring air temperature and relative air humidity at 150 cm above soil level (temperature/RH sensor S-THA), soil temperature at 1 and 15 cm depth (8 Bit temperature sensor S-TMA), photon flux density of photosynthetically active radiation (PPFD) in horizontal position at 2 m above soil level (PAR sensor S-LIA), and precipitation at 1 m height (rain gauge sensor S-RGA). Data were recorded every 10 min and are means of ten iterative measurements. The atmospheric vapor pressure deficit (VPD) was calculated using the equation VPD (kPa) = E_0 (kPa) − E (kPa), with E_0 (kPa) = 0.6108 kPa × exp ((17.27[dew point temperature (°C)])/([dew point temperature (°C)] + 237.3)) and E (kPa) × = 0.6108 kPa × exp ((17.27[air temperature (°C)])/([air temperature (°C)] + 237.3)).

2.4. Shoot water relations

The plant water status was assessed by measuring water potentials (Ψ) in sun-exposed shoots once a month from May to September 2006 in a group of *U. pumila* trees at site A. Five trees were measured as replicates, and from each tree, five twigs were selected as subsamples. The measurements were done on rainless days with clear sky with a Model 1000 Pressure Chamber Instrument (PMS Instrument Company, Albany, OR, USA) applying the method of Scholander et al. (1964). Monthly measurements were limited to the recording of the predawn water potential (Ψ_p), which represents the daily maximum water potential, and of the midday water potential (Ψ_m), which equals the daily minimum water potential. In addition, pressure–volume curves were recorded in triplicate every month in three fully saturated shoots of a single tree. The pressure–volume curves were used to determine the point of zero turgor (Ψ_0) (Roberts et al., 1980). Ψ_0 is of interest insofar as the risk of irreversible cell damage due to cytorrhysis increases with Ψ decreasing below this point. In addition to the monthly measurements of Ψ_p and Ψ_m, the diurnal variation of Ψ was exemplarily measured in the mid of the growing season on 4 August 2006 in one tree. All measurements of Ψ were carried out in 2-year-old twigs collected from the south-facing side of the tree at 1.5–2.0 m above the ground. Measurements comparing twigs from different height and of different age showed that these factors had little influence on Ψ in the small elm trees studied (Table 1).

Table 1
Effects of twig age and height on daily maximum (Ψ_p) and minimum (Ψ_m) shoot water potentials in *U. pumila*. Measurements from south-facing twigs of one tree with five subsamples on 28 July 2006.

Height	Ψ (MPa)		
	1 year	2 years	3 years
Ψ_p			
0.5–1.0 m	−0.13 ± 0.01	−0.15 ± 0.00	−0.17 ± 0.01
1.5–2.0 m	−0.12 ± 0.01	−0.14 ± 0.02	−0.02 ± 0.02
2.0–2.5 m	−0.12 ± 0.01	−0.12 ± 0.01	−0.15 ± 0.00
Ψ_m			
0.5–1.0 m	−1.33 ± 0.06	−1.08 ± 0.01	−1.25 ± 0.02
1.5–2.0 m	−1.55 ± 0.04	−1.30 ± 0.06	−1.43 ± 0.04
2.0–2.5 m	−1.00 ± 0.05	−1.32 ± 0.07	−1.08 ± 0.03

2.5. Light-response curves

Light-response curves were recorded with a Mini-PAM chlorophyll fluorometer (Walz Mess- und Regeltechnik, Effeltrich, Germany) from two groups of *U. pumila* trees growing in a distance of 500 m from each other at site A by measuring the effective quantum yield (Φ_2) of photochemical energy conversion in photosystem II. Twigs used for these light-response curves were adapted to the dark for 30 min. Five individuals of each group of elm trees were measured including one shoot per tree. PPFD was measured with a sensor integrated in the leaf clip holder of the Mini-PAM; these data were used to calculate the electron transport rate (ETR) based on the equation $\mathrm{ETR} = \Phi_2 \times \mathrm{PPFD} \times 0.42$. The non-photochemical quenching (NPQ) was calculated following the equation $\mathrm{NPQ} = (F_M - F'_M)/F'_M$ with F_M being the absolute maximum fluorescence in the dark-adapted state and F'_M being the absolute maximum fluorescence in the light-adapted state (Roháček, 2002).

2.6. Age structure of U. pumila stands

The age structure of a savanna-like *U. pumila* stand at site A was determined by sampling wood cores with an increment borer. Twelve trees were sampled, four of which, however, had rotten stem cores so that their age could not be determined. In addition, height was estimated in steps of 1 m and trunk diameter was measured in all twelve sample trees.

2.7. Herbivory and drought-related damage on mature elm trees

To assess the severity of herbivory and drought-related damage in mature trees of *U. pumila*, 100 elm trees were studied in August 2005. Sample trees were selected by setting up c. 200–300 m long transects running from the top to the bottom of the slope. Each five transects were studied at sites A and B. As *U. pumila* trees occurred in irregularly distributed clusters on the slopes, transects were selected in a way that they included the first five *U. pumila* stands found on the slopes if approached from the west. Minimum distance between the individual transects was 50 m. The first ten trees along each transect below the mountain top were selected as sample trees. On each tree, ten twig sections each of 20 cm length were randomly chosen. Herbivore damage was assessed by estimating the intact leaf area along each twig section as percent of the original undamaged leaf area. In addition, trees were searched for acute infestation by caterpillars. As parts of the tree crowns of *U. pumila* were often dead, the percentage of dead branches related to the entire tree crown was estimated. All estimations were made in steps of 5%.

In addition to *U. pumila*, the most common shrub species of the *U. pumila* stands, *Spiraea aquilegifolia*, was studied for herbivore damage using the same method as for *U. pumila*. As *S. aquilegifolia* is abundant and quite regularly distributed in the *U. pumila* stands of the western Khentey Mountains, ten shrubs (of 1–2 m in height) in a distance of 10 m between neighboring sample shrubs were selected.

2.8. Statistics

Arithmetic means ± standard error are given throughout the paper. Data were tested for normal distribution with the Shapiro-Wilk test. Pairwise comparison of means (Ψ_m vs. Ψ_0) were made with a Bonferroni-corrected Student's *t*-test. Statistical analyses were computed with SAS 6.04 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Vegetation and soil characteristics of U. pumila stands

The *U. pumila* stands are irregularly distributed on the south-facing slopes and mostly consist of less than ten elm trees growing with a distance of several meters to one another. Most individuals of *U. pumila* do not exceed the height of 2 m and are, thus, listed in the shrub layer in Table 2. Maximum height of the elm trees was around 5 m. Trees other than *U. pumila* are absent from the savanna-like elm woodlands, but the shrub layer, covering between 10% and 60%, consisted, in addition to *U. pumila*, of five other species including *Spiraea aquilegifolia* and *Padus asiatica* (Table 2). The herb layer is highly diverse and resembles to mountain steppes that often grow in spatial contact to the elm woodlands (Dulamsuren et al., 2005b). The total cover of the herb layer widely varies between 40% and 80%, mostly amounting to 40–60%. Overall, a total of 77 vascular plant species was found in the 24 plots. Only 61 species growing in more than two plots are listed in the vegetation survey given in Table 2. Typical plants of the elm stands of the south-facing slopes include *Spiraea aquilegifolia*, *Artemisia gmelinii*, *A. frigida*, *Pulsatilla turczaninovii*, *Poa botryoides*, *Agropyron cristatum*, *Carex korshinskyi*, *C. pediformis*, *Potentilla acervata*, *Stipa sibirica* (Table 2) as well as the rare *Rhamnus erythroxylon*, *Rubia cordifolia*, and *Lespedeza dahurica*.

Savanna-like elm stands on the middle slope were more diverse with 30 ± 1 plant species than those on the lower slope with 24 ± 1 plant species. Some herbs, including *Cerastium cerastoides*, *Orostachys malacophylla*, *Iris tigrida*, or *Rhaponticum uniflorum*, were limited to the middle slopes or occurred here more abundant than at the lower slopes (Table 2). A denser and often more diverse shrub layer with higher dominance of, e.g. *Padus asiatica* and *Ribes diacantha*, than on the middle slopes, was characteristic of the lower slopes. Furthermore, species such as *Artemisia mongolica*, *A. scoparia*, *A. dracunculus*, *A. rupestris*, or *Thalictrum foetidum*, indicating occasional disturbance occurred more abundantly or only at the lower slopes (Table 2).

The soil below the *U. pumila* stands has a loose structure and is rich in stones and rocks. Most of these soils can be classified as Eutric Leptosols. The 10- to 20-cm deep Ah horizon of these soils consists of dark brown, gravelly sand or sandy silt with a single-grain texture and is rich in plant roots, humus, mica and small to medium-sized stones. The latter form 30–80% of the soil volume. The Ah horizon is not clearly separated from the underlying soil layer, which is either an AC, BC or directly the C horizon (Table 3). The AC or BC horizons are formed of notably loose, dark brown sands or sandy silts and are located between boulders and large stones. The deep-reaching roots of perennial plants can penetrate through these loose soil fractions between the rocks. Stones and rocks can constitute up to 90% of the soil volume. Alternating BC and B horizons of a total depth of up to 80 cm, sometimes found at the lower slopes, give evidence of occasional deposition events (Table 3).

Soils below the treeless meadow steppe, which covers most of the south-facing slopes, are also Eutric Leptosols, but differ from the soil below *U. pumila* stands by their low rooting depth (Table 3). The often shallow Ah horizon of fine-grained silty or loamy sands intermixed with pepples overlays a dense AC or C horizon, which prevents most roots from penetrating beneath the Ah horizon.

3.2. Water relations and light-response curves in mature elm trees

The shoot water potential of up to 5-m high trees of *U. pumila* naturally growing on the south-facing slopes was significantly above Ψ_0 throughout the growing season (Fig. 1). However, Ψ_p showed a slight trend for decline and Ψ_m was strongly reduced towards the end of the growing season (Fig. 1). No shoot water

Table 2
Vegetation of savanna-like *U. pumila* stands on south-facing slopes at Khonin Nuga, western Khentey.

	Middle slope ($n = 11$)		Lower slope ($n = 13$)	
	Fr. (%)	Cover	Fr. (%)	Cover
Tree layer				
Ulmus pumila	9	3.0	62	2.0 ± 0.3
Shrub layer				
Ulmus pumila	91	3.8 ± 0.8	100	6.9 ± 1.8
Spiraea aquilegifolia	91	10.0 ± 1.4	92	11.5 ± 2.9
Padus asiatica	18	5.3 ± 1.9	62	4.3 ± 1.1
Ribes diacantha	9	1.0	38	1.8 ± 0.3
Cotoneaster melanocarpus	36	7.3 ± 2.5	8	10.0
Potentilla fruticosa	9	15.0	23	6.0 ± 1.0
Herb layer				
Artemisia gmelinii	100	8.5 ± 1.3	92	7.4 ± 0.9
Pulsatilla turczaninovii	91	2.8 ± 0.4	92	3.5 ± 0.7
Potentilla acervata	100	3.0 ± 0.4	77	2.6 ± 0.5
Carex korshinskyi	82	5.7 ± 0.8	77	5.3 ± 1.0
Poa botryoides	82	8.4 ± 1.7	77	9.8 ± 1.3
Artemisia frigida	91	4.1 ± 0.8	69	3.9 ± 0.8
Stipa sibirica	64	3.6 ± 0.4	85	5.4 ± 1.1
Agropyron cristatum	82	9.8 ± 2.1	69	8.1 ± 0.8
Carex pediformis	82	7.6 ± 1.1	69	9.4 ± 0.9
Patrinia sibirica	82	1.2 ± 0.2	62	1.4 ± 0.3
Potentilla acaulis	82	2.6 ± 0.6	62	3.0 ± 0.5
Koeleria cristata	73	4.0 ± 0.3	62	4.6 ± 1.1
Youngia tenuicaulis	73	1.2 ± 0.2	62	1.0 ± 0.0
Bupleurum scorzonerifolium	55	1.0 ± 0.0	69	0.9 ± 0.0
Artemisia commutata	82	3.8 ± 0.8	38	3.0 ± 0.4
Galium verum	82	1.3 ± 0.3	38	1.0 ± 0.0
Artemisia scoparia	27	1.0 ± 0.0	77	3.1 ± 0.8
Allium leucocephalum	55	0.8 ± 0.1	46	0.8 ± 0.1
Thymus gobicus	55	3.8 ± 1.0	46	2.3 ± 0.5
Chamaerhodos erecta	64	0.9 ± 0.1	38	1.7 ± 0.3
Alyssum lenense	64	1.9 ± 0.3	31	1.9 ± 0.4
Goniolimon speciosum	46	0.6 ± 0.1	38	0.7 ± 0.1
Polygonatum odoratum	46	0.8 ± 0.1	38	1.2 ± 0.3
Silene jeniseensis	46	0.8 ± 0.1	38	1.8 ± 0.3
Scorzonera radiata	55	0.9 ± 0.1	31	0.9 ± 0.1
Schizonepeta multifida	73	1.1 ± 0.2	15	1.0 ± 0.0
Rhaponticum uniflorum	91	1.1 ± 0.2	0	
Peucedanum baicalense	55	0.8 ± 0.1	23	0.8 ± 0.1
Vicia amoena	55	0.9 ± 0.1	23	0.8 ± 0.1
Artemisia mongolica	0	.	62	3.8 ± 0.4
Lilium pumilum	55	0.8 ± 0.1	15	0.5 ± 0.0
Aconogonon angustifolium	64	1.5 ± 0.3	8	1.0
Artemisia dracunculus	0	.	54	10.0 ± 0.0
Serratula centauroides	9	1.0	46	1.7 ± 0.5
Adenophora stenanthina	55	1.0 ± 0.0	8	1.0
Heteropappus biennis	27	0.7 ± 0.1	23	2.3 ± 0.3
Heteropappus hispidus	27	0.8 ± 0.1	23	1.0 ± 0.0
Sedum aizoon	46	0.7 ± 0.1	8	0.5
Cerastium cerastoides	55	0.7 ± 0.1	0	
Potentilla fruticosa	9	1.0	31	1.4 ± 0.3
Spiraea aquilegifolia	18	3.0 ± 0.8	23	2.3 ± 0.7
Orostachys spinosa	27	0.7 ± 0.1	15	2.0 ± 0.4
Potentilla bifurca	9	1.0	23	3.0 ± 0.0
Rheum undulatum	9	0.5	23	0.5 ± 0.0
Alyssum obovatum	27	2.3 ± 0.7	8	3.0
Astragalus melilotoides			23	0.7 ± 0.1
Cotoneaster melanocarpus			23	1.0 ± 0.0
Thalictrum foetidum			23	1.7 ± 0.3
Antennaria dioica	9	1.0	15	2.0 ± 0.4
Allium lenense	18	0.8 ± 0.1	8	3.0
Dontostemon integrifolius	18	0.5 ± 0.0	8	0.5
Leontopodium leontopodioides	18	0.8 ± 0.1	8	0.5
Iris tigrida	27	0.8 ± 0.1	0	.
Orostachys malacophylla	27	0.7 ± 0.1	0	.

Frequency (Fr.) in the relevés and mean cover ± S.E. Species are listed in the sequence of decreasing frequency in the 24 plots.

Table 3
Exemplary soil profiles from steep, south-facing slopes at Khonin Nuga, western Khentey. (a, b) Two *U. pumila* stands from the (a) middle or (b) lower slope as well as (c) two meadow steppe grasslands.

Horizon	Depth (cm)	Soil description
(a) *U. pumila* stand on middle slope		
Ah	0–28	Dark brown, gravelly sand filling gaps between stones; single-grain structure; rich in humus, with many roots, rich in mica; stone content up to 70%
AC	>28	Brown fine- and coarse-grained sand in gaps between the stones; loose single-grain structure; moderate humus content; moderate to high root abundance in the upper 50 cm of the horizon, decreasing with increasing depth; stone content up to 70%
(b) *U. pumila* stand on lower slope		
Ah	0–9	Dark brown, sandy silt; crumbly structure; high humus content, with many roots, rich in mica; 30% gravel
BC1	9–83	Light brown to yellowish brown sandy silt in gaps between gravel and stones; single-grain structure; moderately humic, decreasing with increasing depth; abundance of roots moderate to high; rich in mica; with $CaCO_3$ intrusions in the lower parts; 70% medium-sized and small stones and gravel
BC2	>83	Yellowish loamy sand with limestone deposits; single-grain structure; roots rare; rich in mica; 90% medium-sized and small stones and gravel
(c) Meadow steppe		
Ah1-Ah2-C		
Ah1	0–6	Dark brown, silty sand; crumbly structure; strongly humous, with many roots, rich in mica, 10% gravel
Ah2	6–11	Dark brown, silty sand; crumbly structure; strongly humous, with many roots, 70% stones and gravel
C	>11	Dense skeletal soil with a stone content of 70%; in-between yellowish-brown sand, slightly humous, without roots
Ah-C:		
Ah	0–26	Light brown to brown, downwards paler, slightly loamy medium-sized to fine sand; single-grain structure; moderately humous, with many roots, rich in mica; 30–40% stones gravel.
C	>26	Scree with strongly condensed, gravelly coarse sand; a few roots, becoming rarer with depth

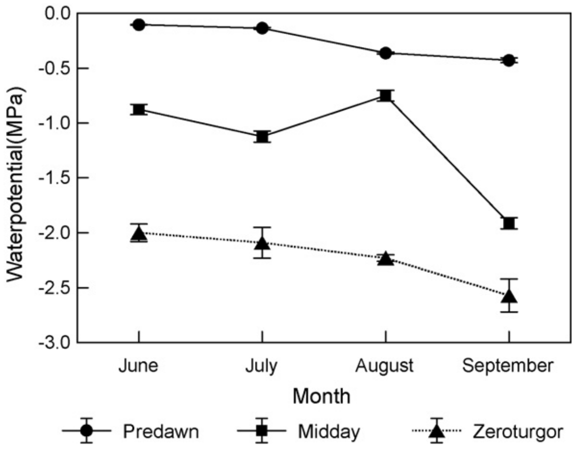


Fig. 1. Shoot water potential of mature *U. pumila* trees of up to 5 m height. Variation during the growing season of the predawn (Ψ_p) and midday potentials (Ψ_m) (5 trees each with 5 subsamples) as well as the zero turgor (Ψ_0; $n = 3$). Ψ_m differed from Ψ_0 on the 5% level throughout the growing season (Bonferroni-corrected *t*-test).

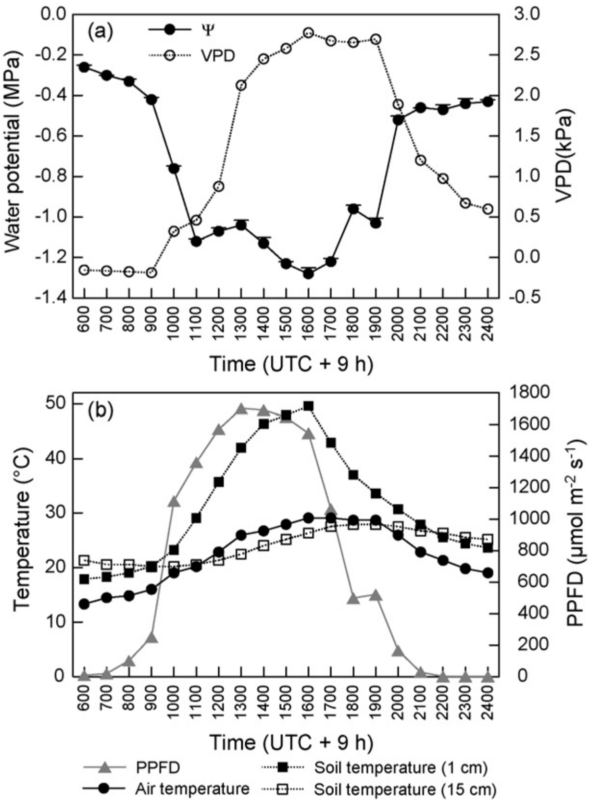


Fig. 2. Shoot water potential of mature *U. pumila* trees of up to 5 m height. Diurnal variation of the shoot water potential and the vapor pressure deficit of the atmosphere (a) and of PPFD, air temperature as well as soil temperature at 1 or 15 cm depth (b) on 4 August 2006.

potential could be measured in May shortly before foliation, because no water could be pressed out of the twigs. During the course of a warm summer day, *U. pumila* was characterized by a rapid decrease of the shoot water potential with increasing VPD, but also by a rapid recovery in the evening (Fig. 2). In the light-response curves, the ETR was saturated between a PPFD of 1500–2000 μmol m^{-2} s^{-1} (Fig. 3a), while NPQ was still increasing at >2000 μmol m^{-2} s^{-1} (Fig. 3b).

3.3. Age structure of *U. pumila* stands

The studied elm trees were between 8 and 35 years old; mean age amounted to 17 ± 3 years (Fig. 4). Neither height (2–5 m) nor diameter (3–11 cm) was significantly correlated with age (Fig. 4).

3.4. Herbivory and drought-related damage in mature elm trees

Between 5% and 10% of the leaf area of native elm trees were consumed by herbivores. The intact leaf area without herbivore damage (as a fraction of the original leaf area) amounted to 94 ± 0% at site A and 93 ± 1% at site B. On, respectively, 16 ± 9% (site A) or 32 ± 9% (site B) of the trees, caterpillars were found. A total of 15 ± 2% (site A) or 11 ± 1% (site B) of the tree crown volume of *U. pumila* was dry and dead. *Spiraea aquilegifolia* was less attacked by herbivores than *U. pumila* with only 1 ± 0% of the leaf area lost due to herbivory at either site. Drought-related damage was virtually absent from *S. aquilegifolia*.

4. Discussion

The data suggest that *U. pumila* trees growing in savanna-like stands on the south-facing slopes of northern Mongolia are usually not limited by drought nor are they sensitive to photoinhibition during summer drought. Resistance to high solar irradiation even in periods of summer drought is inferred from increasing non-photochemical quenching with increasing light influx even at a PPFD > 2000 μmol m^{-2} s^{-1} (Fig. 3). Such adaptation comes up to expectations, as *U. pumila* generally inhabits sun-exposed sites, including deserts (Lindeman et al., 1994). Minimum shoot water potentials far above zero turgor throughout the growing season indicate that the elm trees are not significantly affected by water shortage at the leaf level (Fig. 1). The rapid recovery of the shoot water potential after sunset (Fig. 2) also suggests that the elm trees

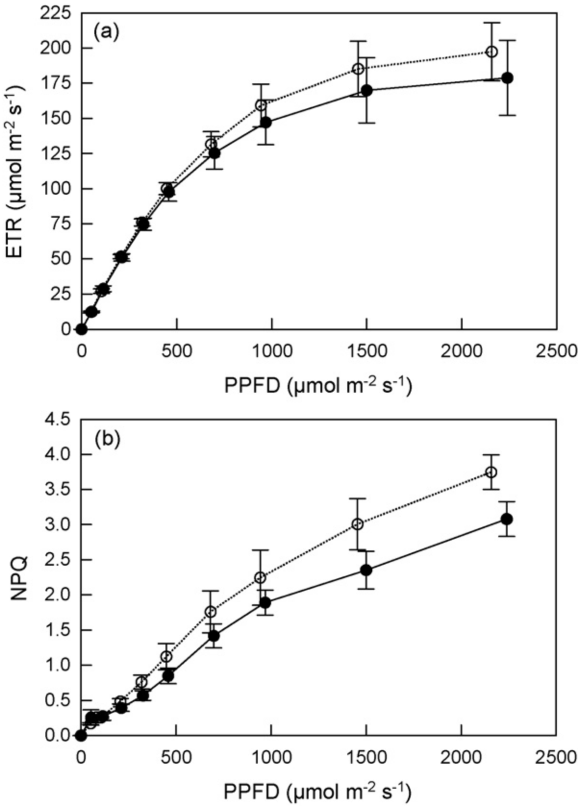


Fig. 3. Light-response curves of ETR (a) and NPQ (b) in mature *U. pumila* trees of up to 5 m height. Different marker and line types represent two collectives each of five trees.

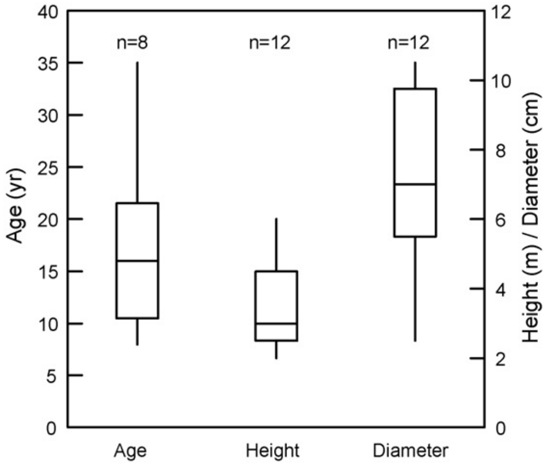


Fig. 4. Age structure, tree height and trunk diameter of a savanna-like *U. pumila* stand at site A. Boxplots with median as well as lower and upper quartiles and extreme values.

trap sufficient water, probably through deep-reaching roots. Since *U. pumila* controls its transpiration less efficiently than many other woody plants of arid environments (Li et al., 2003), as is, for example, shown by high δ ^{13}C values (Chen et al., 2007), the large difference between the daily minimum shoot water potentials and the point of zero turgor can be attributed to the potential of *U. pumila* for effective osmotic adjustment (Liu et al., 2003; Chen et al., 2007).

The poorly regulated transpiration, which is linked with the advantage that leaves are cooled during hot and dry weather (Blažková, 1985; Li et al., 2003), is the cause why, albeit intolerant to water-logging, *U. pumila* requires contact to the groundwater table. Hence, it preferably grows on terraces in floodplains or dry riverbeds which are underlain with water-bearing soil layers (Blažková, 1985; Lindeman et al., 1994). The demand for constant water supply explains why *U. pumila* was found in the present study on deep, loose, stony soils, preferably in the vicinity of rock outcrops. This preference agrees with observations from other sites in Mongolia (Lindeman et al., 1994; Hilbig, 1995) and can be attributed to the ability of *U. pumila* to penetrate with its roots several meters into the earth through rock crevices (Lindeman et al., 1994). Though *U. pumila* has a fast-growing root system, it is hardly conceivable that the roots can always reach the groundwater table below the slopes (Li et al., 2007). Rather, the roots are thought to obtain rainwater that is collected in the rock crevices and from dewfall that precipitates on the rocks at nighttime (Lindeman, 1981).

Though the point of zero turgor was not met in the study period, *U. pumila* growing on rocky slopes can suffer from drought-related damage in extremely hot summers, as demonstrated by Lindeman (1981) for the summers 1972 and 1974. Desiccation is probably the main cause for the withering of crowns and the low age of the *U. pumila* trees on the steppe slopes. The low age of the trees of up to 35 years (Fig. 4) was confirmed with single samples taken from other slopes of the study area where an age of 45 years was never exceeded. Moreover, it agrees with results of Lindeman (1981), who specified the maximum age of *U. pumila* as varying between 20 and 40 years at similar sites. Relatively small xylem vessels in *U. pumila* (Solla et al., 2005) reduce the risk of cavitation and support the conclusion that drought-related damage in *U. pumila* is limited to severe drought periods. Rich, uniform epiphytic lichen vegetation on the dead parts of the tree crowns (Hauck et al., 2007) indicates that these branches died concurrently in the same drought period and thus supports the idea of drought-related damage being a rare, but regular event in *U. pumila*. The lack of sap flow in spring before foliation is probably not an indication of winter desiccation, but owed to the ring-porous wood type of *U. pumila* (Solla et al., 2005).

By its dependence on loose, stony and rocky soils, the limited ability of *U. pumila* to close its stomata at high VPD, once evolved to withstand high solar irradiation (Blažková, 1985), inhibits the encroachment of the whole steppe slopes by *U. pumila*. Although seedlings of *U. pumila* are able to grow on shallow, a few decimeter deep soils, which prevail below most grassland communities of the steppe slopes (Dulamsuren, 2004; Dulamsuren et al., 2005a,b), larger elm trees are excluded from such sites, as cracks where their roots could reach deep water-bearing soil layers are lacking. Indeed, Li et al. (2007) showed that shrubs growing on treeless slopes in northern Mongolia usually depend on the surface water deriving from recent precipitation events. *Padus asiatica*, which is occasionally associated with *U. pumila* (Table 2), pursues a strategy that is different from that of *U. pumila* (Li et al., 2003). *P. asiatica* effectively reduces its transpiration at high VPD. Therefore, the species has lower demands for water, but is exposed to higher leaf temperatures in periods of high solar irradiation (Li et al., 2003). In the study area, this strategy is less successful than that of *U. pumila*, as *P. asiatica*, but not *U. pumila* is restricted to the lowest parts of the sun-exposed slopes, and there especially to gullies.

Herbivory is evidently not a cause of lethal damage in *U. pumila* trees beyond the age of seedlings. Though many trees were infested with caterpillars, damage in the leaves was low, even though trees of *Larix sibirica* at neighboring edges of light taiga forests to the steppe slopes suffered from severe feeding damage by gypsy moth (*Lymantria dispar*) (Hauck et al., 2008) at the same time. Since gypsy moth is a major source of herbivore damage in trees in north-eastern Asia (Gninenko and Orlinskii, 2003) and gypsy moth larvae hatch on rock outcrops that are often found in the vicinity of *U. pumila* stands on sun-exposed slopes (Hauck et al., 2008), woody plants growing on the sun-exposed slopes probably have to be efficient at detracting gypsy moth larvae from feeding. The low feeding damage, despite high density of gypsy moths on the slopes, in *U. pumila* and even more in *Spiraea aquilegifolia* suggests that these species form chemical deterrents making the plants relatively resistant to insect herbivory.

U. pumila is highly resistant to Dutch elm disease caused by the ascomycete *Ophiostoma* spp. (De Rafael et al., 2001; Solla et al., 2005). Therefore, *Ophiostoma* can be ruled out as a cause of early mortality in *U. pumila*, though it is a major threat for other elm species. Despite the low age of the elm stands, the core wood of one third of the trees selected for wood core sampling with the increment borer was rotten. Whether this was caused by fungi or bacteria is unknown. These infections could, of course, cause the death of the elm trees. However, as trees with dead parts of the crowns were also found among the trees with intact stem wood, it is plausible to assume that drought-related damage was at least partly the cause of the withering of major branches.

As a result of the low age and the low vitality of the trees, *U. pumila* is rarely fertile on the south-facing slopes. Palynological work from the study area showed that flowering of *U. pumila* was a rare event throughout the last 2500 years (Schlütz et al., 2008). This result agrees with field observations during ten years of field work in the study area and with results of Lindeman (1981) from other areas of Mongolia. *U. pumila* flowers regularly and copiously in the floodplain of the Kharaa River, 70 km SW of study area, but flowers were never observed on the steppe slopes. In contrast to the Kharaa River, which is located in a wide valley with steppe grasslands, the floodplain of the largest river in the study area, the Eroo River, is relatively narrow and wooded (Dulamsuren et al., 2005a) and therefore not suited for *U. pumila*, which apparently needs light-flooded sites for regeneration. The sparse flowering of *U. pumila* on the sun-exposed slopes certainly is the result of the low age and the low vitality of the trees. Combined with the high mortality, the low fertility is probably a major limiting factor for *U. pumila* on the steppe slopes. Flowering limited to wet periods in the past (Schlütz et al., 2008) also supports the hypothesis that desiccation is the main cause of the crown damage in *U. pumila*.

U. pumila has a high potential for vegetative regeneration (Blažková, 1985). In the study area, the species not only regenerates from stools, but frequently also from pending twigs touching the soil surface especially on the uphill side of the trees growing on very steep slopes. This suggests that vegetative regeneration is the key factor that enables populations of *U. pumila* to persist on the sun-exposed slopes of northern Mongolia for millennia (Schlütz et al., 2008) despite unfavorable growth conditions and high mortality.

5. Conclusions

Our ecophysiological measurements and the examination of soil profiles suggest that the need for access to water-bearing soil layers limits *U. pumila* to the surroundings of rock outcrops and places with deep, loose, stony soil, where the roots can deeply penetrate into the soil, on the south-facing, grassland-dominated slopes of northern Mongolia. Exclusion from sites with shallow and fine-grained soils, which prevail on the slopes and are dominated by meadow steppe, is apparently due to the high water demand of *U. pumila* caused by high transpiration rates at hot weather. The ostensible disadvantage of the ineffective control of transpiration may rather represent a strength of *U. pumila* on steep, sun-exposed slopes as those in the study area, when intensive transpiration helps to cool the leaves.

If the requirements of *U. pumila* for water cannot be fulfilled in drought periods, parts of the tree crowns or the whole trees apparently die from desiccation. As a result of this damage, trees of *U. pumila* do not exceed the age of one to a few decades on the steppe slopes, which is far below the maximum age of *U. pumila* achieved along water-filled or dry streams (Lindeman et al., 1994). The low lifespan of the *U. pumila* on the slopes is certainly not only a matter of the frequency of drought years, but also attributable to an increasing water demand with increasing age and growing canopy surface area. Early mortality of *U. pumila* exacerbates the reproduction of the species, as flowering is limited to rare, unusually wet periods (Schlütz et al., 2008), whereas elm trees in river valleys are regularly fertile (Blažková, 1985; Lindeman et al., 1994). Therefore, *U. pumila* depends on vegetative regeneration for its survival on the steppe slopes, ensuring the long-term survival of the populations despite the low lifespan of the individual trees.

Our results suggest that *U. pumila* is not capable of encroaching on the entire south-facing slopes presently covered with steppe and savanna-like elm woodlands. This is apparently not possible, as the ability of *U. pumila* to withstand high solar irradiation by high transpiration is dearly bought with the requirement for substrata, which allow the roots to penetrate into deep, water-bearing soil layers. Therefore, *U. pumila* cannot form dense forests on the steppe slopes even in areas without livestock grazing and logging. Moreover, its water demand causes the spatial pattern of irregularly distributed patches of single trees or more often groups of trees, as these patches primarily reflect the spatial distribution of suitable soils. Nonetheless, anthropo-zoogenic influences can additionally reduce the density of *U. pumila* on the steppe slopes, as, firstly, *U. pumila* has relatively low growth rates (Blažková, 1985; Lindeman et al., 1994), and secondly, the widespread failure of regeneration of elm forests in Mongolia's river valleys, in spite of richly fertile mature trees, can demonstrably be attributed to overgrazing (Blažková, 1985; Hilbig, 1995).

Acknowledgments

The study was supported by a grant of the German Science Foundation (Deutsche Forschungsgemeinschaft) to Ch. Dulamsuren (Du 1145/1-1). We are thankful to Prof. Dr. Michael Mühlenberg (Center of Nature Conservation, University of Göttingen) to make the facilities of Khonin Nuga Research Station (a biological field station jointly run by the University of Göttingen and the National University of Mongolia, Ulan Bator) available to us. Prof. Dr. Michael Runge (University of Göttingen) helped us with his constant advice. Dr. Doris Möller (University of Göttingen), Dr. S. Byambasuren (Ulan Bator, deceased) and T. Sain-Erdene (National University of Mongolia, Ulan Bator) helped with the measurements of the shoot water potential.

References

Blažková, D., 1985. *Ulmus pumila* L. und ihre Vergesellschaftung in der Nord-Mongolei. Feddes Repert. 96, 433–444.

Chen, S., Bai, Y., Lin, G., Huang, J., Han, X., 2007. Isotopic carbon composition and related characters of dominant species along an environmental gradient in Inner Mongolia. China. J. Arid Environ. 71, 12–28.

De Rafael, M.A., Valle, T., Babiano, M.J., Corchete, P., 2001. Correlation of resistance and H_2O_2 production in *Ulmus pumila* and *Ulmus campestris* cell suspension cultures inoculated with *Ophiostoma novo-ulmi*. Physiol. Plant. 111, 512–518.

Dulamsuren, Ch., 2004. Floristische Diversität, Vegetation und Standortbedingungen in der Gebirgstaiga des Westkhentej. Nordmongolei. Ber. Forschungszentr. Waldökosyst. A 191, 1–290.

Dulamsuren, Ch., Hauck, M., 2008. Spatial and seasonal variation of climate on steppe slopes of the northern Mongolian mountain taiga. Grassland Sci. 54, 217–239.

Dulamsuren, Ch., Hauck, M., Mühlenberg, M., 2005a. Vegetation at the taiga forest-steppe borderline in the western Khentej Mountains, northern Mongolia. Ann. Bot. Fenn. 42, 411–426.

Dulamsuren, Ch., Hauck, M., Mühlenberg, M., 2005b. Ground vegetation in the Mongolian taiga forest-steppe ecotone does not offer evidence for the human origin of grasslands. Appl. Veg. Sci. 8, 149–154.

Dulamsuren, Ch., Hauck, M., Mühlenberg, M., 2008. Insect and small mammal herbivores limit tree establishment in northern Mongolian steppe. Plant Ecol. 195, 143–156.

Gninenko, Yu.I., Orlinskii, A.D., 2003. Outbreaks of Lymantria dispar in Russian forests during the 1990s. Eur. Medit. Plant Protect. Org. Bull. 33, 325–329.

Gubanov, I.A., 1996. Konspekt flory vneshney Mongolii [Survey of the Flora of Outer Mongolia]. Valang Publishers, Moscow (in Russian).

Hauck, M., Dulamsuren, Ch., Mühlenberg, M., 2007. Lichen diversity on steppe slopes in the northern Mongolian mountain taiga and its dependence on microclimate. Flora 202, 530–546.

Hauck, M., Dulamsuren, Ch., Heimes, C., 2008. Effects of insect herbivory on the performance of *Larix sibirica* in a forest-steppe ecotone. Environ. Exp. Bot. 62, 351–356.

Hilbig, W., 1995. The Vegetation of Mongolia. SPB, Amsterdam.

Li, Y.G., Jiang, G.M., Niu, S.L., Liu, M.Z., Peng, Y., Yu, S.L., Gao, L.M., 2003. Gas exchange and water use efficiency of three native tree species in Hunshandak Sandland of China. Photosynthetica 41, 227–232.

Li, S.-G., Romero-Saltos, H., Tsujimura, M., Sugimoto, A., Sasaki, L., Davaa, G., Oyunbaatar, D., 2007. Plant water sources in the cold semiarid ecosystem of the upper Kherlen river catchment in Mongolia: a stable isotope approach. J. Hydrol. 333, 109–117.

Lindeman, G.V., 1981. Estestvenno rastushii vyaz melkolistnii [Naturally Growing Chinese Elm]. Nauka, Moscow (in Russian).

Lindeman, G.V., Enkhsaikhan, D., Zhalbaa, K., 1994. Conditions of natural growth of Chinese elm in the deserts of Mongolia. Lesovedenie 2, 42–53.

Liu, M.Z., Jiang, G.M., Li, Y.G., Niu, S.L., Gao, L.M., Ding, L., Peng, Y., 2003. Leaf osmotic potentials of 104 plant species in relation to habitats and plant functional types in Hushandak Sandland, Inner Mongolia, China. Trees 17, 554–560.

Roberts, S.W., Strain, B.R., Knoerr, K.R., 1980. Seasonal patterns of leaf water relations in four co-occurring forest tree species: parameters from pressure–volume curves. Oecologia 46, 330–337.

Roháček, K., 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. Photosynthetica 40, 13–29.

Schlütz, F., Dulamsuren, Ch., Wieckowska, M., Mühlenberg, M., Hauck, M., 2008. Late Holocene vegetation history suggests natural origin of steppes in the northern Mongolian mountain taiga. Palaeogeogr. Palaeoclim. Palaeoecol. 261, 203–217.

Scholander, P.F., Hammel, H.T., Hemmingsen, E.A., Bradstreet, E.D., 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. Nat. Acad. Sci. U.S.A. 52, 119–125.

Solla, A., Martín, J.A., Corral, P., Gil, L., 2005. Seasonal changes in wood formation of *Ulmus pumila* and *U. minor* and its relation with Dutch elm disease. N. Phytol. 166, 1025–1034.

Chapter 7

The different strategies of *Pinus sylvestris* and *Larix sibirica* to deal with summer drought in a northern Mongolian forest-steppe ecotone suggest a future superiority of pine in a warming climate

The different strategies of *Pinus sylvestris* and *Larix sibirica* to deal with summer drought in a northern Mongolian forest–steppe ecotone suggest a future superiority of pine in a warming climate

Choimaa Dulamsuren, Markus Hauck, Martin Bader, Shagjjav Oyungerel, Dalaikhuu Osokhjargal, Suran Nyambayar, and Christoph Leuschner

Abstract: *Pinus sylvesteris* L. is locally common at the lower forest line to the steppe in Mongolia's taiga forests, which are otherwise dominated by *Larix sibirica* Ledeb. The factors responsible for the occurrence of pine patches in the forest–steppe ecotone are insufficiently studied. We analyzed the response of *P. sylvestris* to summer drought in the western Khentey Mountains, northern Mongolia, by measuring shoot water potentials and exemplary measurements of $\delta^{13}C$ signatures, CO_2–H_2O gas exchange, chlorophyll fluorescence yield, and concentrations of nonstructural carbohydrates. Pine trees growing in the forest–steppe ecotone show conservative water consumption with a sensitive stomatal regulation and relatively constant shoot water potentials under dry conditions. This might be a selective advantage for *P. sylvestris* against increasing aridity driven by global warming, since the main competitor, *L. sibirica*, often suffers from drought stress even under the current climate in the study area.

Résumé : Le *Pinus sylvestris* L. est commun localement à la limite inférieure de la forêt où débute la steppe dans les forêts de la taïga en Mongolie qui sont dans l'ensemble dominées par le *Larix sibirica* Ledeb. Les facteurs responsables de la présence de bouquets de pins dans l'écotone forêt–steppe n'ont pas suffisamment été étudiés. Nous avons analysé la réponse du *P. sylvestris* à la sécheresse estivale dans les Khentey occidentales, dans le nord de la Mongolie, en mesurant le potentiel hydrique des pousses et par des mesures exemplaires des signatures de $\delta^{13}C$, des échanges gazeux CO_2–H_2O, du rendement de la fluorescence de la chlorophylle et de la concentration des hydrates de carbone non structuraux. Les pins qui croissent dans l'écotone forêt–steppe ont une consommation conservatrice de l'eau, une régulation stomatale sensible et un potentiel hydrique relativement constant dans les pousses en période sèche. Cela pourrait constituer un avantage sélectif pour le *P. sylvestris* étant donné l'augmentation de l'aridité due au réchauffement global alors que son principal compétiteur, le *L. sibirica*, souffre souvent de la sécheresse dans le climat actuel de la région à l'étude.

[Traduit par la Rédaction]

Introduction

Forests in Mongolia's ecotone between the Siberian taiga and the central Asian steppe are widely dominated by Siberian larch (*Larix sibirica* Ledeb.), which covers 80% of the forested area (Savin et al. 1978; Tsogtbaatar 2004). The species has recently been shown to suffer regularly from water shortage at the forest line to the steppe, even in years without pronounced drought events (Dulamsuren et al. 2009*a*). This finding was made both in the relatively moist mountain taiga in northernmost Mongolia and at the southernmost limit of the closed taiga forest towards the open steppe. Furthermore, sowing and planting experiments have demonstrated that *L. sibirica* frequently fails to regenerate at the forest line to the steppe because of low water supply, high soil temperatures, and high grazing pressure by native insects and small mammals (Dulamsuren et al. 2008; Hauck et al. 2008). Livestock grazing and timber logging by Mongolia's growing population additionally increase the pressure on the larch forests (Erdenechuluun 2006; Sankey et al. 2006). Against the background of rising temperatures and decreasing precipitation in parts of Mongolia's taiga belt (Sato and Kimura 2006; Sato et al. 2007), the multiple stressors acting upon *L. sibirica* give reason for serious concern about the future prospects of Mongolian larch forests. Retreats of larch forests from the central and southern parts of Mongolia with deteriorating water supply and intensified land use were commonplace throughout the late Holocene (Gunin et al. 1999; Miehe et al. 2007).

Received 17 February 2009. Accepted 22 September 2009. Published on the NRC Research Press Web site at cjfr.nrc.ca on 22 December 2009.

Ch. Dulamsuren, M. Hauck,[1] and C. Leuschner. Department of Plant Ecology, Albrecht von Haller Institute of Plant Sciences, University of Göttingen, D-37073 Göttingen, Germany.
M. Bader. Botanical Institute, University of Basel, CH-4056 Basel, Switzerland.
Sh. Oyungerel, D. Osokhjargal, and S. Nyambayar. Faculty of Biology, National University of Mongolia, 210646 Ulan Batar, Mongolia.

[1] Corresponding author (e-mail: mhauck@gwdg.de).

The current situation of Mongolia's larch forests gives rise to question the extent of drought exposure and drought tolerance in Scots pine (*Pinus sylvestris* L.), which locally replaces *L. sibirica* at forest–steppe borders (Hilbig 1995). *Pinus sylvestris* has never been a focus of forest ecological research in Mongolia because it covers no more than 5% of the country's woodlands (Savin et al. 1978). In the western Khentey Mountains in northeastern Mongolia, where Dulamsuren et al. (2008, 2009*a*) and Hauck et al. (2008) conducted studies of *L. sibirica*, *P. sylvestris* is associated in minor proportions with *L. sibirica* and *Betula platyphylla* Sukaczev in light taiga forests on north-facing slopes and in river valleys on deeper soils (Dulamsuren et al. 2005*a*; Dorjsuren 2009). *Pinus sylvestris* is also found in patches on mountain ridges and on steep slopes with shallow soil facing to the south, west, or east (Dulamsuren et al. 2005*a*, 2005*b*). These pine stands often border on meadow steppe covering the south-facing slopes, although most of the forest line to the steppe is formed by *L. sibirica*. *Larix sibirica* and *P. sylvestris* resemble each other in terms of water demand (Tchebakova et al. 2005). The regular occurrence of *P. sylvestris* at sun-exposed forest edges, however, gives rise to the assumption that *P. sylvestris* is even more drought-tolerant than *L. sibirica*. Therefore, we applied shoot water potential measurements and $\delta^{13}C$ analyses to test the hypothesis that *P. sylvestris* growing at the forest line to the steppe is less affected by summer drought than is *L. sibirica* in the western Khentey. In addition, photosynthetic performance was assessed by means of measurements of CO_2–H_2O gas exchange, chlorophyll fluorescence yield, and concentrations of nonstructural carbohydrates in sun-exposed shoots. Measurements were conducted to compare both pine with larch and mature pine with young pine trees. A better understanding of the capacity of *P. sylvestris* to tolerate water shortage and of the current microclimate in Mongolia's forest–steppe ecotone is important because a clear understanding of these factors forms the basis for predictions on the future development and composition of the southernmost taiga forests and, in particular, answers the question whether larch stands declining as a result of climate warming might be replaced by pine.

Materials and methods

Study sites

The study area is located near Khonin Nuga Research Station in the western Khentey Mountains (49°04′48″ N, 107°17′15″ E), 130 km north of the Mongolian capital Ulan Batar. Khonin Nuga belongs to the mountain taiga, where steppe occurs as insular outposts on south-facing slopes surrounded by woodlands on north-facing slopes and in valleys (Dulamsuren et al. 2005*a*). Studies were carried out on two mountains in the valley of the river Eroo, viz. on Mount Baziin Am (49°2′N, 107°15′E; 1180 m a.s.l.) and Mount Bayantogol (49°5′N, 107°17′E; 1110 m a.s.l.). Mount Baziin Am is named as site A and Mount Bayantogol as site B throughout the paper. Both study sites harbor small stands of *P. sylvestris* growing on the uppermost part of south-facing slopes and on the mountain ridges (Fig. 1). The rest of the south-facing slopes is covered with different communities of meadow steppe and mountain steppe as well as small savanna-like woodlands of *Ulmus pumila* L. (Dulamsuren et al. 2005*a*, 2005*b*, 2009*b*). The north-facing slopes are covered by light taiga forests of *L. sibirica* and *Betula platyphylla*, with *P. sylvestris* as a rare associated species (Tsedendash 1995; Dulamsuren et al. 2005*a*).

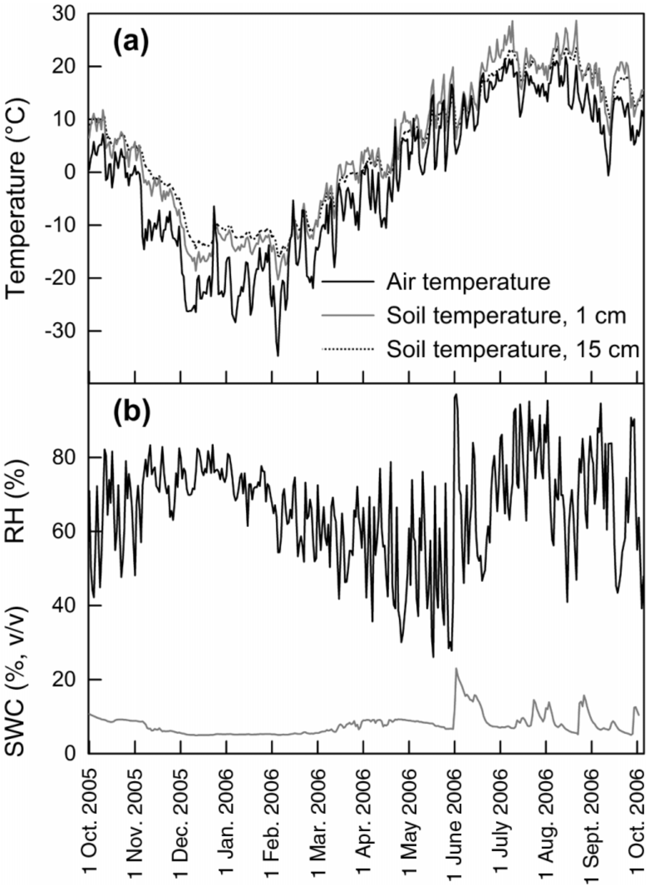
Fig. 1. A *Pinus sylvestris* stand bordering on meadow steppe at (*a*) site A and (*b*) site B, with Fig. 1*a* photographed in the middle of the growing season, illustrating the poor ground vegetation below the pine trees, and Fig. 1*b* photographed from early spring, showing pine saplings encroaching upon the grassland in front of the forest line.

Long-term climate data from the vicinities of the study areas are available from the weather station Eroo, ca. 70 km northwest of the Khonin Nuga Research Station. Microclimate measurements from nine weather stations near Khonin Nuga Research Stations from spring 2005 to fall 2007 suggest temperatures similar to those recorded at the weather station Eroo (Dulamsuren and Hauck 2008). However, precipitation is too variable in Mongolia to deduce long-term annual means from only the weather data recorded from 2005 to 2007.

Weather conditions during the study period

Microclimate was measured at two HOBO weather stations (Onset Computer Corporation, Bourne, Massachusetts, USA) located in the meadow steppe in front of the pine-

Fig. 2. Microclimate in the meadow steppe in front of a *Pinus sylvestris* stand at site B from October 2005 to October 2006. (*a*) Daily means of air temperature and soil temperatures at 1 and 15 cm depths. (*b*) Daily means of relative air humidity (RH) and volumetric soil water content (SWC).



dominated stands of sites A and B (Dulamsuren and Hauck 2008). Data are available for the study period and are also presented for the foregoing months, as the weather condition prior to our investigations might have influenced the water status of the trees. Data for air and soil temperatures, relative humidity, and soil volumetric water content are shown in Fig. 2. The data in Fig. 2 were measured at site B, but the data from site A (not shown) were basically similar. Daily means of air temperatures at 150 cm above the ground varied between –35 and 21 °C, averaging –1.4 ± 0.7 °C at site A and –1.2 ± 0.8 °C at site B. The soil below the steppe along the forest line of the south-facing slopes was extremely dry throughout the winter, with the water content amounting to <5% (*v*/*v*). During the growing season, it usually varied between 5% and 15% (*v*/*v*) and rarely reached 20% (*v*/*v*). The annual precipitation of <300 mm was largely limited to the growing season (Dulamsuren and Hauck 2008).

Measurement of shoot water potential

The plant water status was investigated by analyzing the diurnal and seasonal variation in shoot water potential (Ψ) of *P. sylvestris* on rainless days with clear sky, using a Model 1000 Pressure Chamber Instrument (PMS Instrument Company, Albany, Oregon, USA), applying the method of Scholander et al. (1964). Measurements included the determination of the predawn water potential (Ψ_{p}), which was assumed to represent the daily maximum of Ψ, and the midday water potential (Ψ_{m}), as a measure of the daily minimum of Ψ. These measurements were conducted with freshly cut twigs of 10 cm length from sun-exposed branches at a height of approximately 3 m above the ground. Five replicates were studied per tree and sampling occasion. In addition, pressure–volume curves were recorded from fully water-saturated twigs sampled the day (≤12 h) before the water potential measurements to determine the point of zero turgor (Ψ_0) (Roberts et al. 1980). The cells can withstand Ψ values below Ψ_0 if the stability of the cell wall allows for negative turgor values (Rhizopoulou 1997). Shoots used for the determination of Ψ_0 were re-cut underwater and saturated with water in a pot wrapped in plastic foil for 12 h. Ψ_0 is only weakly affected by the length of the saturation phase prior to the measurements (Parker and Pallardy 1987). Ψ_0 was deduced from plots of $-1/\Psi$ versus the relative water content (RWC) of the sample. RWC was calculated from the equation RWC (%) = [(fresh weight – dry weight)/(turgid weight – dry weight)] × 100. Three twigs per tree were sampled as subsamples for the determination of Ψ_0.

Measurements were taken at sites A and B throughout the growing season 2006 (Table 1). The measurements at site B started 1 month later than at site A, since the river Eroo, which has to be crossed to access the site, was in flood in May 2006. All trees sampled were located directly at the lower forest line to the steppe. Sample trees for monthly measurements of Ψ_{p}, Ψ_{m}, and Ψ_0 included four mature pine trees at site A as well as two mature and five young (stem diameter 3 to 5 cm) pine trees at site B. At site A, Ψ of *P. sylvestris* shoots was directly compared with that of two larch trees growing nearby in the investigated pine stand.

Determination of $\delta^{13}C$ signatures in the needles

The $\delta^{13}C$ signature in the needle mass was determined in 10 mature *P. sylvestris* trees from the forest line to the steppe at both sites A and B (Table 1). Two mature *L. sibirica* trees associated with *P. sylvestris* were additionally sampled at site A. Furthermore, four young trees of *P. sylvestris* (stem diameter <5 cm) growing at the forest line of site B were included. Sun-exposed needles were collected in the afternoon at 3 m height above the ground or in the upper crown of the saplings from the southward side of the trees in August 2006. The samples were dried at 105 °C for 24 h, ground to a fine powder, and weighed in tin capsules. The analyses were conducted with a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), which was combined with an NA 1500 C/N Elementar Analyzer (Carlo Erba Strumentazione, Milan, Italy) and Conflo III interface (Thermo Fisher Scientific). Acetanilide was used as an internal standard. Using this internal standard, the $\delta^{13}C$ signature was related to the Peedee belemnite limestone standard, using the equation $\delta^{13}C$ (‰) = $[(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000$, with $R = {}^{12}C/{}^{13}C$. Enrichment of ^{13}C indicating decreased

Table 1. Numbers of sample trees for ecophysiological studies at sites A and B.

Type of measurements	Trees	Site A	Site B
Shoot water potential, monthly variation	*Pinus*, mature	4	2
	Pinus, young	—	5
	Larix, mature	2	—
Shoot water potential, diurnal variation	*Pinus*, mature	—	1
	Pinus, young	—	2
CO_2–H_2O gas exchange, diurnal variation	*Pinus*, mature	6	—
	Larix, mature	2	—
Chlorophyll fluorescence yield, diurnal variation	*Pinus*, mature	9	—
	Pinus, young	1	—
	Larix, mature	2	—
Needle $\delta^{13}C$	*Pinus*, mature	10	10
	Pinus, young	—	4
	Larix, mature	2	—
Needle and shoot NSC*	*Pinus*, mature	8	10
	Larix, mature	—	7

*NSC, nonstructural carbohydrates — free, intracellular low-molecular weight sugars (glucose, fructose, sucrose) and starch.

CO_2 concentrations in the leaf mesophyll results in high (less negative) values of $\delta^{13}C$.

Gas exchange measurements

Diurnal variation of CO_2–H_2O gas exchange of the needles was measured at site A in August 2006 in shoots of six mature pine trees. Two larch trees in close proximity to the *P. sylvestris* trees were measured in comparison (Table 1). Net photosynthesis, transpiration, stomatal conductance, and photosynthetic photon flux density (PPFD) were determined under ambient conditions with an LCi Ultra Compact Photosynthesis System equipped with a standard conifer cuvette (ADC Bioscientific, Herts, UK). The measurements could not start before 0930, as the needles were covered with a water film from nocturnal fog. Three shoots per tree were measured as subsamples.

Measurement and calculation of chlorophyll fluorescence parameters

The effective quantum yield (Φ_2) of photochemical energy conversion in photosystem II (PS II) was measured with a Mini-PAM chlorophyll fluorometer (Walz Mess- und Regeltechnik, Effeltrich, Germany). Measurements were conducted in August 2006 at site A in nine mature pine trees. Furthermore, two mature larch trees associated with the pine trees and a pine sapling (stem diameter 2 cm) growing just 5 m in front of the forest line, which were also used for recording Ψ, were included in the measurements. Five measurements were made per tree and sampling occasion (Table 1). In addition to the field measurements, shoots of 10 mature pine trees (five growing directly at the forest line to the steppe and five growing about 20 m behind the forest line inside the forest) and of five young pine trees (from the forest line; stem diameter <5 cm) (no subsamples) from site B were used to establish light-response curves. Shoots were collected on the day of the measurements and then stored in water in the sun. Prior to the measurement, the samples were adapted to the dark for 30 min. In addition to Φ_2, PPFD and leaf temperature were measured with sensors integrated in the leaf clip holder of the Mini-PAM. PPFD data were used to calculate the electron transport rate (ETR) applying the equation ETR = $\Phi_2 \times \text{PPFD} \times 0.42$. The nonphotochemical quenching (NPQ) was calculated following the equation NPQ = $(F_M - F_M')/F_M'$, with F_M being the absolute maximum fluorescence in the dark-adapted state (measured at predawn) and F_M' the absolute maximum fluorescence in the light-adapted state (Roháček 2002).

Analysis of nonstructural carbohydrates

Free, intracellular low-molecular weight sugars (glucose, fructose, sucrose) and starch, together subsumed under nonstructural carbohydrates (NSC), were analyzed in needles and branches following Hoch et al. (2002, 2003). Samples of *P. sylvestris* were taken from both sites A and B in August 2006 (Table 1). Furthermore, samples of *L. sibirica* from a stand growing near the studied pine stand of site B were analyzed. The plant material was sampled from south-facing parts of the tree crowns at approximately 3 m above the ground. Bark was peeled off the branch samples, so that only the sapwood was analyzed. Needles and sapwood were dried at 80 °C after sampling and then homogenized in a swing mill. The homogenate was extracted for 30 min in deionized water. In one subsample of the extract, starch, sucrose, and fructose were enzymatically converted into glucose. The total NSC content was measured photometrically as the increase of $NADH^+ + H^+$ while converting glucose with hexocinase to gluconate-6-phosphate. In another aliquot, the content of low-molecular weight sugars alone was analyzed by the addition of invertase and isomerase. The starch content was calculated by subtracting the results from the two subsamples.

Statistics

Arithmetic means ± standard error are given throughout the paper. Data were tested for normal distribution with the Shapiro–Wilk test. Pairwise comparisons of means were made with Student's *t* test with Bonferroni correction for repeated measurements. Duncan's multiple range test was

Fig. 3. Seasonal variation of the shoot water potential in *Pinus sylvestris* growing at the forest line to the steppe during the 2006 growing season. (*a*) Mature trees at site A ($N = 4$), (*b*) mature trees at site B ($N = 2$), and (*c*) young trees at site B ($N = 5$). Midday (Ψ_m) and predawn (Ψ_p) shoot water potentials as well as points of zero turgor (Ψ_0) are given.

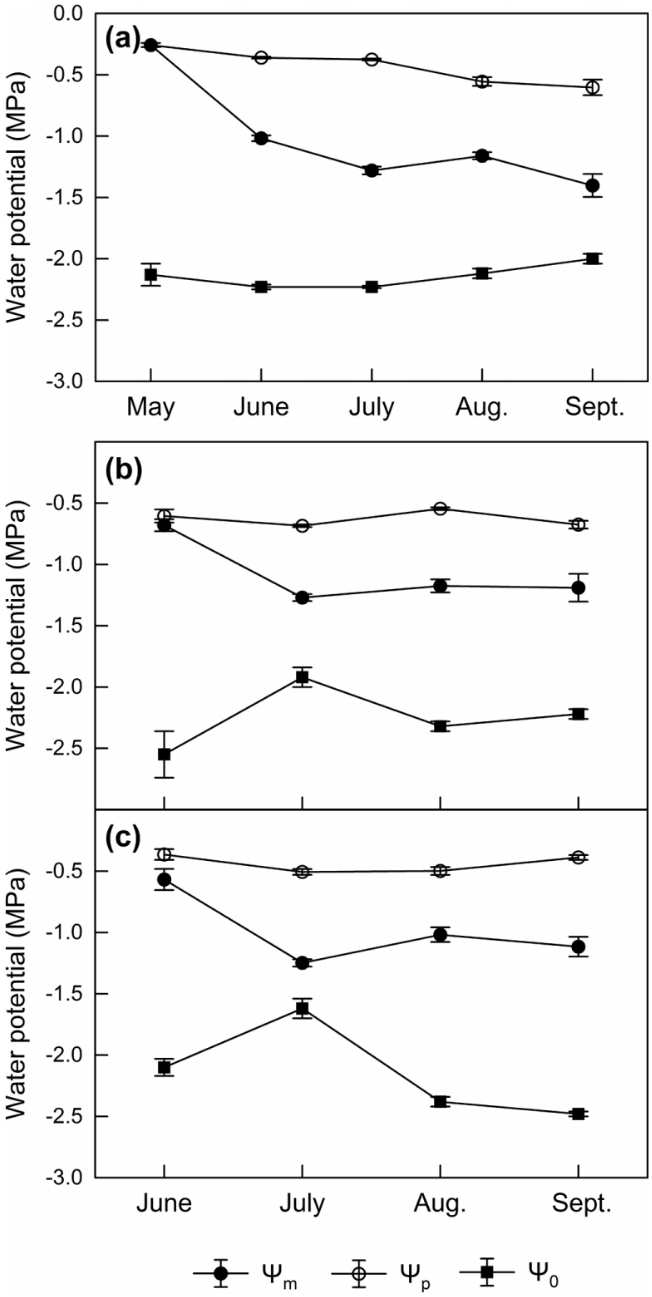
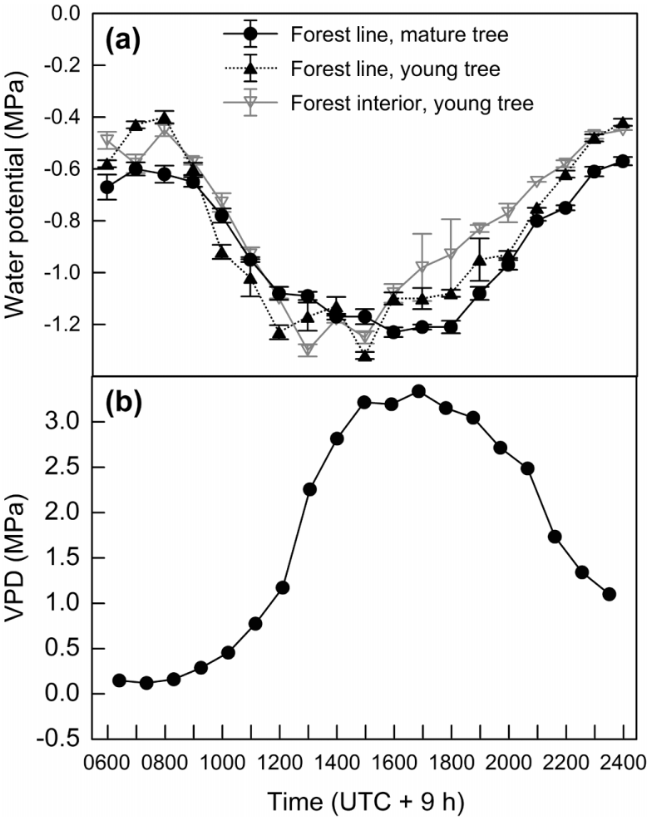


Fig. 4. Diurnal variation of (*a*) the shoot water potential in mature and young *Pinus sylvestris* growing directly at the forest line to the steppe or in young pine growing 20 m behind the forest line in the forest interior and (*b*) atmospheric vapor pressure deficit (VPD) the (site B, 2 August 2006).



used for multiple comparisons. Statistical analyses were computed with SAS version 6.04 software (SAS Institute Inc., Cary, North Carolina, USA).

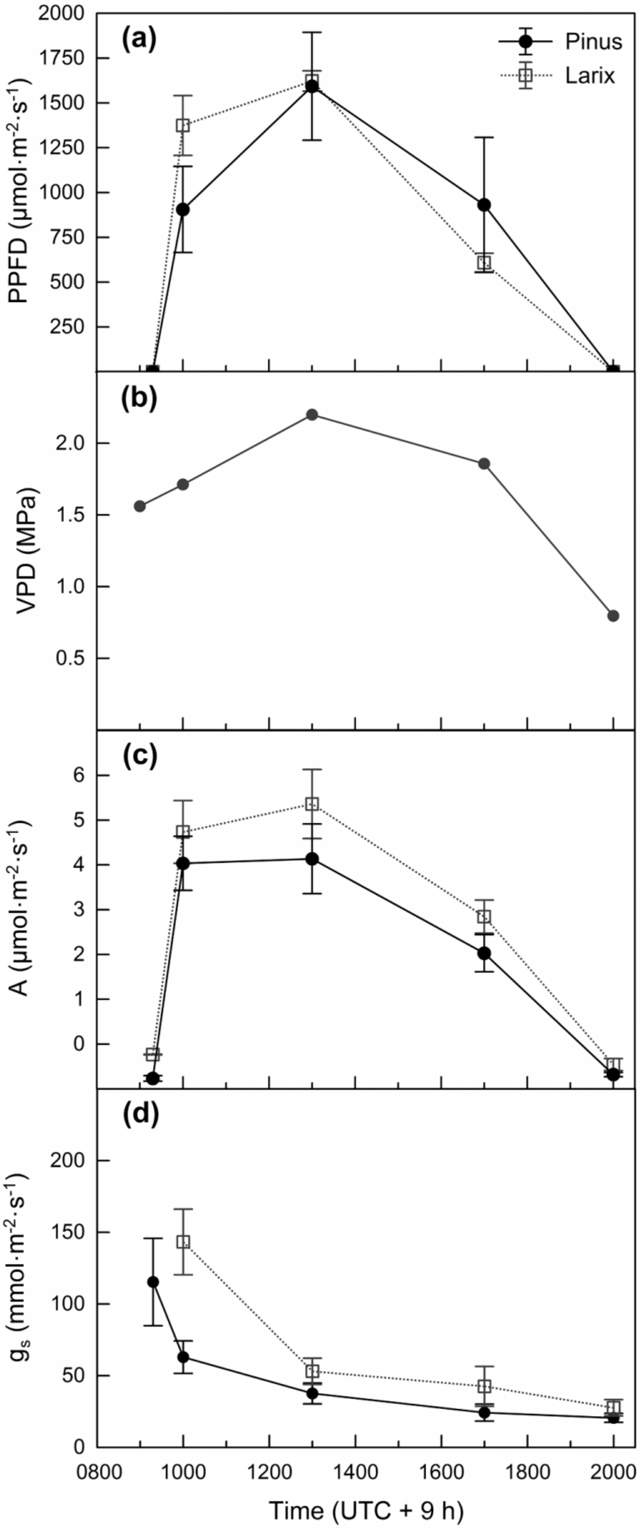
Results

Mature pine trees at sites A and B (Figs. 3*a* and 3*b*) exhibited fairly constant values of Ψ_p of their shoots throughout the growing seasons. The Ψ_m fell below –1.0 MPa but was always significantly distinct from Ψ_0, which varied approximately between –2.0 and –2.5 MPa (Figs. 3*a* and 3*b*). Values of Ψ_p, Ψ_m, and Ψ_0 in mature trees did not differ from those in young pine trees encroaching a few metres in front of the closed forest upon the meadow steppe (Fig. 1); in all cases, the difference between Ψ_m and Ψ_0 was significant ($P \leq 0.05$, Bonferroni corrected *t* test). Diurnal variation of Ψ in pine trees directly from the forest line or growing 20 m behind the forest line inside the forest shows that water relations in pine were largely independent of tree age and the position along the forest–steppe transition, though Ψ in *P. sylvestris* from the forest interior recovered faster from the midday minimum than Ψ in the trees from the forest line (Fig. 4*a*). The diurnal variation of Ψ follows the atmospheric vapor pressure deficit (Fig. 4*b*).

Foliage $\delta^{13}C$ signatures of mature pine trees at the forest line to the steppe were –28.68‰ ± 1.07‰ at site A ($N = 10$) and – 27.49‰ ± 0.35‰ at site B ($N = 10$). Young pine trees at site B showed a tendency for lower $\delta^{13}C$ values (–28.52‰ ± 1.55‰, $N = 4$). Larch trees associated with the pine trees of site A had needle $\delta^{13}C$ values of –29.91‰ ± 0.20‰ ($N = 2$). None of these differences among the $\delta^{13}C$ signatures were significant ($P \leq 0.05$, Duncan's multiple range test).

Net photosynthesis measured in sun-exposed needles at

Fig. 5. Diurnal variation of (*a*) photosynthetic photon flux density (PPFD), (*b*) atmospheric vapor pressure deficit (VPD), (*c*) net CO_2 assimilation rate (A), and (*d*) stomatal conductance (g_s), in sun-exposed shoots of *Pinus sylvestris* ($N = 6$) in comparison with *Larix sibirica* ($N = 2$) growing at the forest line to the steppe at site A on 11 August 2006. PPFD was recorded in close proximity to the twigs measured.



site A followed the same course as that of PPFD (Fig. 5*a*), which in turn correlated with vapor pressure deficit (Fig. 5*b*). Net photosynthesis throughout its diurnal course was lower in pine than in nearby larch trees (Fig. 5*c*), which is consistent with lower stomatal conductance (Fig. 5*d*) and coincides with the smaller NSC pools in *P. sylvestris* than in *L. sibirica*. Total NSC concentrations in branch sapwood were significantly lower in mature pine trees from sites A (2.29% ± 0.31% dry weight, $N = 8$) and B (3.43% ± 0.33% dry weight, $N = 10$) than in mature larch from site B (5.11% ± 0.77% dry weight, $N = 7$) ($P \leq 0.05$, Duncan's multiple range test, df = 22). Similar trends were found for the NSC pool of the needles, but differences were not statistically significant. The chlorophyll fluorescence characteristics of mature *P. sylvestris* at the lower forest line, including Φ_2 and NPQ, were comparable to that of *L. sibirica* at a given PPFD (Fig. 6). The pine sapling growing in the steppe in front of the forest line received more light than the other trees (Fig. 6*a*). This surplus of light was utilized for a higher ETR (Fig. 6*d*). Only at noon, the high NPQ (Fig. 6*e*) reduced the ETR in the pine sapling to the ETR level of mature pine and larch trees growing directly at or shortly behind the forest line (Fig. 6*d*). This behavior matches with the light-response curves showing a steadily increasing ETR until 1500 µmol·m^{-2}·s^{-1} (Fig. 7*a*) and an increasing NPQ even at a PPFD of >2000 µmol·m^{-2}·s^{-1} (Fig. 7*b*). The ETR at a given PPFD decreases from mature *P. sylvestris* at the forest line via mature trees in the forest interior to young trees at the forest line (Fig. 7*a*).

Discussion

Relatively constant shoot water potentials in mature and young trees and minimum potentials significantly higher than the point of zero turgor throughout the studied growing season (Fig. 3) suggest that *P. sylvestris* is well adapted to summer drought in the semiarid forest–steppe ecotone of northern Mongolia. The equal $\delta^{13}C$ signatures in mature and young pine trees agree with this conclusion. A comparison of the CO_2–H_2O gas exchange in *P. sylvestris* with that of *L. sibirica* showed that the stable shoot water potentials in the former are achieved by lower stomatal conductances, associated with lower CO_2 assimilation rates in *P. sylvestris* than in *L. sibirica* (Fig. 5). Stomatal conductance was reduced from early morning onwards in *P. sylvestris* indicating a sensitive regulation, probably mediated through a response of the stomata to the vapor pressure deficit of the atmosphere. This sensitive regulation of stomatal conductance was also indicated by a transient increase of the shoot water potential in the diurnal course of the young pine trees between 12 and 15 h (Fig. 4), a pattern assumed to be typical of feedforward stomatal regulation of plant water relations (Lösch and Franz 1974). The drought response of *P. sylvestris*, thus, fits to a typical water-saving strategy (Badalotti et al. 2000), as the strategy was already demonstrated in drought-stressed, mature *P. sylvestris* trees in Scotland (Irvine et al. 1998; Perks et al. 2004), Spain (Llorens et al. 2008), and Siberia (Sugimoto et al. 2002). The water-saving strategy corresponds to the relatively high vulnerability of *P. sylvestris* to xylem embolism (Martínez-Vilalta and Piñol 2002; Martínez-Vilalta et al. 2004). The fact that pine saplings growing on the steppe in front of the

Fig. 6. Diurnal variation of climatic parameters and chlorophyll fluorescence in sun-exposed shoots of mature *Pinus sylvestris* (N = 9) growing at the forest line to the steppe at site A (11 August 2006). For comparison, data of one sapling of *P. sylvestris* growing in front of the closed forest in the meadow steppe and of two mature *Larix sibirica* associated with pine at the forest line are given. (*a*) Photosynthetic photon flux density (PPFD), (*b*) leaf temperature, (*c*) effective quantum yield of photochemical energy conversion in photosystem II (Φ_2), (*d*) electron transport rate (ETR), and (*e*) nonphotochemical quenching (NPQ).

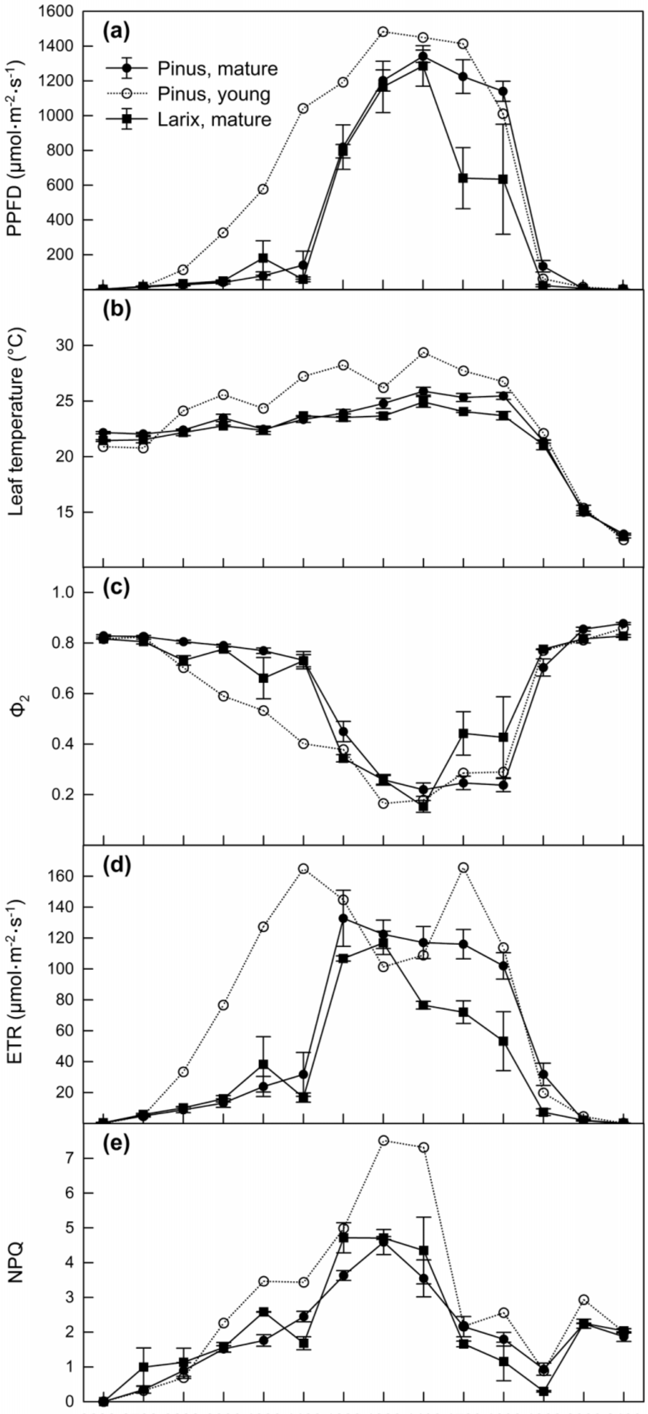
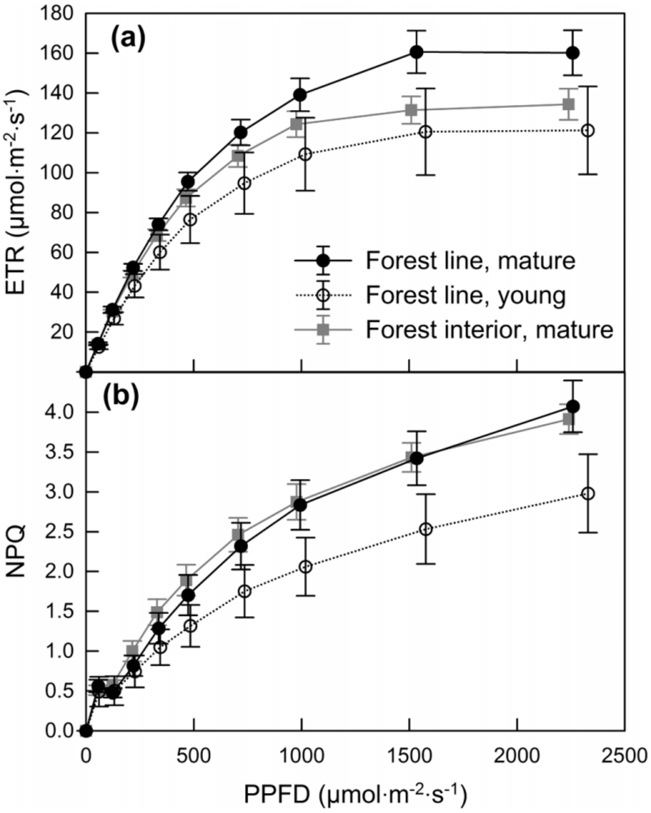


Fig. 7. Light-response curves of (*a*) electron transport rate (ETR) and (*b*) nonphotochemical quenching (NPQ) in sun-exposed shoots of mature and young *Pinus sylvestris* individuals from the forest line to the steppe and from mature *P. sylvestris* growing 20 m behind the forest line in the forest interior at site B (August 2006). PPFD, photosynthetic photon flux density.



closed forest were able to maintain a balanced water budget during dry and hot summer weather (Figs. 3 and 4) by closing their stomata (Figs. 5 and 6) explains the ability of *P. sylvestris* to successfully establish at forest edges. Since the availability of water must be more restricted for saplings than for mature trees, which have a more deep-reaching root system, stable shoot water potentials even in young trees most likely are a consequence of a particularly sensitive stomatal regulation. This sensitive stomatal regulation is also suggested by the lower electron transport rates in young than mature trees in the light-response curves (Fig. 7*a*), as electron transport rate, net photosynthesis, and stomatal conductance typically are closely positively correlated with one another (Luoma 1997).

Young individuals of the main competitor of *P. sylvestris* in Mongolia, *L. sibirica*, are more susceptible to water shortage (Dulamsuren et al. 2009*a*). This explains the disproportionately high abundance of pine at forest edges (Dulamsuren et al. 2005*a*, 2005*b*). Moreover, the establishment of *L. sibirica* at the forest line to the steppe is strongly hampered by herbivorous insects and small mammals (Dulamsuren et al. 2008; Hauck et al. 2008). Herbivore attack is less severe in *P. sylvestris* (Ch. Dulamsuren and M. Hauck, unpublished data), presumably because pine produces more defensive phenolic and terpenic secondary metabolites than larch (Bucyanayandi et al. 1990). Tolerance to the high solar irradiation found at the forest–steppe borderlines seems to be similar in *P. sylvestris* (Fig. 7) and *L. sibirica* (Dulamsuren et al. 2009*a*). That tolerance is apparently not only achieved by NPQ (Figs. 6*e* and 7*b*) but may also result from photorespiration or the water–water cycle (Kozaki and Takeba 1996; Asada 2000), as inferred from stable ETRs (Fig. 6*d*) at declining CO_2 assimilation rates (Fig. 5*c*).

Larix sibirica apparently follows a more risky strategy to withstand the high evaporative demand and the low water availability in northern Mongolia than *P. sylvestris* does. The higher stomatal conductance of larch coincides with it assimilating more CO_2 than pine (Fig. 5). Lower NSC pools in sun-exposed needles and branch sapwood of *P. sylvestris* than that of *L. sibirica* can be interpreted as the result of the lower photosynthetic carbon gain (Gibon et al. 2009). This assumption matches with growth reductions in *P. sylvestris* due to stomatal limitation found by Irvine et al. (1998). Higher leaf conductance in periods of low soil moisture, as they often occur in the study area (Fig. 2*b*), inevitably lead to a decline of shoot water potentials during drought periods (Dulamsuren et al. 2009*a*). This is not critical as long as the point of zero turgor drops along with soil moisture, probably as a result of active osmotic adjustment. This latter process is not evident from the measurements in *P. sylvestris* but occurs in *L. sibirica* (Dulamsuren et al. 2009*a*). In the forest–steppe ecotone of the study area, however, where trees must fulfill their water demand primarily from the current precipitation of the summer months (Li et al. 2006), the minimum shoot water potential of *L. sibirica* was frequently found to drop close to the point of zero turgor indicating severe drought stress (Dulamsuren et al. 2009*a*). Similarly critical water relations, which might be fatal for *L. sibirica* if aridity increases in future (Sato and Kimura 2006; Sato et al. 2007), have not been observed in *P. sylvestris*. This implies that the latter might cope better with global warming than larch.

Conclusions

Pinus sylvestris responds to the frequent summer droughts in Mongolia's forest–steppe ecotone by a water-saving strategy that apparently permanently ensures high shoot water potentials through a sensitive stomatal regulation. In this sense, pine differs from larch (Dulamsuren et al. 2009*a*), which is characterized by higher stomatal conductance and lower shoot water potentials. Local dominance of *P. sylvestris* at the forest line to the steppe can be explained by the low success of *L. sibirica* to establish offspring in these habitats (Dulamsuren et al. 2008). The water-saving strategy of *P. sylvestris* might be a selective advantage, as it might enable the species to tolerate increasing aridity as a result of global warming (Sato and Kimura 2006; Sato et al. 2007).

Acknowledgements

The study was supported by grants from the German Science Foundation (Deutsche Forschungsgemeinschaft) to Ch. Dulamsuren (Du 1145/1-1, 1-2). We are thankful to Prof. Dr. Michael Mühlenberg (Center of Nature Conservation, University of Göttingen) for making the facilities of Khonin Nuga Research Station (a biological field station jointly run by the University of Göttingen and the National University of Mongolia, Ulan Bator) available to the authors. We thank Dr. Doris Möller, Denise Schwerdtner (University of Göttingen), Dr. S. Byambasuren (Ulan Bator; deceased), and T. Sain-Erdene (National University of Mongolia, Ulan Bator) for their help with the measurements of the shoot water potentials. Prof. Dr. M. Runge (Department of Plant Ecology, University of Göttingen) is thanked for his constant advice. We thank Prof. Dr. Rainer Lösch (formerly University of Düsseldorf, Germany) and Prof. Dr. Frank Thomas (University of Trier, Germany) for their helpful comments on the manuscript.

References

Asada, K. 2000. The water–water cycle as alternative photon and electron sink. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. **355**(1402): 1419–1431. doi:10.1098/rstb.2000.0703.

Badalotti, A., Anfodillo, T., and Grace, J. 2000. Evidence of osmoregulation in *Larix decidua* at alpine treeline and comparative responses to water availabity of two co-occurring evergreen species. Ann. For. Sci. **57**(7): 623–633. doi:10.1051/forest:2000146.

Bucyanayandi, J.-D., Bergeron, J.-M., and Menard, H. 1990. Preference of meadow voles (*Microtus pennsylvanicus*) for conifer seedlings: chemical components and nutritional quality of bark of damaged and undamaged trees. J. Chem. Ecol. **16**(8): 2569–2579. doi:10.1007/BF01017479.

Dorjsuren, Ch. 2009. Anthropogenic succession of the larch forests in Mongolia. Nauka, Moscow. (In Russian with English summary.)

Dulamsuren, Ch., and Hauck, M. 2008. Spatial and seasonal variation of climate on steppe slopes of the northern Mongolian mountain taiga. Grassl. Sci. **54**(4): 217–230. doi:10.1111/j.1744-697X.2008.00128.x.

Dulamsuren, Ch., Hauck, M., and Mühlenberg, M. 2005*a*. Vegetation at the taiga forest–steppe borderline in the western Khentej Mountains, northern Mongolia. Ann. Bot. Fenn. **42**: 411–426.

Dulamsuren, Ch., Hauck, M., and Mühlenberg, M. 2005*b*. Ground vegetation in the Mongolian taiga forest–steppe ecotone does not offer evidence for the human origin of grasslands. Appl. Veg. Sci. **8**(2): 149–154. doi:10.1111/j.1654-109X.2005.tb00640.x.

Dulamsuren, Ch., Hauck, M., and Mühlenberg, M. 2008. Insect and small mammal herbivores limit tree establishment in northern Mongolian steppe. Plant Ecol. **195**(1): 143–156. doi:10.1007/s11258-007-9311-z.

Dulamsuren, Ch., Hauck, M., Bader, M., Osokhjargal, D., Oyungerel, Sh., Nyambayar, S., Runge, M., and Leuschner, C. 2009*a*. Water relations and photosynthetic performance in *Larix sibirica* growing in the forest–steppe ecotone of northern Mongolia. Tree Physiol. **29**(1): 99–110. doi:10.1093/treephys/tpn008. PMID:19203936.

Dulamsuren, Ch., Hauck, M., Nyambayar, S., Bader, M., Osokhjargal, D., Oyungerel, S., and Leuschner, C. 2009*b*. Performance of Siberian elm (*Ulmus pumila*) on steppe slopes of the northern Mongolian mountain taiga: drought stress and herbivory in mature trees. Environ. Exp. Bot. **66**(1): 18–24. doi:10.1016/j.envexpbot.2008.12.020.

Erdenechuluun, T. 2006. Wood supply in Mongolia: the legal and illegal economies. Mongolia Discuss. Pap. World Bank, Washington, D.C.

Gibon, Y., Pyl, E.-T., Sulpice, R., Lunn, J.E., Höhne, M., Günther, M., and Stitt, M. 2009. Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when *Arabidopsis* is grown in very short photoperiods. Plant Cell Environ. **32**(7): 859–874. doi:10.1111/j.1365-3040.2009.01965.x. PMID:19236606.

Gunin, P.D., Vostokova, E.A., Dorofeyuk, N.I., Tarasov, P.E., and Black, C.C. 1999. Vegetation dynamics of Mongolia. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Hauck, M., Dulamsuren, Ch., and Heimes, C. 2008. Effects of insect herbivory on the performance of *Larix sibirica* in a forest–steppe ecotone. Environ. Exp. Bot. **62**(3): 351–356. doi:10.1016/j.envexpbot.2007.10.025.

Hilbig, W. 1995. The vegetation of Mongolia. SPB Academic Publishing, Amsterdam, the Netherlands.

Hoch, G., Popp, M., and Körner, C. 2002. Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. Oikos, **98**(3): 361–374. doi:10.1034/j.1600-0706.2002.980301.x.

Hoch, G., Richter, A., and Körner, C. 2003. Non-structural carbon compounds in temperate forest trees. Plant Cell Environ. **26**(7): 1067–1081. doi:10.1046/j.0016-8025.2003.01032.x.

Irvine, J., Perks, M.P., Magnani, F., and Grace, J. 1998. The response of *Pinus sylvestris* to drought: stomatal control of transpiration and hydraulic conductance. Tree Physiol. **18**(6): 393–402. PMID:12651364.

Kozaki, A., and Takeba, G. 1996. Photorespiration protects C_3 plants from photooxidation. Nature, **384**(6609): 557–560. doi:10.1038/384557a0.

Li, S.-G., Tsujimura, M., Sugimoto, A., Sasaki, L., Yamanaka, T., Davaa, G., Oyunbaatar, D., and Sugita, M. 2006. Seasonal variation in oxygen isotope composition of waters for a montane larch forest in Mongolia. Trees (Berl.), **20**(1): 122–130. doi:10.1007/s00468-005-0019-1.

Llorens, P., Poyatos, R., Latron, J., Delgado, J., and Gallert, F. 2008. Analysis of three severe droughts (1995–2006) and their effect on *Pinus sylvestris* transpiration and physiological response in a montane Mediterranean research catchment (Vallcebre, Spain). Geophys. Res. Abstr. **10**: EGU2008–A-07353.

Lösch, R., and Franz, N. 1974. Tagesverlauf von Wasserpotential und Wasserbilanz bei Pflanzen verschiedener Standorte des fränkischen Wellenkalkes. Flora, **163**: 466–479. (In German with English summary.)

Luoma, S. 1997. Geographical pattern in photosynthetic light response of *Pinus sylvestris* in Europe. Funct. Ecol. **11**(3): 273–281. doi:10.1046/j.1365-2435.1997.00089.x.

Martínez-Vilalta, J., and Piñol, J. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage. **161**(1-3): 247–256. doi:10.1016/S0378-1127(01)00495-9.

Martínez-Vilalta, J., Sala, A., and Piñol, J. 2004. The hydraulic architecture of Pinaceae — a review. Plant Ecol. **171**: 3–13.

Miehe, G., Schlütz, F., Miehe, S., Opgenoorth, L., Cermak, J., Samiya, R., Jäger, E.J., and Wesche, K. 2007. Mountain forest islands and Holocene environmental changes in Central Asia: a case study from the southern Gobi Altay, Mongolia. Palaeogeogr. Palaeoclimatol. Palaeoecol. **250**(1-4): 150–166. doi:10.1016/j.palaeo.2007.03.022.

Parker, W.C., and Pallardy, S.G. 1987. The influence of resaturation method and tissue type on pressure–volume analysis of *Quercus alba* L. seedlings. J. Exp. Bot. **38**: 535–549. doi:10.1093/jxb/38.3.535.

Perks, M.P., Irvine, J., and Grace, J. 2004. Xylem acoustic signals from mature *Pinus sylvestris* during an extended drought. Ann. For. Sci. **61**(1): 1–8. doi:10.1051/forest:2003079.

Rhizopoulou, S. 1997. Is negative turgor fallacious? Physiol. Plant. **99**(3): 505–510. doi:10.1111/j.1399-3054.1997.tb00566.x.

Roberts, S.W., Strain, B.R., and Knoerr, K.R. 1980. Seasonal patterns of leaf water relations in four co-occurring forest tree species: parameters from pressure–volume curves. Oecologia (Berl.), **46**: 330–337.

Roháček, K. 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. Photosynthetica, **40**(1): 13–29. doi:10.1023/A:1020125719386.

Sankey, T.T., Montagne, C., Graumlich, L., Lawrence, R., and Nielsen, J. 2006. Lower forest–grassland ecotones and 20th century livestock herbivory effects in northern Mongolia. For. Ecol. Manage. **233**(1): 36–44. doi:10.1016/j.foreco.2006.05.070.

Sato, T., and Kimura, F. 2006. Regional climate simulations to diagnose environmental changes in Mongolia. Bull. Terr. Environ. Res. Center. Univ. Tsukuba, **7**: 59–69.

Sato, T., Kimura, F., and Kitoh, A. 2007. Projection of global warming onto regional precipitation over Mongolia using a regional climate model. J. Hydrol. (Amst.), **333**(1): 144–154. doi:10.1016/j.jhydrol.2006.07.023.

Savin, E.N., Ogorodnikov, A.V., Semechkin, I.V., Dugarzhov, Ch., and Korotkov, I.A. 1978. Forests of the Mongolian People's Republic. Nauka, Moscow. [In Russian.]

Scholander, P.F., Hammel, H.T., Hemmingsen, E.A., and Bradstreet, E.D. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. Natl. Acad. Sci. U.S.A. **52**(1): 119–125. doi:10.1073/pnas.52.1.119. PMID:16591185.

Sugimoto, A., Yanagisawa, N., Naito, D., Fujita, N., and Maximov, T.C. 2002. Importance of permafrost as a source of water for plants in east Siberian taiga. Ecol. Res. **17**(4): 493–503. doi:10.1046/j.1440-1703.2002.00506.x.

Tchebakova, N.M., Rehfeldt, G.E., and Parfenova, E.I. 2005. Impacts of climate change on the distribution of *Larix* spp., and *Pinus sylvestris* and their climatypes in Siberia. Mitigat. Adapt. Strat. Glob. Change, **11**: 861–882.

Tsedendash, G. 1995. The forest vegetation of the Khentey Mountains. Dissertation, National University of Mongolia, Ulan Bator. (In Mongolian.)

Tsogtbaatar, J. 2004. Deforestation and reforestation needs in Mongolia. For. Ecol. Manage. **201**(1): 57–63. doi:10.1016/j.foreco.2004.06.011.

Chapter 8

General summary and Conclusions

General summary and conclusions

Summary

This PhD thesis addressed several major aspects of the carbon (C) cycle in a *c.* 100-year-old, mixed deciduous forest under elevated CO_2 with an emphasis on below-ground processes. The aim was to assess the responses of tree fine roots and soil respiration to canopy CO_2 enrichment (~ 550 ppm) in this tallest forest studied to date. Furthermore, leaf gas-exchange of the five study species was examined to ascertain the long-term response of photosynthetic carbon uptake to elevated amospheric CO_2. Investigations at the Swiss Canopy Crane (SCC) experimental site were guided by the following key questions: (1) Does below-ground C allocation to fine root production increase in response to CO_2 enrichment in order to acquire more nutrients to match the enhanced C supply in the forest canopy? (2) Is below-ground metabolism enhanced and therefore forest soil respiration stimulated by canopy CO_2 enrichment? (3) Is leaf-level photosynthesis persistently stimulated by elevated CO_2 in this stand or had these mature broad-leaved trees reduced their carbon uptake by photosynthetic down-regulation under long-term CO_2 enrichment?

Findings from earlier studies at the SCC site, including ^{13}C isotope tracing, all point towards an increased flux of C through CO_2-enriched trees to the soil but neither fine root biomass nor soil respiration were stimulated by elevated CO_2. Surprisingly, fine root biomass in bulk soil and ingrowth cores showed strong reductions by $\sim 30\%$ in year five and six but were unaffected in the following seventh year of CO_2 enrichment. Given the absence of a positive biomass response of fine roots, we assumed that the extra C assimilated in the CO_2-enriched forest canopy was largely respired back to the atmosphere via increases in fine root and rhizosphere respiration and the metabolization of increased root derived exudates by soil microbes. Indeed, 52% higher soil air CO_2 concentration during the growing season and 14% greater soil microbial biomass both indicated enhanced below-ground metabolism in soil under CO_2-enriched trees. However, this did not translate into a persistent stimulation of soil respiration. At times of high or continuous precipitation soil water savings under CO_2-exposed trees (resulting from reduced sapflow) led to excessive soil moisture (> 45 vol.-%) impeding soil gas-exchange and thus soil respiration. Depending on the interplay between soil temperature and the consistently high soil water content in this stand, instantaneous rates of soil respiration were periodically reduced or increased under elevated CO_2 but on a diel scale and integrated over the growing season soil CO_2 emissions were similar under CO_2-enriched and control trees. Soil respiration could therefore not explain the fate of the extra C. The lacking sink capacity for additional assimilates led us to assume downward adjustment of photosynthetic capacity in CO_2-enriched trees thereby reducing carbon uptake in the forest canopy. Photosynthetic acclimation cannot completely eliminate the CO_2-driven stimulation in carbon uptake, but a reduction could hamper the detection of a CO_2 effect considering the low statistical power inevitably involved with such large-scale experiments. However, after eight years of CO_2 enrichment we found sustained stimulation in leaf photosynthesis (42-49%) indicating a lack of closure in the carbon budget for this stand under elevated atmospheric CO_2.

Conclusions

Since our measurements at the SCC stand failed to identify the sink(s) that could account for the extra C persistently assimilated under elevated CO_2, we must assume that the extra C entered pools that remained unexplored such as coarse roots, which have been shown to increase biomass in response to elevated CO_2 at the Duke-FACE pine stand (+200%, Jackson et al., 2009) and in a scrub-oak ecosystem (+37%, Stover et al., 2007). Alternatively, the extra C may, at least in part, leave the system via leaching of dissolved organic C (from CO_2-enriched litter, Hagedorn & Machwitz, 2007) and in the form of dissolved inorganic C as indicated by preliminary data provided by our collaboration partners (pers. comm. F. Hagedorn).

References

Hagedorn, F. & Machwitz, M. (2007) Controls on dissolved organic matter leaching from forest litter grown under elevated atmospheric CO_2. *Soil Biology & Biochemistry*, **39**, 1759-1769.

Jackson, R.B., Cook, C.W., Pippen, J.S. & Drake, B.G. (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.

Stover, D.B., Day, F.P., Butnor, J.R. & Palmer, S.M. (2007) Effect of elevated CO_2 on coarse-root biomass in Florida scrub detected by ground-penetrating radar. *Ecology*, **88**, 1328-1334.

Curriculum vitae

Martin K.-F. Bader

Insitute of Botany, University of Basel, Switzerland

Nationality	German
Address	Leimenstrasse 16, 4051 Basel, Switzerland
E-mail	martin.bader@unibas.ch

Academic education

2006 – 2009	PhD at the Institute of Botany, University of Basel, Switzerland. Title: “Carbon fluxes in a mature deciduous forest under elevated CO_2”, supervised by Prof. Christian Körner
2006	MSc at the University of Waikato (1st class honours, Plant Ecology), New Zealand, supervised by Prof. Allan Green (University of Waikato, NZ) and Prof. Burkhard Büdel (University of Kaiserslautern, Germany)
2003	BSc in biology at the University of Kaiserslautern, Germany
2002	MSc in geography at the University of Saarbrücken, Germany
1998	BSc in geography at the University of Saarbrücken, Germany

Professional experience

since 2009	Post-doctoral researcher at the Institute of Botany, University of Basel, Switzerland Project: “Drought-sensitivity of deciduous forest trees” funded by the National Centre of Competence in Research (NCCR) Climate
2002	Environmental impact assessment – fixed-term contract as geographer with a company for outdoor planning and landscaping in Saarbrücken, Germany

Scholarships

2005 – 2006	Excellency grant of the federal state of Rheinland-Pfalz, Germany
2005	DAAD grant (German Academic Exchange Service)

Teaching

2010	Co-lecturer for the course “Introduction to R” held at the Swiss Federal Institute of Technology Zürich (ETH Zürich)
2009	Coordinator and co-leader of the field course/excursion ‘Teneriffa – Ecology and Evolution of Subtropical Island Vegetation’ together with Prof. Jürg Stocklin, University of Basel, Switzerland

2009	Co-lecturer for the lab course “Stable Isotopes in Ecology” at the Paul Scherrer Insitute, Villigen, Switzerland
2006 – 2009	Lecturer for the lab and field course ‘Ecology & Conservation’ at the University of Basel, Switzerland
2006 – 2009	Lecture ‘Principles of Gas-exchange and Chlorophyll Flourescence Measurements’ at the University of Basel, Switzerland
2006 – 2009	Lecture ‘Plant-Insect Interactions’ as part of the ‘Ecosystems and Population Processes’ lecture series of the master and PhD programme at the University of Basel, Switzerland
2004	Initiation and realization of the lab and field course “Ecology and Biodiversity of Beetles”, biology department of the University of Kaiserslautern, Germany

Scientific assistances and extramural internships

2005	Graduate assistant at the biology department of the University of Waikato, New Zealand
2003 – 2004	Graduate assistant at the Research Institute of Forest Ecology and Forestry Rheinland-Pfalz (Department of entomology), Trippstadt, Germany. Insect identification and data analysis
2003	Tropical practical course (plant & insect ecology) in Panama (Barro Colorado Island) at the Smithsonian Tropical Research Institute (STRI) (2 months)
2002 – 2003	Graduate assistant at the biology department of the University of Kaiserslautern
1999 – 2000	Lab assistant at the ‘Environmental laboratory’ of Kaiserslautern, Germany

Stays abroad

2005 – 2006	Master of Science in plant ecology at the University of Waikato, New Zealand
2006	Invited research on tree responses to drought at the forest-steppe ecotone in northern Mongolia (University of Goettingen, Germany)

Statistical education

2007	‘Advanced Statistics using R’, held by T. Fabbro at the University of Basel, Switzerland
2007	‘Planning and statistical analysis of experiments in ecology’, held by P. Niklaus at the University of Basel, Switzerland
2008	‘Contemporary Applied Statistics for Ecology’, held by A. Hector at the University of Zürich, Switzerland

Languages

German native speaker, near-native level of English, medium level of French and basic knowledge of Russian.

Peer-reviewed publications

Bader, M. K.-F., Siegwolf, R. & Körner, C. Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment (FACE). *Planta*, online, doi: 10.1007/s00425-010-1240-8.

Bader, M. K.-F. & Körner, C. (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. *Global Change Biology*, in press (early view, doi: 10.1111/j.1365-2486.2010.02159.x).

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

Dulamsuren, C., Hauck, M., Bader, M., Osokhjargal, D. Oyungerel, S., Nyambayar, S., Runge, M. & Leuschner, C. (2009) Water relations and photosynthetic performance in *Larix sibirica* growing in the forest-steppe ecotone of northern Mongolia. *Tree Physiology*, **29**, 99-110.

Dulamsuren, C., Hauck, M., Nyambayar, S., Bader, M., Osokhjargal, D., Oyungerel, S. & Leuschner, C. (2009) Performance of Siberian elm (*Ulmus pumila*) on steppe slopes of the northern Mongolian mountain taiga: Drought stress and herbivory in mature trees. *Environmental and Experimental Botany*, 66, 18-24.

Dulamsuren, C., Hauck, M., Bader, M., Oyungerel, S., Osokhjargal, D., Nyambayar, S. & Leuschner, C. (2009) The different strategies of *Pinus sylvestris* and *Larix sibirica* to deal with summer drought in a northern Mongolian forest-steppe ecotone suggest a future superiority of pine in a warming climate. *Canadian Journal of Forest Research*, **39**, 2520-2528.