In situ effects of elevated CO_2 on plants and insects

INAUGURALDISSERTATION

zur Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

PHILOSOPHISCH-NATURWISSENSCHAFTLICHEN
FAKULTÄT
der Universität Basel

von

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WALDBRÖL, BRD

Basel, 2005

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

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Basel, den 7. Juni 2005

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"Die Veränderung des aufsteigenden
Saftes, das sind die Blätter"

Aristoteles,
peri phyton, II c.7 827a, 37 (Bekker)

Danksagung

ZurErstellung der Dissertation haben zahlreiche Personen beigetragen, denen an dieser Stelle herzlich gedankt sein soll.

Christian Körner und Stephan Hättenschwiler für die Möglichkeit diese Arbeit in zwei faszinierenden Versuchen durchführen zu dürfen. Ich danke beiden, dass ich meinen Interessen weitestgehend frei wählend nachgehen durfte, und bei Schwierigkeiten und Unklarheiten wiederum jederzeit ein offenes Ohr fand. Die kritische Rezensionen meiner Manuskripte, die in zum Teil hingebungsvollen Korrekturarbeiten seitens der Betreuer mündeten, haben entscheidend zum Werdegang der Publikationen beigetragen.

Am Botanischen Institut herrschte eine freundliche und offenherzige Atmosphäre und somit ein ideales Arbeitsumfeld. Besonders danke möchte ich an dieser Stelle Erwin Amstutz, Tanya Handa, Günter Hoch, Sonja Keel, Sebastian Leuzinger, Thomas Zumbrunn und Gerhard Zotz.

Zur ehemaligen Arbeitsgruppe 'Populationsökologie' der Uni Jena bestand im Lauf der Dissertation immer Kontakt und ich erfuhr auch hier Hilfe, Anregungen und Aufmunterungen, insbesondere von Günter Köhler, Klaus Reinhardt und Gerd Wagner.

Für die nicht zu unterschätzende Hilfe bei der Jahrringanalyse danke ich Dieter Eckstein und Werner Schoch, Werner Baltenweiler für die Mithilfe bei der Suche nach Lärchenwicklerraupen und für geduldiges Beantworten jeglicher Fragen rund um den Versuch. Armin Coray, Beat Wermelinger, Denise Wyniger und Roland Mühlethaler erteilten Auskunft zu einer Vielzahl entomologischer Fragen.

Nachdem der FC Carl Zeiss Jena zum Beginn der Dissertation (2001) aus der dritten Liga in die Oberliga (NOFV) abgestiegen ist, wahren sie als diesjähriger Meister der NOFV-Süd Staffel, die Chance, zum Ende der Disseration (2005) erneut in die 3. Liga aufzusteigen.

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1 General introduction

Rising atmospheric CO_2 is almost unanimously identified as the main driver of global warming (Rohde 1990, Crowley 2000). It has been suggested that changes in climate and in atmospheric carbon dioxide concentrations have modified the carbon cycle, which render terrestrial ecosystems as substantial carbon sinks, but direct evidence for this is very limited (Cao and Woodward 1998). Whereas climate change can also directly affect plants and animals and their interaction (Parmesan et al. 1999, Warren et al. 2001, Bale et al. 2002, Root et al. 2003), the effect of rising CO_2 on insects occurs indirectly through changes in leaf chemistry and thus nutritional quality. In this thesis I focus on the impact of elevated CO_2 on plants and insects.

1.1 Plants and insects in a CO_2 enriched world

The current increase in atmospheric concentration of CO_2 is expected to cause drastic, indirect and direct, changes in the biosphere. The transfer from inorganic carbon (CO_2) to organic substrate (carbohydrates) via photosynthesis is the fundamental biological process. Carbon is the material basis of life on earth: 45% to 60% of the biomass of any living organism is made up of the element C. The provision of more C to plants thus represents a fundamental change in their diet, and will affect their metabolism, growth and tissue quality, and consequently, their interaction with animals and microbes. Enhanced carbon supply may cause ecosystems to become more productive and perhaps to store more carbon in living biomass or soil humus. This process will tie up other essential chemical elements and produce negative feedback on growth and plant performance, except for luxurious growth conditions. It is the overall growth environment, their nutrition in particular, which drives CO_2-responses of plants (Körner 2000). If growth would be stimulated, this would not necessarily cause carbon stocks to increase in the landscape, because faster growth may simply enhance tree turnover (shorter tree life span), with no sustained net effect on the C-balance. However, over a limited time, biomass increments could help ‘buying time‘ with regard to CO_2 measures of society. Higher net productivity may also feed more carbon into the recalcitrant soil humus fraction. But this is a very slow process and has not yet been shown to happen.

The question of carbon sequestration is of overwhelming importance for any prediction about future changes in atmospheric CO_2 and the responses of the biosphere. Unfortunately, most of the available information comes from studies conducted with seedlings or saplings, mostly growing in some sort of enclosures, for a short period of time only. Scaling up the results of these experiments to the real world is problematic. In the past 20 years many FACE (Free Air CO_2 Enrichment) experiments were established, primarily in grasslands and agronomic crops. There are only two FACE studies on larger, albeit still relatively young (app. 15-20-year old) trees, namely the one in a *Pinus taeda* plantation at Duke in North Carolina (e.g. deLucia et al. 1999, Hamilton et al. 2002) and in a *Liquidambar styraciflua* plantation in Oak Ridge, Tennessee (e.g. Norby et al. 2001 and 2003). Two FACE experiments use plantations of young *Populus* saplings, one in Wisconsin with *Populus tremuloides* (e.g. Karnosky et al. 2003), and one in Italy with two *Populus* species and one hybrid (e.g. Wittig et al. 2005). The only test-site with mature trees is at the Swiss Canopy Crane (SCC) experimental site in Hofstetten near Basel.

It has been emphasized that the more “natural” growth conditions are, the more likely are the observed growth and tissue quality changes representative of what a future CO_2-rich world will induce. Among the globe’s biota, forests play a particularly prominent role in carbon cycle because close to 90% of all carbon stored in vegetation (ca. 600 Gt C) is tied to forests (Olsen et al. 1983). Thus, it is largely a question of forest tree responses, whether more carbon can be accumulated into biomass in a CO_2 enriched world. It is widely assumed that the amount of carbon sequestered by forests will increase as CO_2 concentrations rise. However, carbon sequestration at the ecosystem level is the complex result of plant photosynthesis, respiration, growth, litter production, root exudation, decomposition, and other processes. Considering this complexity, it is not surprising that there is much dispute about the changes in the forest carbon cycle in response to rising atmospheric CO_2 although an increasing body of literature on tree biomass responses in elevated CO_2 is now available. Growth or biomass increase of young plants generally is stimulated in a CO_2-enriched atmosphere (e.g. Ceulemans and Mousseau 1994, Wullschleger et al. 1997, Curtis and Wang 1998, Saxe et al. 1998, Norby et al. 1999, Körner 2000). This stimulation depends on a suite of factors, e.g., light patterns in the forest understorey (Hättenschwiler and Körner 2000) or nutrient availability (Spinnler et al. 2002). For example, biomass increase of *Picea* was enhanced in elevated CO_2 on nutrient-poor acidic and on a more fertile calcareous soil, whereas *Fagus* responded negatively to CO_2 on acidic but positively on calcareous soil. An important conclusion from all these studies is that the effects of elevated CO_2 are highly species-specific and depend on general growth conditions, and thus, have to be studied in the respective context.

Rising CO_2 concentrations can further affect directly the nutrient concentrations of plants and thus herbivore performance feeding on this plants. Different nutritional quality has huge effects on herbivores. In the first instance pests, mainly insect pests might change ecosystem structure through altered feeding behaviour or altered performance (e.g. Percy et al. 2002) with rising atmospheric CO_2. There are different scenarios how insects can react when faced with different nutritional quality induced by CO_2. A CO_2 induced reduction in host plant quality can result in increased larval consumption in insects species (Fajer 1989, Lincoln et al. 1984, Osbrink et al. 1987, Johnson and Lincoln 1990), whereas in other insects no difference was observed when feeding in elevated CO_2, indicating that increased consumption rates probably compensate for poorer nutritional quality (mainly higher C:N ratio through dilution of N). But results depend highly on the specific plant-insect interaction studied. Leaf-chewers generally seem able to compensate for the decreased nitrogen levels in the plant tissues by increasing their food consumption (by 30%) and with no adverse effects on pupal weights, in contrast leaf-miners only slightly increase their food consumption (Bezemer and Jones 1998). However, it appears that in most cases, increased feeding rates do not compensate fully for reduced nutritional quality (Watt et al. 1995). Apart from nitrogen dilution an increase of carbon-based compounds in plants growing in elevated CO_2 can impact insect herbivores, but it depends upon which type of carbon-based compound increases in concentration. An increase of starch may be beneficial because this component can enhance digestion, but structural carbohydrates, such as cellulose, secondary compounds or carbon-based defense compounds can limit insect feeding. Plant-insect interactions in a CO_2 enriched world have been a focus of the research activities at the Botanical Institute in Basel during the last decade. For example, Zaller and Arnone (1999) have shown that elevated CO_2 does not necessarily impact size and composition of earthworm communities. The slug *Derocera reticulatum* served as a study organism in another study (Peters et al. 2000): the authors documented a preference shift from non-legumes to legumes in elevated CO_2. Similarly, the common woodlouse *Oniscus asellus* may alter food selection in a CO_2 enriched word, which could in turn affect the composition of the forest litter layer (Hättenschwiler and Bretscher 2001). Caterpillars of *Lymantria monacha* showed higher consumption rates in elevated CO_2 but still reached a lower biomass compared to caterpillars feeding on control plants. However increasing N deposition might mitigate these effects (Hättenschwiler and Schafellner 1999). A second lymantriid *L. dispar* may alter feeding behavior in a CO_2 rich world due to changing nutritional quality of host leaves (Hättenschwiler and Schafellner 2004).

Moreover, elevated CO_2 has been shown to alter insect communities. For example, of the 59 morpho-species of insects identified on *Quercus* and *Fagus* (31% bugs, 24% beetles, 18% cicadas as the largest groups) at the SCC site, chewing feeding guilds gained in abundance on *Fagus* and sap-sucking guilds gained on *Quercus* growing in elevated CO_2 (Altermatt 2003). Overall diversity of insect taxa declined in the CO_2 enriched zone according to this study. In contrast to the results of a study conducted at the Oak Ridge FACE site, where arthropod abundance and richness across all trophic groups did not differ between ambient and elevated CO_2 plots (Sanders et al. 2004). At the Duke Forest FACE facility in North Carolina herbivory decreased in a naturally established forest understorey exposed to elevated CO_2. The question is how this discrepancy can be explained. Did insects migrate to non-CO_2 enriched areas in these experiments or if they do not will poorer food quality lead to higher mortality? This is definitely a question of insect mobility. However, in the future there will be no narrow CO_2 enriched zones and control zones as inevitably the case in FACE experiments nowadays.

1.2 This doctoral thesis

Rising CO_2 will affect biotic processes at multiple levels, of which I studied the effects on growth of mature forest trees, changes in leaf chemistry and herbivore behaviour. The fieldwork for this doctoral thesis contributed to two ongoing long-term studies conducted by the Institute of Botany, Basel in a lowland temperate forest near Basel and in trees at treeline near Davos, Switzerland. The first site is in a 100-year old deciduous forest with canopy access possible with a 45-m tall tower crane (Swiss Canopy Crane, SCC, since 1999). Beginning in late September 2000, a group of 14 ca. 35-m tall forest trees (belonging to 6 species) received canopy CO_2-enrichment using a new, pure CO_2 release technique called web-FACE (Pepin and Körner 2002). The key question of this project is, whether mature forest trees are carbon limited in growth.

The second study site is located at Stillberg, Davos in the Swiss Central Alps at an elevation of 2180 m a.s.l. In a large afforestation experiment the tree species *Larix decidua, Pinus cembra* and *Pinus uncinata* were planted across an area of 5 ha in 1975. At this site our group has installed another FACE experiment. At the upper limit of this large-scale plantation (2180 m a.s.l.), we have chosen a total of 40 individual trees (on average 1.5 m tall, 30 years old) from the two species *Larix decidua* and *Pinus uncinata* together with a typical alpine dwarf shrub vegetation to establish experimental plots. One half of the trees (20 trees and their understorey) were enriched with elevated CO_2 using FACE from the beginning of the 2001 vegetation period (Hättenschwiler et al. 2002). The remaining 20 trees served as control trees. Similar to the SCC project near Basel, the question here is again whether growth of trees at treeline is carbon limited, how tissue quality will change in elevated CO_2 (Hättenschwiler et al. 2002, Handa et al. 2005) and which effect this will have on plant-animal interactions.

Chapter 1 of this thesis addresses the issue, whether mature trees, the largest biomass pool on earth will respond to the continued rise in atmospheric CO_2 concentrations. Is there a potential for more growth, and perhaps more carbon storage due to CO_2-fertilization? Are trees in natural forests already carbon saturated or will they soon be, given that current CO_2 concentrations have reached already twice the glacial minimum concentration.

Chapter 2 describes the effect of elevated CO_2 on larvae of the grey larch bud moth (*Zeiraphera diniana*). This lepidopteran is the most important leaf-chewing insect herbivore of the larch-cembran pine forests in the central European Alps causing large-scale defoliations in these forests. As explained above, elevated CO_2 is known to affect leaf or needle tissue quality of plant species. These induced alterations in leaf chemistry can in turn affect insect herbivores feeding on these leaves.

Chapter 3 deals with the performance of the alpine grasshopper (*Miramella alpina*), feeding on CO_2 enriched dwarf shrubs at treeline. The alpine grasshopper, a widespread generalist herbivore in the Alps at altitudes between 1000 m and 2500 m a.s.l., preferentially feeds on different species of the genus *Vaccinium* (Harz 1957, Illich and Winding 1989). In Ticino (Switzerland) the genus *Miramella* tends to population explosions (outbreaks), associated with huge defoliation on broadleaved trees (Asshoff et al. 1999).

Chapter 4 While chapters 2 and 3 explored herbivore insect responses to elevated CO_2 exposed foliage in captivity, chapter 4 addresses a very basic question related to any study in free-living herbivore insects under FACE, namely insect mobility. For this study a stable carbon isotope tracer ($\delta^{13}C$) was used to establish movement patterns in the canopy. The chapter thus describes the ability of arthropods to migrate to different trees or different areas in a forest or whether wingless arthropods, larvae or nymphs are restricted to the particular region in the forest canopy where they have hatched. This study gives some insight to which extend insects move in the canopy and thus their ability to react to the future changes in nutritional quality of food plants.

Chapter 5, **6 and 7** Parts of my doctoral works become contributions to joint publications of our group in Basel. These publications offer new perspectives (environmental stress, cross-cutting synthesis, methods) and strongly depended on my doctoral thesis. In the study by Leuzinger et al. (2005) plant water relations under elevated CO_2 were studied in the drought year 2003. I contributed a set of phenology data and growth data (basal area increment) to this paper (Chapter 5). Chapter 6 synthesized the results of 4 years of research at the SCC. This manuscript is under review in Science magazine. I will only show the abstract, text passages and the figure I am responsible for. Finally, Hättenschwiler et al. (2002) provide information about the performance of the treeline FACE at the Stillberg site and summarize results obtained during the first year of CO_2 enrichment (Handa et al. 2005, Chapter 7).

Chapter 8 In this concluding chapter, I am appending titles and summaries of a few studies I conducted during my doctoral works, which are not linked directly to the CO_2 experiments, but nevertheless contribute to herbivorous insect ecology, which forms the major part of this thesis. These papers are written in German and for a wider audience and may stimulate the interest of a more general readership in getting engaged in biological issues. One of these papers contributed to the faunistic inventory of the SCC forest (bush-crickets and grasshoppers), another publication relates to a historical dispute about forest pest insects of the late 18th century. To protect the forests adequately in those times, information was required about the biology of insect pests, which was rare at that time. The authors contributing to this discussion are arguing which insect is responsible for the huge defoliation in parts of middle Germany: *Lymantria monacha* or *Lymantria dispar*? The latter herbivore species was also used in an experiment on the effects of elevated CO_2 on its performance at the SCC site (Hättenschwiller and Schafellner 2004). It is a curious thought to imagine these authors seeing a canopy crane with scientists in a gondola studying plant-insect interaction in a CO_2 enriched world 200 years later.

Formally, each of the chapters is structured as an independent contribution, with the references given in the end of the thesis.

2 Growth and phenology of mature temperate forest trees in elevated CO_2

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Abstract

Are mature forest trees carbon limited at current CO_2 concentrations? Will 'mid-life', 35-m tall deciduous trees grow faster in a CO_2-enriched atmosphere? To answer this question we exposed ca. 100-year-old temperate forest trees at the Swiss Canopy Crane site (SCC) near Basel, Switzerland to a 530 ppm CO_2 atmosphere using web-FACE technology. Fourteen trees out of 5 different species were exposed to elevated CO_2 and 36 trees served as controls in ambient CO_2. Here we report growth data for the initial four treatment years. At the end of the first treatment season (2001), the increment of stem basal area (BA) was significantly enhanced in *Fagus sylvatica.* This species exhibited a second significant BA stimulation in 2003, when Europe experienced a centennial drought (no response in 2002 and 2004). None of the other dominant species (*Quercus petraea*, *Carpinus betulus*) showed a growth response to CO_2 in any of the 4 years or for all years together. Tested across all species, there was no CO_2 effect either (neither when tested per year nor cumulative for four years). The inclusion of individual trees of *Prunus* and *Tilia* did not change the picture. In elevated CO_2 lateral branching was significantly higher in *Fagus* in 2002 when shoots developed from buds that were formed during the first season of CO_2 enrichment (2001), but there was no effect in later years. In *Quercus*, there was a steady stimulation in leading shoot length in high CO_2 trees resulting in significant longer leading shoots after 4 years of CO_2 enrichment. Phenological variables (bud break, leaf fall, leaf duration) were highly species-specific and were not affected by elevated CO_2 in any consistent way. Our four-year data set reflects a highly dynamic and species-specific response of tree growth to a step change in CO_2 supply. Except for some transient growth stimulation the CO_2 response of these relatively fast growing trees do not support the notion that mature forest trees will grow faster in a CO_2 enriched world.

Keywords: basal area increment, branching, FACE, leaf duration, stable carbon isotopes, Swiss canopy crane, tree rings

Introduction

Atmospheric CO_2 concentration is now higher than it has ever been during the last 26 million years and has reached more then twice of the glacial minimum concentration (Petit *et al.*, 1999; Pearson & Palmer, 2000). Forest trees store close to 90 % of all carbon fixed in plant biomass. It is still unclear how this largest biomass pool on earth will respond to the continued rise in atmospheric CO_2 concentrations (Norby *et al.*, 2001a). Is there a potential for more growth, and perhaps more carbon storage, as a result of CO_2-fertilization (Canadell & Pataki, 2002; Joos *et al.*, 2002), or are mature trees already carbon-saturated (Prentice *et al.*, 2000; Körner, 2003a,b)? It has been proposed that rising CO_2 will increase the net primary productivity of forest ecosystems (e.g. Mellilo *et al.*, 1993) and also stimulate the rate of biomass accumulation of forest trees (Wullschleger *et al.*, 1995). Recently, Norby *et al.*, (2002) provided the first evidence that CO_2 enrichment can increase net primary productivity in a closed-canopy deciduous forest. However, the increase in productivity reported in this study resulted in faster cycling of C through the system rather than increased C storage in wood. Elevated atmospheric CO_2 concentrations can indeed induce an initial stimulation of plant growth, as was demonstrated in many studies (Lee & Jarvis, 1995; Drake *et al.*, 1997; Hättenschwiler *et al.*, 1997a; Cenrtitto *et al.*, 1999; Norby *et al.*, 1995, 1999; Oren *et al.* 2001). These studies focused predominately on young plants whereas the age structure of tree populations in Europe (and possibly also elsewhere) reveals that 80% of all forest trees are older than 20 years (United Nations, 2000). Until now experiments investigating the growth responses of trees to elevated CO_2 are limited to smaller sized trees and mature trees in a natural forest have never been studied before (Norby *et al.*, 1999). Data from the Duke FACE site in North Carolina revealed, that growth of *Pinus taeda* trees (currently 22 years old) was continuously stimulated during three years of CO_2 enrichment (Hamilton *et al.*, 2002). In contrast, *Liquidambar styraciflua* trees (currently 17 years old) in the Oak Ridge FACE showed more stem growth only in the first year of CO_2-enrichment. In the following years, stimulation was restricted to faster fine root production (Norby *et al.*, 2002, 2004). Trees grown under high CO_2 for 30 years (1964-1993) in two different natural CO_2 springs in Italy showed a 12% greater final radial stem width over these 30 years compared to controls growing at ambient CO_2. However, this stimulation was largely due to responses when trees were young, i.e. this stimulation decreased with age. By the time trees reached an age of

25-30 years the annual difference in tree ring width between low and high CO_2 grown trees had disappeared (Hättenschwiler *et al.*, 1997a). Tognettti *et al.*, (2000), who studied only a single CO_2 spring and a different set of taxa found no significant increase in radial stem growth. In general, extrapolations from these earlier conducted experiments with maturing trees (some of which were very fast growing) are at risk to overestimate responses because of their vigor and high nutrient supply (Loehle, 1995; Lee & Jarvis, 1995; Körner, 1995). Further complications arise from pronounced interspecific variation, i.e. species responded very differently to elevated CO_2 for unknown reasons (Norby, 1996; Körner, 2000; Tangley, 2001; Craine *et al.*, 2003; Handa *et al.*, 2005, Körner, 2005), and even genotypic differences are possible (Wang *et al.*, 2000). The study of tree growth should not be confused with the issue of carbon sequestration and carbon pool size. Even if mature trees accreted more biomass carbon in trunks in response to elevated CO_2, such a stimulation acting upon a large area and a broad spectrum of age classes would simply accelerate the trees' life-cycle and not necessarily enhance C pools in the long run. Hence, enhanced carbon turnover is a realistic alternative. For example, *Pinus taeda* trees in the Duke FACE were twice as likely to be reproductively mature and produced three times as many cones and seeds as trees at ambient CO_2 concentration (LaDeau & Clark, 2001), signaling accelerated tree development.

It is still unclear whether elevated CO_2 affects leaf duration and leaf phenology of trees. As an results of increasing temperatures the average annual growing season has lengthened by 11 days since the early 1960s in Europe (Menzel & Fabian, 1999); spring events, such as leaf unfolding, have advanced by 6 days, whereas autumn events, such as leaf coloring, are delayed by 5 days, which might be another way to an increase in plant productivity and perhaps temporarily higher C pools. Rising atmospheric CO_2 is almost unanimously identified as the main driver of global warming (Rohde, 1990; Crowley, 2000), but there may also be a direct effect of elevated CO_2 on leaf phenology parameters, which had not been explored for adult trees. In very young trees spring phenology had not been found to change in CO_2 enriched atmospheres in several experiments (Sigurdsson, 2001; Norby *et al.*, 2003; Calfapietra *et al*., 2003; Badeck *et al*., 2004) Yet, some other authors report that bud break is delayed (Murray *et al.*, 1994, Repo *et al.*, 1996) or advanced (Karnosky, 2003). *Liquidambar* trees growing in elevated CO_2 in the Oak Ridge FACE did not show any differences in phenology parameters (leaf longevity, emergence date or abscission date) compared to controls (Herrick & Thomas 2003).

The Swiss Canopy Crane project (SCC) was specifically designed to test how mature trees will react to future CO_2 concentrations. A new CO_2-enrichment technology (Pepin and Körner, 2002) permitted us to simulate an atmosphere containing ca. 530 ppm CO_2 in the crowns of 30 - 35 m tall forest trees. Since recent studies from FACE experiments, indubigous have shown a persistent enhancement in leaf level photosynthesis in response to elevated CO_2 (Oak Ridge FACE, Sholtis *et al*., 2004; Swiss Canopy Crane, Zotz *et al*., 2005), one might expect this to facilitate more growth.

In this paper we addressed this major question together with an assessment of changes in leaf phenology. It is a great advantage of this project that the tank CO_2 used is $\delta^{13}C$ depleted, a signal that can be used to trace new carbon in the tree-soil continuum and back in time through tree ring analysis. We measured tree rings and the increment of tree basal area (BA), calculated a branching index to establish an allometric relationship in the canopy, determined a leaf area/shoot length ratio for trees growing either in ambient or elevated CO_2. Phenology was documented to estimate leaf duration. Our main aim was to determine whether biomass accumulation would increase in mature temperate forest trees exposed to elevated CO_2.

Material and methods

Study site (Swiss canopy Crane) and trees

The experimental facilities are located in a mature temperate deciduous forest (ca. 100 years old) close to Hofstetten, 15 km south of Basel, Switzerland 47° 28 ′ N. 7 ° 30 ′ E, 550 m a.s.l.). The stand is characterized by a dominance of *Fagus sylvatica* (L.) and *Quercus petraea* (Matt.) Liebl. Broad-leafed *Carpinus betulus* (L.), *Tilia platyphyllos* (Scop.), *Acer campestre* (L.) and *Prunus avium* (L.) and three species of conifers (not in the CO_2 enriched zone) occur as companion species. All trees have heights between 30 to 35 m. The forest has a comparatively high growth rate, a basal area of ca. 46 m^2 ha^{-1}, and is well supplied with mineral nutrients based on standard forestry criteria (S. Braun, pers. comm.). It stocks on shallow soils of the rendzina type on Jurrasic calcareous bedrock, with ground cover dominated by herbaceous plants (e.g. *Helleborus foetidus*, *Mercurialis perennis* and *Paris quatrifolia*) and understory shrubs (e.g. *Daphne mezereum* and *Lonicera xylosteum*). *Herdera helix* plays a prominent role as grand cover as well as canopy climber. The climate is oceanic with an annual precipitation of 900-1000 mm and usually only a few weeks of slight snow cover during mid-winter. The mean air-temperature over the growing season from mid April to mid October is 16.5 °C, with a mean temperature for the warmest month (August) of 19.2 °C (1989 to 2000, eleven years mean, weather station Metzerlen, C. Trefzger and J. Paulsen, pers. com.). The winters are mild (dormant season mean: 4.5 °C) with a mean temperature of the coldest month (January) of 2.1 °C. In all tree species leaf-bud break occurs within 3 to 4 weeks between the first week in April and at end of April. In deciduous trees leaf-yellowing starts at the beginning of October. A total of 62 canopy size trees are within the crane perimeter, which covers a ground area of 2800 m^2 (Pepin & Körner, 2002; Körner & Zotz 2003). The 45 m high construction crane provides access to every position in the forest canopy using a gondola. A total of 14 trees belonging to six different tree species have been exposed to elevated CO_2 during the growing season. Three individuals had to be disregarded during the analysis, because they yielded no or very little growth and were at a declining stage, as is normal in such a near natural forest. In the CO_2-enriched zone, we lost 1 *Quercus*, 1 *Carpinus*, and 1 *Acer* individual, reducing the number of useful trees to 11, and for similar reasons we had to disregard 7 control trees (1 *Carpinus*, 2 *Fagus*, 3 *Quercus*, and 2 Acer) leading to a number of

31 trees from current ambient CO_2.

CO_2-*enrichment*

Since late September 2000 trees were exposed to elevated CO_2, using web-FACE (Pepin & Körner, 2002). The start in September exposed trees to a 'warm up' CO_2 treatment, somewhat mitigating the step nature of the treatment impact (Luo & Reynolds, 1998). The web-FACE method uses 4 mm wide plastic tubes woven into the tree crowns (300-1000 m tubing per tree). Pure CO_2 is released through laser-punched wholes. The rate of CO_2 emission is controlled by a computer, connected to an infrared gas analyzer (IRGA), which receives air samples from 75 sampling points in the canopy. Within the upper crown of treated trees the daily mean concentration of CO_2 in the atmosphere is between 526 and 566 µmol mol^{-1} (seasonal mean ca. 530 µmol mol^{-1}), compared to control trees at current ambient CO_2 (concentration of ca. 370 µmol mol^{-1}). Three individuals of *Fagus*, four of *Quercus* and *Carpinus* (n=4 per species), and one individual of each *Tilia*, *Acer,* and *Prunus* are growing in elevated CO_2. Concentrations of CO_2 are independently assessed through C4-grass “iso-meters” in the canopy, which integrate the stable carbon isotope signal introduced by the pure CO_2 release, which come from fossil sources ($\delta^{13}C$ -30‰). This isotope signal is also used as a tracer of the fate of new carbon assimilated into the whole forest.

Leaf Phenology

Phenology and leaf duration were documented from 2002 to 2004 for *Carpinus*, *Fagus* and *Quercus*, from bud break in spring to leaf fall in autumn. Two persons independently estimated events as bud break (defined as 75% of all buds broken) in four-day intervals, for the top canopy area of the trees. The time of leaf loss in autumn was assessed by counting leaves of four different branches per tree until a branch had lost 75% of the original leaf number (similar to Augspurger & Bartlett, 2003).

Shoot measurements

Annual length increment of tagged leading shoots of *Carpinus*, *Fagus* and *Quercus* was measured for the years 2002-2004 and reconstructed for the years 1998-2001 for four different branches per tree using the crane gondola. Annual lateral shoot increment was measured and reconstructed from bud scars for 1998 to 2003. A branching index defined by Hättenschwiler *et al.* (1997b) as the cumulative length (cm) of all lateral branches of one year divided by the length of the main leading branch (cm) of the same year was calculated for the years 1998 - 2003.

Leaf/area shoot ratio

In 2003, the third full season of CO_2-enrichment, the mean total leaf area per branch was calculated from mean individual leaf area multiplied by the number of all leaves of the respective branch (on average 291±21 leaves per branch). We determined a leaf area/ shoot ratio for *Carpinus*, *Fagus* and *Quercus* by dividing total leaf area (on average 6734±744 cm^2) of a branch by the length of the leading shoot (1998-2003, on average 92±4 cm).

Basal area (BA) increment

Tree girth-tapes (D1-L, UMS GmbH, Munich, Germany; accuracy of measurement 0.1 mm) attached at breast height were used to determine radial increment of 63 trees (*Carpinus*: n=13 (4 in elevated CO_2), *Fagus*: n= 21 (3 in elevated CO_2), *Quercus*: n=17 (2001: 4 in elevated CO_2, since 2002: 3 in elevated CO_2), *Prunus, Acer, Tilia*: (n=4 each, 1 each in elevated CO_2). Monthly measurements started in April of 2001. From 2002 onwards we also took dendrometer readings from a subsample of trees (all trees in elevated CO_2 and control trees accessible from the crane) in the upper stem region before the first major branching in ca. 15-20 m above the ground (*Querucs*: n=7, *Carpinus*: n=8. *Fagus*: n= 6) in order to assess any differences in growth with height along the trunk. Basal area (BA), which is defined as the area in square centimeters of a cross section taken at standard breast height, including the solid part of the bark, was calculated from the absolute values of stem diameter. Subsequently, BA increment (%) was calculated for each year separately (2001 - 2004) with the prior year BA as reference value, and cumulative BA was calculated for the years 2001 to 2004 with BA 2000 as reference value.

Tree ring measurements and $\delta^{13}C$ *analysis*

In order to minimize damage to our experimental trees, we punched trees in 2004 with a custom-made 4 mm diameter stainless-steel core-puncher (Asshoff *et al.*, in prep.). A conventional drill type, 5 mm corer, would have produced a major wound with uneven edges. Punches cores had a length of 30 mm. These cores were split in two halves parallel to fiber direction. One half was used for tree ring analysis and the other one for $\delta^{13}C$ analysis (see below). For tree ring measurement a mechanical, computer linked measurement platform was used under a binocular microscope (LINTAB and TSAP software (Frank Rinn, RINNTECH, Heidelberg, Germany). For diffuse-porous tree species (*Acer*, *Carpinus*, and *Tilia*) microsections were prepared and stained and samples were measured with digital image analyses (Olympus). Any new tissue growth under elevated CO_2 should carry the stable carbon isotope signal of the difference of natural (ambient) CO_2 (-8‰) and the mixture of CO_2 released from the CO_2 tank and atmospheric CO_2 (ca. -15‰). Stem wood should carry a highly integrated $\delta^{13}C$ signal. Therefore we cut 0.6 to 0.8 mg of tiny wood pieces with a scalpel from each year's growth ring, rapped it in tin cups and analyzed it for $\delta^{13}C$. The samples were then combusted in an elemental analyzer (EA-1110, Carlo Erba Thermoquest, Italy) and transferred via an open split interface (Conflo II, Finnigan Mat, Germany) to a mass spectrometer (Delta S, Thermo Finnigan Mat, Germany). Unfortunately, we were not able to obtain samples for *Prunus*, *Carpinus* and *Acer* from these cores, because the tree ring width was too narrow (<0.5mm)

Reconstruction of pre-treatment basal area growth

Dendrometer measurements, which average growth over the entire trunk, were available for four years (2001-2004). In order to account for tree specific vigor before the treatment, we needed pre-treatment basal area increments. This was achieved by the following procedure using the tree ring data for each individual tree for the last 5 years before treatment. Dendrometer increment data (2001-2004) were transformed to an average tree ring width for each year. Tree ring widths from single wood cores would be identical with the radial increment of girth tapes if tree growth would be identical across the whole stem, which is unlikely. However for a all trees combined, these two estimates of tree ring width indeed yielded a 1:1 match (slope: 1.09, 95% confidence interval: 0.95-1.23) for the 4 years for which both measurements were available). For each individual, however, the slope of the regression differed substantially from 1 (i.e. the coring position was not representative of radial tree growth in absolute terms), which would result in an overestimate or underestimate of basal area growth during the pre-treatment period. To account for this, we corrected all tree ring widths as determined from wood cores by the core versus dendrometer ratio of the 2001-2004 data. These corrected pre-treatment tree ring widths of each individual tree were transformed to BA and BA increment (%) from 1996-2000. The mean BA increment of these five pre-treatment years served as a reference for each individual tree. The two basic assumptions for this procedure are that the single-point wood cores represent a good relative measure of year-to-year growth, but an unsecured basis for absolute diameter growth of the whole stem, and that the core versus girth tape increment ratio does not differ between treatment and pre-treatment years.

Tree ring analysis

Tree ring measurements enabled us to establish a short tree ring chronology (9 years: 1996-2004) for each individual tree. We tested if there was a significant difference in tree ring widths after the beginning of the experiment (2001 to 2004) compared to pretreatment growth (1996-2000). For this purpose, absolute tree ring width was standardized, i.e. expressed as a percentage of the widest tree ring width (=100%) in this chronology of each individual.

Statistical analysis

Analyses of variance (ANOVA) were applied to test for differences in basal area (BA) increment with CO_2 treatment and tree species as fixed factors. Additionally, we analyzed the time series data set for each tree species separately with CO_2 treatment as fixed factor. To account for the varying vigor of each tree individual before the experiment started, BA data were standardized by dividing BA increment values (%, 2001-2004) by the average BA increment (%) of the years 1996 to 2000 (pre-treatment growth, see above). Standardized tree rings were analyzed using a Wilcoxon Rank test to compare annual increments during the pretreatment period (1996-2000) with annual increment since CO_2-enrichment (2001-2004) for each individual tree.

Different phenology and shoot growth data were tested with ANOVA, again with CO_2 treatment as fixed factor. To account for individual differences in tree vigor the four measured branches of the tree were nested in the respective tree individual. Shoot length data were standardized with the average shoot length of the pre-treatment (1998-2000). Because all these parameters were species-specific they were analyzed separately using CO_2 as fixed factor. All ANOVAs were conducted with type I Sum of Squares (Sequential) using JMP 3.2.2. (SAS Institute).

Results

$\delta^{13}C$ *analysis*

Stable isotope data clearly document the influence of CO_2-enrichment. While $\delta^{13}C$ values of the tree rings from 1997 to 2000 (pre-treatment) did not differ significantly between individuals of *Fagus, Querucs* and *Tilia,* there was an immediate and sharp $\delta^{13}C$ signal after the onset of the CO_2 enrichment ($P<0.001$) in the new tissue produced in 2001 (Fig. 1). The signal was always strongest in *Tilia* and weakest in *Fagus*. By the end of 2004, the mean across trees was 5.8‰ and closely approached the iso-meter signal of 5.8‰. Control trees showed no signal, underlining that canopy CO_2-enrichment was confined to the treatment trees.

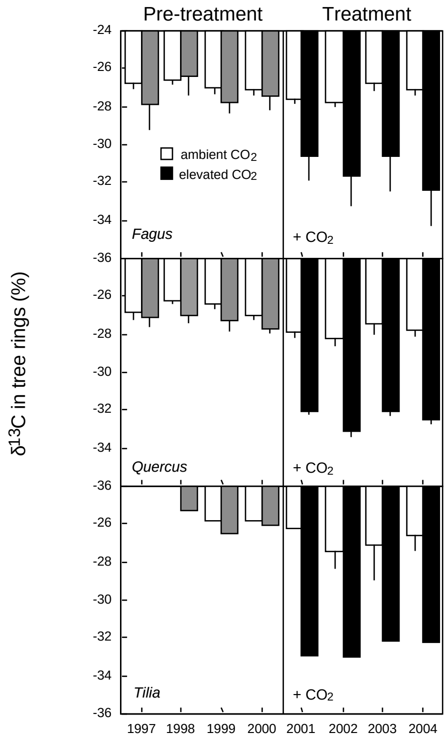

Fig. 1: $\delta^{13}C$ of tree rings in the years before CO_2-enrichment (1997-2000) and during the experiment (2001-2004) for the tree species *Fagus*, *Quercus,* and *Tilia* (Means ± SE). Treatment trees and controls did not differ significantly in their $\delta^{13}C$ value before the onset of the experiment (2000, $P = 0.45$). Grey bars (1997 - 2000) are trees, which were chosen for the CO_2-enrichment since 2001.

Phenology

Phenology variables were highly species-specific (Table 1). Total leaf duration was similar in *Carpinus* (208 days) and *Quercus* (207 days), but about 10% shorter in *Fagus* (188 days). No CO_2 effect on bud break was observed in any of the three species, but exposure to elevated CO_2 increased mean leaf duration for 2002 - 2004 in *Carpinus* and *Fagus* by 5-6 days and reduced it by 5 days in *Quercus*. Statistically, these effects are marginally significant when tested for the species x CO_2 interaction of *Quercus* and *Fagus* (P=0.068) and the difference of the *Quercus* response from the *Fagus* or *Carpinus* response (P=0.018 and P=0.057). In the drought year 2003 there was a trend towards longer leaf duration in trees in elevated CO_2 (P=0.069) Leaf duration was prolonged in *Carpinus* by 11 days, in *Fagus* by days, whereas no effect was observed in *Quercus* growing in elevated CO_2.

Shoot growth

Shoots of *Carpinus* trees in elevated CO_2 grew significantly more in 2001 compared to controls (+ 48%), but showed no response thereafter (2002 to 2004, Table 2, Fig. 2). Annual length increment of *Fagus* in elevated CO_2 tended to be higher from 2001 to 2004 (on average 11%), although these differences were not significant. Interestingly, *Quercus* shoots showed a marginally significantly smaller length growth in elevated CO_2 in the years 2001 and 2002 compared to controls, but this trend was reverted in 2003 and in 2004. In 2004 *Quercus* shoots were significantly longer (+37%) in elevated CO_2, compared to controls (significant CO_2 x time effect, RM ANOVA, P=0.006). In summary, the length increment of shoots in the canopy did not show any consistent response to 4 years of CO_2-enrichment.

The branching index did not reveal a consistent response to elevated CO_2 either, although we observed a transient effect in some species. For example, the index was significantly reduced in CO_2 exposed *Carpinus* compared to controls in 2001 (Table 2, Fig. 3), whereas there were no significant differences in the following years. This reduction of branching in 2001 resulted primarily from the significantly greater length increment in the main shoots of *Carpinus* growing in elevated CO_2. In contrast, the branching index in *Fagus* was significant higher in elevated CO_2 in 2002, when the buds for shoot growth were formed under the first season of CO_2 enrichment (2001). However, the branching index of individual *Fagus* trees already differed before the treatment started (Fig. 3). The same was true for *Quercus* trees. Thus, most of the differences in branching most likely reflect individual peculiarities and cannot be attributed unambiguously to an influence of elevated CO_2. During the 4-year treatment period branching peaked in different years for different species and when CO_2 effects occurred in a given year, they were still within the range seen during the pre-treatment (except for *Fagus* in 2002).

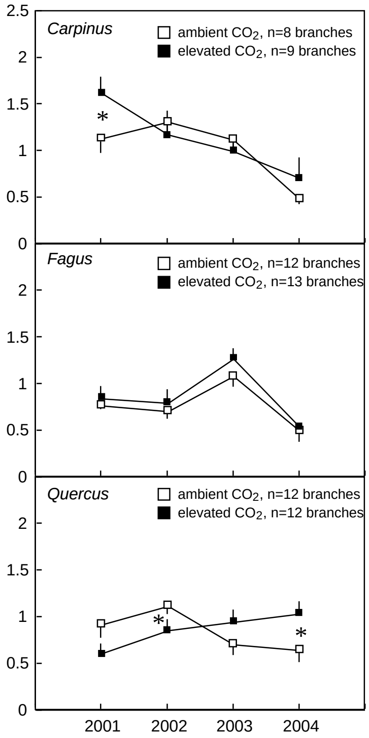

Fig. 2: Average length of leading shoots 2001 to 2004 (standardized) for the three tree species *Carpinus*, *Fagus* and *Quercus* (Means ± SE).

Leaf area/shoot ratio for 2003

The leaf area/shoot ratio was measured in 2003 (third year of treatment) and was significantly (-25%) lower in *Carpinus* trees growing in elevated CO_2 (P=0.03). In contrast, *Quercus* and *Fagus* trees showed a higher leaf area/shoot length ratio in elevated CO_2 (+110%, P=0.016 and +25%, P=0.012) compared to controls. We have no data for other years; hence these trends need further study.

Basal area (BA) increment (2001-2004)

BA increment at breast height correlated very well with BA increment in the upper canopy before branching (R^2=0.72, P<0.001 for all trees and years). Increments between trees in ambient and elevated CO_2 did not differ between breast height and ca. 15 m height, so we used breast height data only. *Fagus* was the only species, which showed a significant growth stimulation (Fig. 4, Table 3). The effect was strongest in 2001, disappeared in 2002, resumed during a heat wave summer with drought in 2003 and vanished again in 2004. *Fagus* is also the only species that revealed at least a trend towards higher cumulative BA increment after four years of CO_2 enrichment (Fig. 5, Table 3). When testing all individuals of *Carpinus*, *Fagus* and *Quercus* together year-by-year we did not find a significant CO_2 effect in any of the years 2001 to 2004, and this did not change when we included *Tilia* and *Prunus* in the analysis. Testing the three replicated species together or all species (including *Tilia* and *Prunus*, Fig. 4, Table 3) we did also not find significant growth stimulation after 4 years of CO_2 enrichment. In line with this we did not find any consistent growth stimulation by CO_2-enrichment from 2001 to 2004 when comparing the standardized annual tree ring width for each individual before and after the initiation of the experiment (1996-2000 vs. 2001-2004).

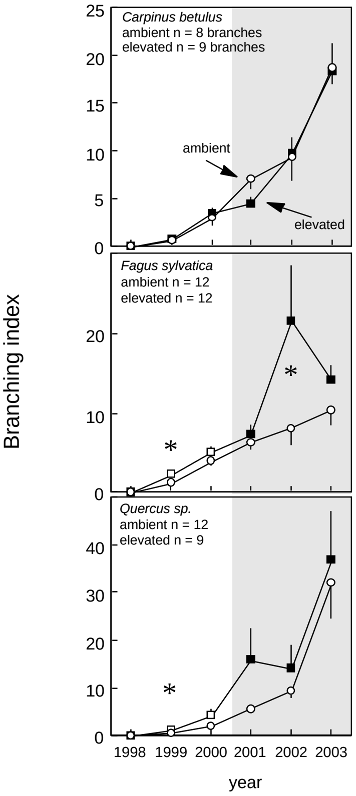

Fig. 3: Branching index of *Carpinus*, *Fagus* and *Quercus* in the years 1998 to 2003 (Mean ± SE). Shaded area: CO_2-enrichment.

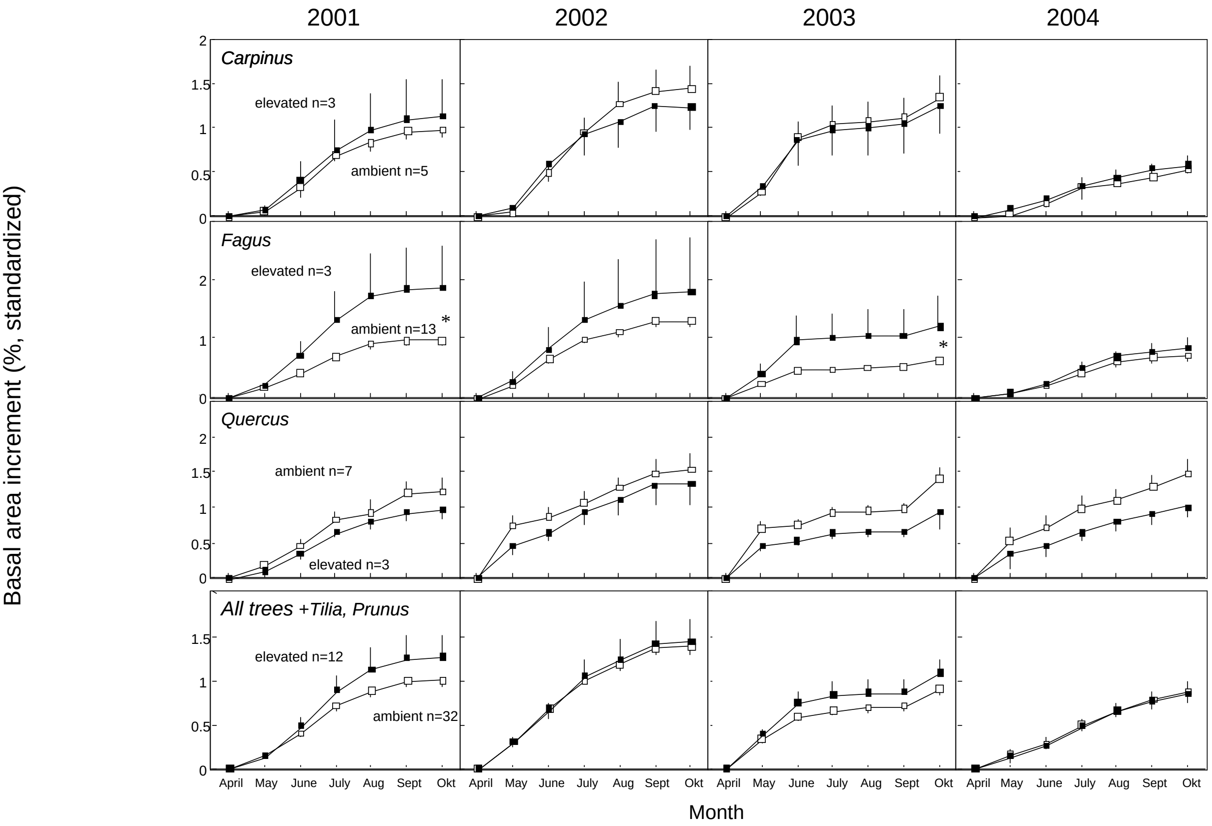

Fig. 4: Seasonal BA increment (Mean ± SE) for three tree *Carpinus*, *Fagus* and *Quercus* and all trees (including *Tilia* and *Prunus*) for the years 2001 through 2004, n = number of trees.

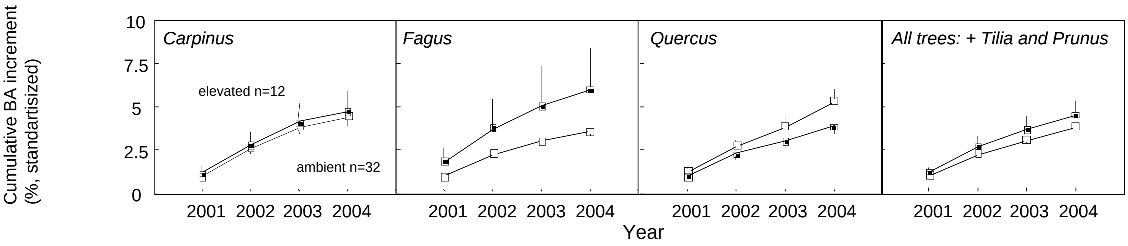

Fig. 5: Cumulative BA increment (Means ± SE) for three tree species *Carpinus*, *Fagus*, and *Quercus* and all trees (including *Tilia* and *Prunus*) for the years 2001 – 2004, n = number of trees.

Discussion

The 'web-FACE' method of CO_2-enrichment made it possible for the first time to study growth responses to elevated CO_2 in tall forest trees. Carbon isotope signals evidenced a rapid incorporation of new carbon into wood tissue. Given the 35 m height of the study trees and crown diameters of up to 15 m, the experiment was, however, limited in the number of study trees. Tree ring based pre-treatment growth helped to account for tree-to-tree variation in vigor, so that treatment effects could be separated to a large extent from individual variation.

The four-year study of these mostly very productive ‘mid-life’ stage hardwood trees provides no support for the notion of a sustained stimulation of tree growth under elevated CO_2. However, at least one species (*Fagus sylvatica*) showed a pronounced initial growth stimulation, a response, which was robust across all statistical tests, including or excluding pre-treatment growth data. Interestingly, this stimulation of *Fagus* surfaced again during a drought summer (the third year) for which we have circumstantial evidence that a CO_2 driven mitigation of drought stress played a role (Leuzinger *et al.* 2005). *Fagus* trees exposed to elevated CO_2 showed less negative pre-dawn water potential in the canopy during this period. The cumulative effect of positive growth responses in *Fagus* in two years and none in two other years was only marginally significant. Analyzing all trees together, we found no growth stimulation after 4 years of CO_2-enrichment. In a similar way, with one-year delay because of bud pre-formation, the branching index of *Fagus* was transitorily higher in elevated CO_2 in the second year of CO_2-enrichment.

The initial stimulation of shoot length in *Carpinus* also vanished later. By year four, *Quercus* still showed a consistent stimulation of leading shoot length: one could speculate that this will eventually lead to a stimulation of BA increment in this species. Until now, however, the main stems, which represent more than 80% of a mature tree’s C-pool, are unresponsive. We conclude in accordance with Hoch *et al*. (2003), who found very high and constant non-structural carbon concentrations across different tree tissues, that the growth of these trees is carbon saturated at current atmospheric CO_2-concentrations.

Other forest FACE experiments with younger trees have either shown a continuous growth stimulation or a transitory response. Growth rates of *Pinus taeda*, growing in elevated CO_2 in the Duke FACE experiment were still 23% higher compared to controls in the fourth year of the experiment (DeLucia *et al*., 1999; Naidu & DeLucia, 1999; Hamilton *et al*., 2002). In contrast, *Liquidambar styraciflua* trees in the understory did not show a response to elevated CO_2 in this experiment. In monospecific stands, *L. styraciflua* trees in the Oak Ridge FACE revealed a similar transitory growth response to elevated CO_2 as the *Fagus* trees in our experiment (Norby *et al*. 2001b, 2002, 2004). Wood increment increased significantly during the first year of exposure, but subsequently most of the extra C was allocated to the production of leaves and fine roots, which represent relatively small C pools. An initial stimulation in growth of *Populus* saplings was also observed in the POPFACE experiment in Italy (Miglietta *et al*., 2001, Wittig *et al*., 2005). In this fast growing, young plantation, gross primary production was stimulated by elevated CO_2 in all 3 years tested, although it decreased in time. It was greatest in the first year (223–251%), but markedly lower in the second (19–24%) and third years (5–19%). These studies show the high inherent species-specificity in responses to elevated CO_2 (Körner, 2005), although, in the case of the poplar plantation, the very young age and the luxurious growth conditions also contributed to the response.

Another major determinant of plant responses to elevated CO_2 is soil quality. In an open top chamber study, biomass of *Fagus* responded negatively to CO_2 on nutrient poor acidic but positively on more fertile calcareous soil (Spinnler *et al*., 2002). Moreover, there was also intraspecific variation in the sensitivity of growth to elevated CO_2 (Spinnler *et al*., 2003). Total biomass of a spruce model ecosystem was not affected by CO_2 concentration, but significantly by N deposition (Hättenschwiler & Körner 1998). The lack of a consistent positive growth response in our study can hardly be explained by nitrogen shortage, given the overabundance of N in this region (mean annual wet N-deposition of ca. 30 kg N ha^{-1} a^{-1}).

The transitory branch response in *Fagus* trees in elevated CO_2 in the second year of CO_2-enrichment agrees with other cases where elevated CO_2 was shown to modify crown structure and increase branch biomass (e.g. Jach & Ceulemans, 1999, Pritchard *et al*., 1999; Gielen *et al*., 2002). Changes in branching patterns as a response to elevated CO_2 may result in changes in crown architecture that could alter vertical light attenuation pattern and possibly affect recruitment (Hättenschwiller & Körner, 2000). Interestingly, *Quercus ilex* trees growing around natural CO_2 springs in Italy showed a significant lower branching (fewer lateral branches) and as a consequence total branch leaf area was reduced compared to control trees (Hättenschwiler *et al*., 1997b). The authors interpreted the reductions in leaf assimilatory surface under elevated CO_2 as morphological adjustment to enhanced carbon availability. The trees studies here did not show such morphological adjustments.

Rising atmospheric CO_2 concentrations can affect leaf phenology, which in turn can affect leaf duration and thus biomass production. Phenological responses (in both spring and autumn) to elevated CO_2 in other field studies included accelerated development, delayed development, or (probably most commonly) no response

(Norby et al., 1999; Murray & Ceulemans, 1998). In our trees, leaf duration was somewhat prolonged in *Carpinus* and *Fagus* growing in elevated CO_2, in contrast it was reduced in *Quercus*. A trend needed to be verified over more years. We did not find an effect of elevated CO_2 on bud break, which is in line with other studies (*Acer rubrum* and *A. saccharum*, Norby *et al.*, 2003; *Fagus sylvatica*, Forstreuter, 2002; *Liquidambar styraciflua*, Herrick & Thomas 2003; *Populus trichocarpa*, Sigurdsson, 2001). The release of vegetative buds from winter dormancy is controlled by a complex suite of environmental signals, of which temperature and photoperiod are most important (Kaszkurewicz & Fogg, 1967; Campbell & Sugano, 1979; Wielgolaski, 2001). A delay of autumnal senescence has been reported for *Populus* trees growing in elevated CO_2 (Tricker et al. 2004), and a reduced leaf duration for *Rumex obtusifolius* (Pearson and Brooks 1995). These results contrasts with our findings in mature forest trees. However, we found a trend towards a prolonged leaf duration in trees growing in elevated CO_2 in the drought year 2003.

Several more years are needed to ascertain longer-term trends, but the outcome of the first four years of treatment provide little evidence for any changes in tree growth and phenology in elevated CO_2. Thus, we assume no carbon limitation of growth in these trees under current CO_2 concentrations. However, enhanced below ground C-turnover indicates a rapid flux of new carbon through this system (Steinmann *et al.*, 2004), which led us to conclude that elevated CO_2 enhance fluxes rather than pools of carbon (Körner *et al.*, in preparation).

Acknowledgements

We thank Steeve Pepin for installing girth tapes in spring 2001. Dieter Eckstein and Werner Schoch provided indispensable help with tree ring analysis. Erwin Amstutz for constructing the puncher and crane operations. Tanya Handa, Günter Hoch, Sonja Keel, Sebastian Leuzinger, and Dieter Spinnler are acknowledged for discussion and statistical advice. This research was supported by the Swiss National Science Foundation (grants 3100-059769.99 and 3100-067775.02 to Christian Körner). The Swiss Federal Office for the Environment (BUWAL) and the University of Basel sponsored the crane and siteinfrastructure. R.A was funded by COST E21/Proj. 69 and Freiwillige Akademische Gesellschaft Basel (FAG).

Table 1: Leaf phenology from bud break (BB) to leaf fall (LF), and resulting in leaf duration (Mean ± SE) for the second to the fourth year of CO_2-enrichment (days of years are given for BB and LF, number of days for leaf duration (LD)), A: ambient CO_2, E: elevated CO_2.

	2002			2003			2004			Mean 2002-2004		
	BB	LF	LD	BB	LF	LD	BB	LF	LD	BB	LF	LD
Carpinus												
E (n=4)	97±2	296±0	200±2	89±1	317±2	228±2	94±2	297± 2	203± 2	93±1	303±2	210±3
A (n=3)	90±4	290±3	200±4	93±1	320±2	217±2	91±0	288±14	197±15	91±1	299±6	205±6
Fagus												
E (n=3)	115±0	295±1	180±1	115±1	314±2	199±2	112±1	306±1	193±1	114±0	305±0	191±0
A (n=3)	118±0	291±2	173±2	113±2	308±7	195±6	112±0	300±4	188±4	114±1	300±4	185±4
Quercus												
E (n=3)	111±2	299±1	186±1	108±1	320±1	212±2	115±1	333±1	218±1	112±0	317±1	205±1
A (n=3)	111±2	306±1	194±2	110±1	321±3	211±3	115±2	338±2	223±3	112±1	322±1	210±2

Table 2: Statistical results of shoot responses. Shoot length increment (leading shoot) is standardized by the average shoot length increment (1998-2000). N.d.: not determined.

	1999		2000		2001		2002		2003		2004	
CO_2	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Shoot length increment (standardized)												
Carpinus					$F_{1,11}=4.9$	0.048	$F_{1,11}=0.7$	0.43	$F_{1,11}=0.8$	0.40	$F_{1,11}=1.7$	0.22
Fagus					$F_{1,18}=0.2$	0.63	$F_{1,18}=0.1$	0.77	$F_{1,18}=2.0$	0.18	$F_{1,18}=0.0$	0.76
Quercus					$F_{1,17}=1.6$	0.08	$F_{1,17}=4.1$	0.06	$F_{1,17}=3.2$	0.09	$F_{1,17}=4.5$	0.049
Branching index												
Carpinus	$F_{1,12}=0.2$	0.68	$F_{1,12}=0.1$	0.75	$F_{1,12}=6.2$	0.029	$F_{1,12}=0.0$	0.90	$F_{1,12}=0.0$	0.90	N.d.	N.d.
Fagus	$F_{1,18}=6.8$	0.02	$F_{1,18}=1.6$	0.23	$F_{1,18}=0.60$	0.45	$F_{1,18}=3.9$	0.06	$F_{1,18}=1.7$	0.21	N.d.	N.d.
Quercus	$F_{1,17}=5.0$	0.04	$F_{1,17}=3.1$	0.096	$F_{1,17}=3.1$	0.099	$F_{1,17}=0.9$	0.36	$F_{1,17}=0.1$	0.71	N.d.	N.d.

Table 3: Statistical analyses of BA increment (%). Results for *Fagus*, *Carpinus*, and *Quercus* and for all species are shown. *T*: *Tilia platyphyllos*, *Pr*: *Prunus avium*.

Factor	2001		2002		2003		2004	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Full Model (+*T*,*Pr*)[a]								
Tree species	$F_{4,33}=0.9$	0.50	$F_{4,33}=4.6$	0.02	$F_{4,33}=10.2$	<0.0001	$F_{4,33}=6.8$	0.0004
CO_2	$F_{1,33}=1.7$	0.21	$F_{1,33}=0.3$	0.61	$F_{1,33}=0.1$	0.72	$F_{1,33}=0.0$	0.91
Species x CO_2	$F_{4,33}=1.6$	0.19	$F_{4,33}=0.7$	0.57	$F_{4,33}=2.2$	0.09	$F_{4,33}=1.3$	0.31
Full Model (-*T*,*Pr*)								
Tree species	$F_{2,28}=0.1$	0.91	$F_{2,28}=0.1$	0.91	$F_{2,28}=7.1$	0.003	$F_{2,28}=10.3$	0.0004
CO_2	$F_{1,28}=2.1$	0.16	$F_{1,28}=0.0$	0.84	$F_{1,28}=0.1$	0.76	$F_{1,28}=0.3$	0.62
Species x CO_2	$F_{2,28}=2.9$	0.07	$F_{2,28}=1.0$	0.39	$F_{2,28}=3.6$	0.04	$F_{2,28}=1.5$	0.24
Fagus								
CO_2	$F_{1,15}=6.1$	0.026	$F_{1,15}=1.3$	0.28	$F_{1,15}=5.9$	0.028	$F_{1,15}=0.2$	0.64
Carpinus								
CO_2	$F_{1,6}=0.2$	0.68	$F_{1,6}=0.3$	0.62	$F_{1,6}=0.1$	0.81	$F_{1,6}=0.0$	0.85
Quercus								
CO_2	$F_{1,8}=0.7$	0.43	$F_{1,8}=0.4$	0.56	$F_{1,8}=3.1$	0.12	$F_{1,8}=2.2$	0.18
Cumulative (CO_2)[b]								
Full Model (+*T*,*Pr*)								
Tree species			$F_{4,33}=2.4$	0.07	$F_{4,33}=3.6$	0.01	$F_{4,33}=3.3$	0.02
CO_2			$F_{1,33}=1.3$	0.25	$F_{1,33}=1.2$	0.29	$F_{1,33}=0.7$	0.40
Species x CO_2			$F_{4,33}=1.1$	0.36	$F_{4,33}=1.5$	0.22	$F_{4,33}=1.0$	0.21
Full Model (-*T*,*Pr*)								
Tree species			$F_{4,29}=0.0$	0.99	$F_{4,29}=0.5$	0.63	$F_{4,29}=0.1$	0.91
CO_2			$F_{1,29}=1.1$	0.30	$F_{1,29}=0.9$	0.35	$F_{1,29}=0.2$	0.63
Species x CO_2			$F_{4,29}=1.9$	0.17	$F_{4,29}=2.6$	0.09	$F_{4,29}=2.7$	0.09
Fagus			$F_{1,15}=3.7$	0.07	$F_{1,15}=4.4$	0.05	$F_{1,15}=3.7$	0.07
Carpinus			$F_{1,6}=0.1$	0.81	$F_{1,6}=0.0$	0.87	$F_{1,6}=0.0$	0.85
Quercus			$F_{1,8}=0.6$	0.45	$F_{1,8}=1.6$	0.24	$F_{1,8}=1.8$	0.21

[a] +*T*,*Pr*/-*T*,*Pr* refers to the inclusion or exclusion of *Tilia* and *Prunus* individuals. Full model (+*T*,*Pr*) refers to n=11 trees (5 species) growing in ambient CO_2 and 32 trees (5 species) growing in ambient CO_2.

[b] Cumulative refers to the 2001-2004 sum of BA increment assessed by the end of 2004 compared to the year 2000 for each individual tree.

3 Changes in needle quality and larch bud moth performance in response to CO_2-enrichment and defoliation of treeline larches

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submitted to *Ecological Entomology*

Abstract

1. The larch bud moth cycle is hypothesized to be under control of host tree foliage quality. In a Free Air CO_2 Enrichment (FACE) experiment at the swiss alpine treeline (2180 m a.s.l.), we investigated the effects of elevated CO_2 and previous year defoliation on larch needle quality and larch bud moth performance.

2. Starch and lignin concentration increased and water content decreased in elevated CO_2-grown needles compared to ambient CO_2. Defoliation resulted in reduced nitrogen, water, starch and sugar concentration in needles of the next year generation. We observed no interactions between elevated CO_2 and defoliation on needle quality.

3. Needle quality changes due to needle maturation over the course of the experiment, however, were much larger than the effects of elevated CO_2 and defoliation. For example, N concentration was on average 38% lower and lignin concentration 55% higher at the end of the experiment (early July 2003) than at the beginning (mid June 2003).

4. Larch bud moth larvae grew somewhat slower under elevated CO_2 compared to ambient CO_2 when trees remained undefoliated. If, however, trees have been defoliated, this response was reversed, with a faster growth of larch bud moth on high CO_2-exposed trees than on control trees. Pupal weight was not affected by either CO_2 or defoliation.

5. Our results suggest that the larch bud moth has to cope with large changes in food quality due to needle maturation during its development, and that additional CO_2- and defoliation induced alterations in needle chemistry have comparatively minor influences on larch bud moth performance at treeline.

Key words: global change, *Larix decidua*, larval relative growth rate, needle chemistry, plant-herbivore interaction, *Zeiraphera diniana*

Introduction

The larva of the grey larch bud moth (*Zeiraphera diniana* GN.), a lepidopterous species (Tortricidae), is the most important leaf chewing insect herbivore of the larch-cembran pine forests in the central European Alps, causing large scale defoliations in these forests. The larch bud moth population cycle is well studied, showing a quite regular oscillation with population peaks every 7 to 10 years accompanied with severe defoliation of entire larch stands (Baltensweiler *et al.*, 1977; Baltensweiler, 1993; Baltensweiler & Fischlin, 1988; Baltensweiler & Rubli, 1999). Dendroecological studies documented, that this phenomenon of periodic outbreaks occurred at least since the 16th century (Weber, 1995; 1997). Changes in larch foliage quality in response to heavy defoliation have been discussed as a potential control mechanism of larch bud moth population dynamics. It was found that larch needle quality declined in the years following larch bud moth outbreaks. Needle fibre concentration increased whereas protein concentration decreased for two or even more years after defoliation (Benz, 1974; 1977). These changes in needle chemistry are believed to impair larval survival and reproduction (Benz, 1974; 1977; Omlin & Herren, 1976; Omlin, 1977; Fischlin, 1982). However, it has recently been suggested that larch bud moth population cycles might be under parasitoid control rather than needle quality control (Turchin *et al.*, 2003). The autors showed that roughly 90% of the variance in larch bud moth population densities can be accounted for by parasitoids, and only 10% by host quality. In a paper of Johnson *et al*. (2004) it was proposed that the spatial arragement of landcape mosaics is responsible for initiating travelling waves of the larch bud moth.

There is clear evidence from both laboratory experiments and long-term field experiments that plant tissue quality will change with the ongoing rise in atmospheric CO_2 concentration (Körner & Arnone, 1992; Körner & Miglietta, 1994; Kinney *et al.*, 1997; Poorter *et al.*, 1997; Cotrufo *et al.*, 1998). Such CO_2-induced leaf quality changes have important consequences for herbivores (Watt *et al.*, 1995; Hunter, 2001; Coviella *et al.*, 2002). In different studies herbivores responded with slower larval growth rates and longer development time to CO_2-induced changes in food quality (Roth & Lindroth, 1995; Roth *et al.*, 1998; Williams *et al*., 1998; Williams *et al*., 2000; Hättenschwiler & Schaffelner 1999; 2004; Veteli *et al.*, 2002; Asshoff & Hättenschwiler, 2005). These findings suggest that elevated CO_2 could further enhance any negative effect of defoliation on food quality. Such reinforced control of defoliation induced resistance by increasing atmospheric CO_2 concentration might fundamentally change the larch bud moth cycle with far-ranging consequences for the structure and function of the alpine forest ecosystems. We know of only two studies, investigating the combined effects of elevated CO_2 and defoliation on insect performance. Growth of gypsy moth (*Lymantria dispar* L.) larvae feeding on maple seedlings was not negatively affected by elevated CO_2, whereas it was significantly reduced on high CO_2-grown aspen seedlings (Lindroth & Kinney, 1998). Defoliation, on the other hand, did not affect larval growth in either tree species and there were no CO_2 x defoliation interactions (Lindroth & Kinney, 1998). Within the same experiment the performance of a second lepidopteran species, the forest tent caterpillar (*Malacosoma disstria* Hübner), was investigated (Roth *et al*., 1998). In line with gypsy moth, the growth rate of forest tent caterpillars in elevated CO_2 was reduced on aspen, but not on maple. In addition, defoliation had a negative effect on *M. disstria*, but on maple only. These findings provide little evidence for additive negative effects of elevated CO_2 and defoliation, but the data basis is strongly limited. Moreover, responses of insects to elevated CO_2 and defoliation are in general highly host-plant specific (Lindroth & Kinney, 1998; Roth *et al*., 1998; Asshoff & Hättenschwiler, 2005), and can even vary widely among host-plant genotypes (Osier & Lindroth, 2004).

In this study we aimed to test the hypothesis of a reinforced negative impact of combined effects of elevated CO_2 and defoliation on the larch needle quality - larch bud moth system in the Central Alps, using the in situ CO_2-enrichment experiment at a Swiss alpine treeline (Hättenschwiler *et al*., 2002).

Material and methods

Study site and experimental setup

The experimental area is located at Stillberg, Davos in the Swiss Central Alps at an elevation of 2180 m a.s.l. The long-term annual precipitation of the NE exposed study site is 1050 mm; the average temperature is -5.8°C in January and 9.4°C in July (Schönenberger & Frey, 1988). The soil is classified as a Ranker (U.S. system: Lithic Haplumbrept) with 10-cm-deep organic topsoil underlain by siliceous bedrock (Paragneis, Schönenberger & Frey, 1988). In 1975, in the context of a large afforestation experiment, the treeline species *Larix decidua* L., *Pinus cembra* L., and *Pinus uncinata* Ramond were planted across an area of 5 ha. At the upper end of this plantation we established our CO_2-enrichment experiment. The vegetation is dominated by the dwarf shrubs *Vaccinium myrtillus*, *Vaccinium uliginosum*, and *Empetrum hermaphroditum*. *Gentiana punctata*, *Homogyne alpina*, and *Melampyrum pratense* are the most common herbaceous species. A total of 40 plots, 20 of each with one individual tree of either one of the two species *Larix* *decidua* and *Pinus uncinata* in the centre of the plot were established in early June 2001. Using a split-plot design two *L. decidua* and two *P. uncinata* trees each were assigned to ten groups (5 groups in elevated CO_2, and 5 control groups). Trees and understorey plants have been exposed to elevated CO_2 concentration since the beginning of the vegetation period in 2001 (seasonal means±SD of 566±42 (2001), 569±25 (2002), 568±45 (2003). Elevated CO_2 was applied using Free Air CO_2 Enrichment (FACE) with pure CO_2 release technology. The CO_2-enrichment system and the experimental setup are explained in detail in Hättenschwiler *et al*., (2002). In late June 2002, simulating a larch bud moth outbreak, half of all trees were assigned to an 80% defoliation treatment (one larch and one pine individual of each group), yielding a replication of 5 trees per CO_2 treatment, defoliation treatment and tree species. Larches were defoliated by manually cutting four out of every five short shoots above the meristem throughout the entire tree (c.f. Handa, Körner & Hättenschwiler, 2004). We used all *Larix decidua* plots to establish the larch bud moth experiment.

Larch bud moth performance

Larch bud moth populations in the Swiss Alps had very low densities in 2002/ 2003, and thus, we collected the larvae in the western part of the Alps near Briançon (France, Bois les Ayes and Bois les Combes, c.f. Emelianov *et al*., 1995) on the 10th of June 2003. Larvae were subsequently transferred to the study site at Stillberg. Due to the particularly warm summer in 2003, larval development of the larch bud moth was more advanced than expected, and we mainly found early L5 larvae (83% L 5, 17 % L4). After weighing, one larva that appeared unparasitized by visual inspection was placed on each of three lateral branches per larch tree and was enclosed with a mesh bag on 12 June 2003 (i.e. a total of 60 larvae). We checked mesh bags for pupae by opening every 4 to 6 days. We decided not to check more often because of the disturbance during mesh bag removal for the search of the relatively small (ca. 8 mm length) pupae. Pupae were removed, sexed, dried and weighed. The last pupa was removed 28 days after the experiment started. Initial dry mass, pupal mass and the time interval until pupation was used to calculate the relative growth rate (RGR), defined as biomass gained per gram initial caterpillar biomass and per day (mg g^{-1} day-^{1}). For the determination of initial dry mass 10 unused larvae were taken to establish a fresh to dry mass ratio (average caterpillar water content was 85%).

Leaf nutritional analyses

From each of the 20 trees included in the experiment, needles were sampled from branches adjacent to those used for larval feeding at the beginning (13 June 2003) and at the end (2 July 2003) of the experiment. The bulk of this leaf material was dried immediately after sampling, ground and analyzed for starch, sugar (sucrose, glucose and fructose), nitrogen, carbon and lignin. From a needle subsample projected area and the water content (difference between fresh and dry mass) was determined. Starch and sugar were analyzed with an enzymatic starch digestion and a spectrophotometric glucose test after invertase and isomerase addition (Hoch, Popp & Körner 2002). Total N and carbon were measured using a CHN analyzer (Model 900, LECO Instruments, St. Joseph, Mich., USA). lignin concentration was determined from 50 to 100 mg samples with the thioglycolic acid method (Bruce & West, 1989; Hirschel *et al.*, 1997).

Statistical analyses

Differences in larval RGR were tested using ANOVA with CO_2, defoliation and sex as fixed factors. According to our split plot design the factor “CO_2” varied among groups and “defoliation“ and its interaction with CO_2 among plots. The effect of CO_2 was, thus, tested against the group error term, and the effect of defoliation and its interactions with CO_2 against the plot error term. Sex and its interactions were tested versus the residual. To test for differences in pupal weight we performed an ANOVA adjusted for initial weight as a covariate in the same way as described above. Differences in needle quality were tested using ANOVA with CO_2 and defoliation as fixed factors similar to what is described above. Data were tested for normality and whenever necessary log transformed. All ANOVA were conducted with type I SS (Sequential) using JUMP 3.2.2., SAS Institute.

Results

Needle Quality

We did not find a significant effect of elevated CO_2 or defoliation on SLA, but there was a trend for lower SLA in defoliated trees (Table 1). In June, at the beginning of the experiment, needle water content was lower at elevated CO_2 compared to control trees when they have been defoliated, but there was no CO_2 effect in undefoliated trees. In July, however, both elevated CO_2 and defoliation decreased needle water content (Table 1), resulting in the lowest water content in defoliated high CO_2-exposed larch trees. Sugar concentration did not change in response to elevated CO_2, but it was significantly reduced by previous year defoliation in June and July. In July, but not in June, high CO_2-grown needles had significantly higher starch concentrations compared to needles from ambient CO_2. Defoliation reduced starch concentration in June, but this initially lower starch concentration in defoliated trees vanished by the end of the experiment. Elevated CO_2 did not significantly affect needle N concentration, although there was a trend for reduced N at elevated CO_2 in July (Table 1). However, nitrogen concentration was significantly reduced by defoliation and accordingly the C/N ratio was higher in defoliated trees. There were no consistent effects of either CO_2 or defoliation on lignin concentration in June. In July, however, lignin concentration was significantly higher in trees grown at elevated CO_2, but defoliation had no effect. In general needle chemistry changed strongly over the three-week observation period in all treatments. Sugar, starch and lignin concentration increased substantially during the experiment, while water content, SLA and nitrogen decreased strongly.

Larch bud moth performance

At the end of the experiment 55% of the initial 60 larvae pupated, 25% died and the remaining 20% were not accounted for. It is possible, that dead shrivelled caterpillars have been overseen when mesh bags were removed and checked, or that some of the larvae were able to escape during mesh bag removal. Exactly 50% of the pupated caterpillars were females.

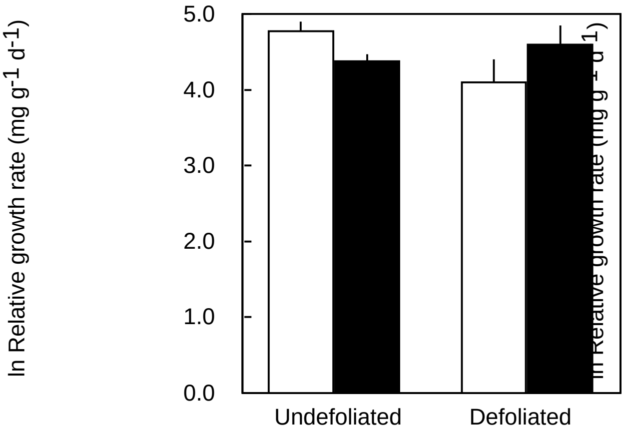

Fig. 1: Relative growth rate (RGR, Mean ± SE) of *Zeiraphera diniana* larvae feeding on *Larix decidua* exposed to elevated CO_2 for three years. Half of all trees have been subjected to heavy defoliation (80% of all needles removed) the year before the feeding experiment started. Open bars represent trees at ambient CO_2 and black bars represent trees at CO_2.

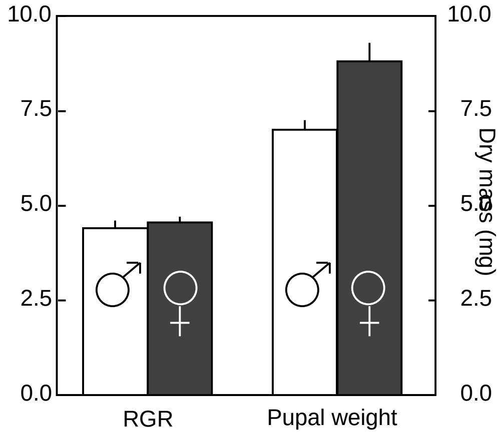

Fig. 2: Caterpillars relative growth rate (Mean ± SE) and pupal dry mass (Mean ± SE) of *Zeiraphera diniana* pooled across all treatment combinations. Open bars represent male caterpillars and grey bars represent female caterpillars.

Table 1: Needle quality of *Larix decidua* grown at two different atmospheric CO_2 concentrations and two levels of defoliation (Mean ± SE) along with results from ANOVA. Leaf water content is given in percent of total leaf fresh mass (*f.m.*). All other values are expressed on a dry mass basis (*d.m.*). Levels of significance are: $^{(*)}$ < 0.1, * < 0.05, ** < 0.01, *** < 0.001.

		Undefoliated		Defoliated				
Parameter	Date	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	*F* (CO_2)	*F* (defoliation)	*F* (CO_2 x defol.)
SLA[1]	June	173.3 ± 6.6	158.0 ± 10.2	150.3 ± 11.2	149.4 ± 9.3	$F_{1,8}$ = 0.6	$F_{1,8}$ = 3.4	$F_{1,8}$ = 3.4
	July	42.0 ± 1.4	38.6 ± 1.7	37.2 ± 1.6	38.2 ± 1.2	$F_{1,8}$ = 0.6	$F_{1,8}$ = 2.9	$F_{1,8}$ = 2.1
Water	June	74.8 ± 0.47	74.7 ± 0.44	76.9 ± 0.82	74.0 ± 0.73	$F_{1,8}$ = 5.9*	$F_{1,8}$ = 1.0	$F_{1,8}$ = 4.1$^{(*)}$
	July	64.8 ± 0.30	63.7 ± 0.61	63.5 ± 0.62	61.3 ± 0.24	$F_{1,8}$ = 13.6*	$F_{1,8}$ = 21.7**	$F_{1,8}$ = 1.7
Sugar	June	4.21 ± 0.32	4.98 ± 0.27	2.79 ± 0.31	3.10 ± 0.33	$F_{1,8}$ = 1.0	$F_{1,8}$ =14.2**	$F_{1,8}$ = 1.1
	July	11.19 ±0.47	10.89 ±0.67	8.87 ± 0.37	8.71 ± 0.90	$F_{1,8}$ = 0.2	$F_{1,8}$ = 8.7*	$F_{1,8}$ = 0.3
Starch	June	8.44 ± 1.39	8.47 ± 0.85	5.15 ± 0.68	6.99 ± 0.97	$F_{1,8}$ = 1.1	$F_{1,8}$ = 6.8*	$F_{1,8}$ = 0.1
	July	9.09 ± 0.70	13.51 ± 0.90	8.27 ± 1.21	11.03 ± 1.04	$F_{1,8}$ = 16.8**	$F_{1,8}$ = 3.5$^{(*)}$	$F_{1,8}$ = 0.2
Nitrogen	June	2.78 ± 0.13	2.8 ± 0.11	2.28 ± 0.07	2.24 ± 0.06	$F_{1,8}$ = 0.0	$F_{1,8}$ = 24.3***	$F_{1,8}$ = 0.1
	July	1.83 ± 0.11	1.75 ± 0.08	1.4 ± 0.09	1.26 ± 0.07	$F_{1,8}$ = 2.2	$F_{1,8}$ = 22.1**	$F_{1,8}$ = 0.1
C/N ratio	June	17.36 ± 0.91	19.52 ± 0.58	21.38 ± 0.66	22.01 ± 0.48	$F_{1,8}$ = 0.2	$F_{1,8}$ = 39.7***	$F_{1,8}$ = 0.2
	July	25.15 ± 1.75	25.15 ± 1.75	33.27 ± 2.0	35.89 ± 1.27	$F_{1,8}$ = 1.3	$F_{1,8}$ = 30.2***	$F_{1,8}$ = 0.3
lignin	June	7.95 ± 0.56	7.16 ± 0.42	7.26 ± 0.54	8.38 ± 0.35	$F_{1,7}$ = 0.1	$F_{1,6}$ = 0.3	$F_{1,6}$ = 4.8$^{(*)}$
	July	9.99 ± 0.41	11.02 ± 0.41	9.59 ± 0.41	12.44 ± 1.27	$F_{1,8}$ = 8.3*	$F_{1,5}$ = 1.9	$F_{1,5}$ = 1.7

[1] The leaf area devided by the dry weight of resepective leaf/ needle. A higher SLA generally indicates that leaves/ needles are softer.

Table 2: Effects of CO_2 treatment and defoliation on RGR and pupal weight of *Zeiraphera diniana*. Initial weight was used as covariate (pupal weight).

	RGR		Pupal weight	
Parameter	*F*-value	*P*-value	*F*-value	*P*-value
Initial weight	Not tested		$F_{1,7}$ = 2.08	0.19
CO_2	$F_{1,7}$ = 0.03	0.88	$F_{1,7}$ = 1.08	0.33
Defoliation (D)	$F_{1,5}$ = 0.31	0.60	$F_{1,5}$ = 0.43	0.54
CO_2 x D	$F_{1,10}$ = 5.78	0.06 $^{(*)}$	$F_{1,9}$ = 0.84	0.40
Sex (S)	$F_{1,10}$ = 0.38	0.55	$F_{1,9}$ = 8.28	0.02*
CO_2 x S	$F_{1,10}$ = 0.29	0.60	$F_{1,9}$ = 0.47	0.51
D x S	$F_{1,10}$ = 2.24	0.17	$F_{1,9}$ = 0.17	0.69
CO_2 x D x S	$F_{1,10}$ = 0.05	0.83	$F_{1,9}$ = 0.63	0.45

There were no significant main effects on larval growth, but we found a marginally significant CO_2 x defoliation interaction (Fig. 1, Table 2) on larval RGR, which is significant if the factor sex is excluded from the model ($F_{1,\ 19}$ = 5.77, P = 0.03). When feeding on ambient CO_2-grown trees larvae grew faster on undefoliated trees compared to defoliated trees. In contrast, larvae grew faster on high CO_2-exposed trees that had been defoliated the previous year (Fig. 1). Pooled across all treatments RGR did not differ between male and female larvae, but the pupal dry mass was significantly higher in females (Fig. 2, Table 2). Pupal weight, however, was not significantly affected by either CO_2 or defoliation (Table 2).

Discussion

Elevated atmospheric CO_2 concentration caused a decrease in water content and an increase in lignin concentration of larch needles in our study. The initially higher starch concentration was of only transitory nature and was not found in older needles of the same year (Handa *et al*., 2005). Previous year defoliation of the larch trees studied here led to a decrease in the concentration of non-structural carbohydrates (starch and sugar) and nitrogen, a response that was maintained throughout the growing season (Handa *et al*., 2005). These effects of elevated CO_2 and defoliation are largely consistent with previous studies (Lindroth & Kinney, 1998; Kaitaniemi *et al*., 1998; Roth *et al*., 1998; Hättenschwiler & Schaffelner, 1999; Blaschke *et al*., 2002; Coviella *et al*., 2002).

However, defoliation does not necessarily induce a decrease of nitrogen concentration in remaining or next seasons leaves as observed for lodgepole pine (*Pinus contorta*) or larch in another study (Vanderklein & Reich, 2000). Volin *et al*., (2002) showed, that current year defoliation had little effects on maple leaf N. An important conclusion from previous studies is that the effects of both elevated CO_2 and defoliation are highly species-specific and depend on general growth conditions, and thus, need to be studied in the respective context. In the case of larch trees in their natural environment at treeline, the CO_2 and defoliation responses in needle quality were quite independent with no interactive effects on none of the parameters tested. There were still some additive negative effects of elevated CO_2 and defoliation. The combined effects of elevated CO_2 and defoliation resulted in the lowest N concentration, the lowest water content, the highest lignin concentration and highest C/N ratio of all treatments. This at least in part supports our initial hypothesis of a reinforced negative impact of the combination of an elevated CO_2 atmosphere and defoliation on needle quality of treeline larches.

The consequences of the reported needle quality changes for larch bud moth performance were less clear. There was a trend for reduced larval RGR in response to elevated CO_2 in undefoliated trees and in response to defoliation at ambient CO_2 concentrations matching the respective changes in needle chemistry and supporting our initial predictions of impaired insect growth due to CO_2 and defoliation induced food quality changes. Counter expectation the combined effects of elevated CO_2 and defoliation resulted in similar RGR as in larvae feeding on undefoliated trees at ambient CO_2. A causal explanation for this CO_2 x defoliation interaction on RGR on the basis of measured needle quality parameters is difficult. We might have missed some other important needle traits that may have contributed to the observed larch bud moth responses. For example, phenolic compounds such as hydrolyzable or condensed tannins might have been affected by either elevated CO_2 (Traw *et al*., 1996; Hättenschwiler & Schaffelner, 2004) or defoliation (Osier & Lindroth, 2001). Furthermore, depending on the relative nutritional composition of the larch needles caterpillars might have shown different physiological responses to regulate their protein and carbohydrate intake.

Such responses might further be influenced by the developmental stage of caterpillars. Some earlier experiments reported distinct CO_2 effects on RGR beetwen early and late instars of butterfly larvae and grasshopper nymphs (Fajer, 1989; Asshoff & Hättenschwiler, 2005). Unfortunately, we were not able to distinguish between early and late instar caterpillars in this study, and it is possible that early instar larvae might have been more responsive to the treatment factors. In line with our results, other studies on the combined effects of elevated CO_2 and defoliation on herbivores did also not find additive negative impacts for insect growth (Roth *et al*., 1998; Lindroth & Kinney, 1998).

We consider the pronounced changes in nutritional quality of larch needles over the course of the experiment and independently of treatments a very important result of this study. When caterpillars started feeding, larch needles were not mature and water content decreased by 16%, SLA by 75% and nitrogen concentration by 38% during the experiment. The C/N ratio increased by 54%, sugar concentration by 160%, starch concentration by 44% and lignin concentration by 55%. Compared to our treatment-induced changes in needle tissue quality these changes over time were much larger. The timing of hatching appears to be essential for an optimal larval nutrition and development. The synchronization of larval emergence and needle flushing is of great importance for the larch bud moth system in the Alps (Baltensweiler, 1984; 1992).

Both CO_2 and defoliation may affect tree phenology. Elevated CO_2 was shown to delay bud burst of sitka spruce (Murray *et al*., 1994) and Norway spruce (Hättenschwiler & Körner, 1996), and *Zeiraphera canadiensis* herbivory modified the inition of budburst of white spruce (Carroll & Quiring, 2003). In our study trees, needles unfolded 5 days (2002) and 7 days (2003) earlier on trees growing in elevated CO_2, but defoliation had no effect (Handa *et al*., 2005). Our reported CO_2-induced differences in needle quality may in part result from this slight shift in phenology. Considering these possible future changes in phenology at treeline due to rising atmospheric CO_2 and rapid changes in needle tissue chemistry during maturation in the early vegetation period, larch bud moth may show reduced growth, a shift in development or a combination of both. However, long-term records of the larch bud moth cycle revealed no clear trends due to rising atmospheric CO_2 or recent climate warming with a quite stable frequency of the periodic larch bud moth outbreaks over centuries (Weber, 1995; 1997). Larch bud moth populations might rather show an altitudinal shift in their distribution

as indicated during the cooler period around the penultimate turn of the century (1780-1830) followed by an altitudinal shift of maximum larch bud moth activity from their common optimal zone above 1800 m a.s.l. to lower altitudes (Weber, 1995; 1997).

In conclusion, our results suggest that elevated CO_2 and defoliation induced changes in larch needle quality have only little impact on larch bud moth performance at the alpine treeline, and, in particular, indicate no reinforced negative effect of these two factors. The pronounced changes of needle quality during needle maturation might suggest that any shifts in tree phenology due to global change may be of greater importance for alpine larch bud moth populations than the direct impact of CO_2 on needle quality.

Acknowledgements

Werner Baltensweiler organized the larch bud moth collecting trip to France and was ready to answer any questions occurring before, during and after the experiment. Tanya Handa joined the trip to Briançon and provided field support. Laurent Dormont took the time cruising with us around Briançon until we collected enough larch bud moth larvae. We thank Luca Egli and Alain Studer for construction and maintenance of the FACE setup and technical support, Oliver Bignucolo for chemical analyses, Pascal Niklaus and Dieter Spinnler for statistical advice. This research was funded through the Swiss National Science Foundation grant 31-061428.00.

4 Growth and reproduction of the alpine grasshopper Miramella alpina feeding in CO_2-enriched dwarf shrubs at treeline

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***Oecologia* 142: 191-201**

Oecologia (2005) 142: 191–201
DOI 10.1007/s00442-004-1714-0

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Growth and reproduction of the alpine grasshopper *Miramella alpina* feeding on CO_2-enriched dwarf shrubs at treeline

Received: 29 July 2004 / Accepted: 25 August 2004 /

Abstract The consequences for plant-insect interactions of atmospheric changes in alpine ecosystems are not well understood. Here, we tested the effects of elevated CO_2 on leaf quality in two dwarf shrub species (*Vaccinium myrtillus* and *V. uliginosum*) and the response of the alpine grasshopper (*Miramella alpina*) feeding on these plants in a field experiment at the alpine treeline (2,180 m a.s.l.) in Davos, Switzerland. Relative growth rates (RGR) of *M. alpina* nymphs were lower when they were feeding on *V. myrtillus* compared to *V. uliginosum*, and were affected by elevated CO_2 depending on plant species and nymph developmental stage. Changes in RGR correlated with CO_2-induced changes in leaf water, nitrogen, and starch concentrations. Elevated CO_2 resulted in reduced female adult weight irrespective of plant species, and prolonged development time on *V. uliginosum* only, but there were no significant differences in nymphal mortality. Newly molted adults of *M. alpina* produced lighter eggs and less secretion (serving as egg protection) under elevated CO_2. When grasshoppers had a choice among four different plant species grown either under ambient or elevated CO_2, *V. myrtillus* and *V. uliginosum* consumption increased under elevated CO_2 in females while it decreased in males compared to ambient CO_2-grown leaves. Our findings suggest that rising atmospheric CO_2 distinctly affects leaf chemistry in two important dwarf shrub species at the alpine treeline, leading to changes in feeding behavior, growth, and reproduction of the most important insect herbivore in this system. Changes in plant-grasshopper interactions might have significant long-term impacts on herbivore pressure, community dynamics and ecosystem stability in the alpine treeline ecotone.

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Keywords Elevated CO_2 · Global change · Herbivory · Leaf chemistry · *Vaccinium* sp.

Introduction

Rising atmospheric CO_2 has been shown to influence insect herbivore performance (Watt et al. 1995; Lindroth 1996; Bezemer and Jones 1998; Whittaker 1999). The CO_2 effects typically result from changes in food quality, such as decreased nitrogen concentration or increased concentrations of carbon-based defense compounds. Insect responses to these changes include compensatory feeding (Fajer 1989; Docherty et al. 1996), slower growth rates (Fajer 1989; Traw et al. 1996; Hättenschwiler and Schafellner 1999), reduced final weight, prolonged development time (Goverde and Erhardt 2003), increased mortality (Fajer 1989), and reduced reproduction (Buse et al. 1998; Brooks and Whittaker 1998). At the level of the individual, these responses may negatively influence fecundity and population dynamics of insect herbivores. Alternatively, compensatory feeding in a CO_2-enriched atmosphere could lead to greater damage of food plants. Both these responses may alter plant-herbivore interactions, and possibly community dynamics and ecosystem functioning.

However, extrapolations from experiments commonly restricted to particular larval stages under predominantly greenhouse conditions to natural ecosystems are difficult (Goverde et al. 2002). Moreover, different plant species respond distinctly to atmospheric CO_2 enrichment (Cotrufo et al. 1998; Körner 2000), as do herbivores feeding on them (Traw et al. 1996; Kinney et al. 1997; Veteli et al. 2002). A possible consequence of species-specific responses to elevated CO_2 could be a switch to other, more suitable food plants by generalist herbivores, which might induce changes in plant species composition (Hättenschwiler and Schafellner 2004).

Climatic conditions are particularly severe and variable at the alpine treeline, and are hard to simulate in the greenhouse. Grasshoppers are the dominant insect herbivores in alpine ecosystems (Illich and Winding 1999; Blumer and Diemer 1996). The alpine grasshopper (*Miramella alpina* Fruhstorfer) is a wide-spread generalist herbivore in the Alps at altitudes between 1,000 and 2,500 m a.s.l., and preferentially feeds on different species of the genus *Vaccinium* (Harz 1957; Illich and Winding 1989). Compared to other Mid-European Acrididae, the genus *Miramella* is known for its particularly long adult phase combined with a high reproductive potential (Asshoff and Köhler 2003; Köhler et al. 1999). However, at high elevations grasshoppers generally experience a shorter growing season and cooler temperatures, meaning that they have to develop, mature, and reproduce in a shorter period of time and under less favorable conditions than grasshoppers at low elevations. An optimal resource availability, and hence high food quality, may thus be of particularly high importance for successful grasshopper development and reproduction in high altitude environments. Changes in plant tissue quality in response to elevated atmospheric CO_2 may therefore have serious consequences for high altitude populations of insect herbivores. To our knowledge the impact of elevated CO_2 on plant-herbivore interactions, however, has never been tested in alpine ecosystems.

The objective of this study was to assess how *M. alpina* might be affected by the projected higher CO_2 concentration in the atmosphere. Specifically, we quantified the direct effects of elevated CO_2 on leaf quality of major host plants of *M. alpina* and determined the impact of leaf quality changes on nymph growth, final adult biomass and reproduction of *M. alpina*. We hypothesized that elevated CO_2 reduces leaf quality and that these changes will negatively affect grasshopper performance leading to a reduced reproductive output under the harsh conditions at the alpine treeline. A second hypothesis was that grasshopper responses to elevated CO_2 depend on plant species-specific changes in leaf chemical composition, which might indicate the potential for compensatory responses of grasshoppers by switching host plant species.

Materials and methods

Study site and experimental setup

The experimental area is located at Stillberg, Davos, in the Swiss Central Alps at an elevation of 2,180 m a.s.l. The long-term annual precipitation of the NE exposed study site is 1,050 mm; the average temperature is −5.8°C in January and 9.4°C in July (Schönenberger and Frey 1988). Compared to the long-term average, precipitation was somewhat higher during 2002 with a particularly wet August (Table 1). Temperatures were similar to the long-term average during the first part of the vegetation period with a warm June, but tended to be somewhat lower during late summer and early fall. The soil is classified as a Ranker (US system: Lithic Haplumbrept) with 10 cm deep organic topsoil underlain by siliceous bedrock (Paragneis, Schönenberger and Frey 1988). In a large afforestation experiment the tree species *Larix decidua* L., *Pinus cembra* L. and *P. uncinata* Ramond were planted across an area of 5 ha in 1975. At the upper limit of this large-scale plantation (2,180 m a.s.l.), we have chosen a total of 40 individual trees (on average 1.5 m tall) from the two species *L. decidua* and *P. uncinata* together with the accompanying vegetation to establish our experimental plots. Due to their small size and scattered distribution, the trees form an open canopy and the site is actually dominated by the dwarf shrubs *Vaccinium myrtillus*, *V. uliginosum*, and *Empetrum hermaphroditum*. *Gentiana punctata*, *Homogyne alpina*, and *Melampyrum pratense* are the most common herbaceous species. Half of all plots, each of an area of 1.1 m^2, have been randomly assigned to an elevated CO_2 concentration (550 $\mu mol\ mol^{-1}$), and the remaining 20 plots served as controls at ambient CO_2. Elevated CO_2 was applied using free air CO_2 enrichment (FACE) with pure CO_2 release technology from the beginning of the vegetation period in 2001 (Hättenschwiler et al. 2002).

A total of 20 plots showing a good dwarf shrub coverage ($n = 10$ per CO_2 treatment) were used to establish the grasshopper experiment. Mesh bags (volume 520 cm^3, mesh size: 280 µm, Sefar Propyltex 05 280/41, Sefar Holding, Rüschlikon, Switzerland) were constructed to place grasshoppers onto their respective food plants. The relatively small mesh size was chosen because initial tests for light transmission showed the best performance compared to a number of other mesh sizes (85–100% of transmitted light at 280 µm mesh width, depending on the angle of light incidence). Third instar nymphs of *M. alpina* were collected close to the study site on 20 June 2002. Before distributing a total of 160 individuals (average fresh weight of 43 mg per individual) to the mesh bags on 22 June 2002, the initial biomass of each individual was determined. To avoid crowding effects, only two individual nymphs were placed in each of four mesh bags per plot. The mesh

Table 1 Mean temperature(T; 50 cm above ground) and precipitation during the *Miramella alpina* experiment (2002) and the long term average (LTA) at the site (1975–1984; Schönenberger and Frey 1988)

	June		July		August		September		October	
	LTA	2002	LTA	2002	LTA	2002	LTA	2002	LTA	2002
T (air mean; °C)	6.9	10.5	9.4	9.4	8.7	8.5	6.8	4.4	3.5	2.4
Precipitation (mm)	110	105	131	138	125	208	127	92	82	113

bags contained shoots of either *V. myrtillus* or *V. uliginosum* plants (two mesh bags of each plant species per plot). Because mesh bags may alter microclimatic conditions we measured air temperature and air humidity within and outside the bags, but found no overall significant differences over the entire vegetation period. The largest differences were observed on clear days. For example, on a clear day in June we measured temperatures and humidities of 24.8°C and 33.2% within bags compared to 23.7°C and 35% outside bags.

Leaf nutritional analysis

From each of the 20 plots (10 elevated CO_2 and 10 ambient CO_2) leaves of both *Vaccinium* species were collected from different shoots at the beginning (27 June 2002) and at the end (26 August 2002) of the experiment. An intermediate sample was taken on 28 July ($n = 5$). The bulk of this leaf material was dried immediately after sampling, ground and analyzed for starch, sugar (sucrose, glucose and fructose), nitrogen, carbon and lignin. From a subsample of six leaves per *Vaccinium* species, projected area and water content (difference between fresh and dry mass) were determined. Starch and sugar were analyzed with an enzymatic starch digestion and a spectrophotometric glucose test after invertase and isomerase addition (Hoch et al. 2002). Total N and carbon were measured using a CHN analyzer (Model 900, LECO Instruments, St. Joseph, Mich.). Lignin concentration was determined on 50–100 mg samples using the thioglycolic acid method (Bruce and West 1989; Hirschel et al. 1997).

Performance of grasshopper nymphs

Grasshopper biomass was measured three times during the experiment, 32 and 52 days after the experiment started, and directly after individuals eclosed to the adult stage. After 32 days most of the grasshoppers were in the fourth instar, and after 52 days most of them had reached the fifth instar. Relative growth rate (RGR) of nymphs was calculated as the biomass gained per gram initial nymphal biomass per day (mg g^{-1} day^{-1}). Mesh bags were checked every fourth day for dead individuals and to assess food supply. Food shortage was not frequently observed and over the entire duration of the experiment a total of 29 mesh bags had to be switched to a new neighboring shoot to assure adequate food supply. The experiment was terminated as soon as the last individual molted into the adult stage. Biomass of freshly molted adults and development time (from instar 3 to the adult stage) was determined. Relative consumption rate (RCR), defined as milligram leaf biomass ingested per milligram mean nymphal biomass and per day (mg mg^{-1} day^{-1}), was estimated using specific leaf area (SLA) and the difference of the total leaf area within each mesh bag measured at the start (total number of leaves per *Vaccinium* shoot multiplied by the average area per leaf) and at the end of the experiment. This relatively crude estimate of consumed leaf biomass was the only possible method in this field experiment. However, the results obtained are quite robust because (1) *Vaccinium* leaves vary little in size within shoots, (2) there is a large number of leaves within a single bag (about 170 leaves) averaging out size differences, (3) there is no current-year regrowth in *Vaccinium*, and (4) *M. alpina* produces very little frass.

Reproduction

As soon as individuals from the nymphal trial reached the adult stage, pairs of them were placed into newly installed mesh bags according to their previous CO_2 treatment and plant species for assessment of reproduction. Because the final molt did not occur synchronously among all individuals and treatments, the setup was time-lagged. The reproductive output in female grasshoppers is determined by environmental conditions, feeding activity, and nutritional quality during the adult phase, but may also be influenced by nymphal performance (Ingrisch and Köhler 1999). If, for example, grasshoppers are switched from high to low protein nutrition, oocytes may be resorbed. The number of ovarioles in the ovarian determines the egg number in one egg pod. Each ovariole contributes one egg to the clutch. After the final molt, the ovaries of grasshoppers are still relatively small, and egg maturation starts normally after a further week (Uvarov 1966; Joern and Gaines 1990; Ingrisch and Köhler 1999).

To distinguish the impact of feeding on high-CO_2-exposed plant material during the adult phase only from that during the entire development, we set up an additional experiment with newly molted adults: 28 female adults were collected in the field and were then placed individually in a total of seven newly installed mesh bags per *Vaccinium* species and CO_2 treatment (a total of 28 mesh bags) in 14 experimental plots (similar to the nymphal trial setup). An individual field-collected male and a vial filled with soil as substrate for egg deposition were added. An additional control with a more diverse diet (*V. myrtillus*, *V. uliginosum*, *V. vitis idaea*, *Adenostyles alliariae*, *Gentiana punctata*) was established in cages adjacent to the study site (five replicates), with individuals collected and treated in the same way as described above. This reproduction trial started on 3 August 2002 and grasshoppers were kept in the mesh bags until they died (end of September). Since this experiment was aimed at understanding how elevated CO_2 and host plant species will affect the quality of egg pods rather than total egg pod production, data evaluation focused on the first egg pod deposited. Most of our grasshoppers died after depositing the first egg pod. Egg pod quality commonly declines with repeated depositions (shown for *Oedipoda germanica* by Wagner 2000; G. Köhler, personal communication), as was observed in our study, with substantially smaller second egg pods. Egg pods consist of a certain amount of eggs and a glue-like secretion serving as protection from desiccation and mechanical damage. Consequently, we measured total pod mass, egg mass, secretion mass, and number of eggs of the first pod.

Food preference

An additional cafeteria experiment was designed to test whether adult males and females of *M. alpina* change their food preference in response to plant growth under elevated CO_2. Adult individuals collected in the field were transferred to cages and were offered four different plant species (*V. myrtillus*, *V. uliginosum*, *V. vitis idaea*, *G. punctata*) to choose from. Similar amounts of leaf material (an area of approximately 10 cm^2) from each plant species taken from either control plots or CO_2-enriched plots, were added to Petri dishes and kept moist. Each individual Petri dish contained plant material from one individual plot, and grasshoppers fed either on plant material from CO_2-enriched or control plots. Leaf consumption was calculated from the difference in leaf area before and after grasshopper feeding and was converted to leaf mass, using the SLA. Individuals were first kept without any food for 24 h until their guts were empty and were then allowed to feed for 24 h. This experiment was repeated three times, twice with females (one individual per Petri dish, $n=5$) and once with males (two individuals per Petri dish, $n=5$).

Statistical analysis

Analyses of variance were applied to test for differences in leaf quality traits, nymphal RGR and RCR using a split-plot design with the factors "CO_2" varied among plots and "plant species" nested within individual plots. Accordingly, the effect of CO_2 was tested against the plot error term, and the effect of host plant species and its interactions with CO_2 against the residual. Due to grasshopper mortality during nymphal development we lost about 45% of the original ten replicates before the end of the experiment. However, remaining replicates were distributed equally among plant species and CO_2 treatments. Analyses of RGR and RCR were based on plot means. Possible differences in nymphal mortality rates were tested using the likelihood ratio test statistic (−2 log likelihood). Simple linear regression was used to test for possible correlations between RGR and leaf quality parameters.

Statistical tests for differences in adult body mass (final weight) and development time included initial biomass as a covariable. According to the split-plot design the covariable and the factor CO_2 were tested against the plot error term, and host plant, sex and interactions were tested against the residual. Because of the reduced replicates for adult grasshoppers, it was not always possible to test for all possible interactions. Reproduction parameters were also tested using a split-plot design as described above. For the food preference test we used a three-way ANOVA with CO_2, host plant and sex as factors. All data were tested for normality and when appropriate were log transformed or arcsine-square root (RGR) transformed. All ANOVA were conducted with type I SS (Sequential) using JUMP 3.2.2., SAS Institute.

Results

Leaf chemistry

Leaf water content was significantly lower in *V. myrtillus* compared to *V. uliginosum* at the beginning of the experiment, not different in July, and significantly higher in *V. myrtillus* than in *V. uliginosum* in August. There was no difference between CO_2 treatments, except in the final measurement in August when leaves in high-CO_2 plots had a higher water content compared to those at ambient CO_2 in both species (Table 2). The SLA was significantly lower in *V. uliginosum* than in *V. myrtillus* throughout the entire vegetation period and tended to be lower at elevated CO_2 in both species and at all dates (Table 2).

Nitrogen concentration remained significantly higher in *V. uliginosum* than in *V. myrtillus* during the whole summer. Elevated CO_2 resulted in a significantly lower N concentration in both species, but this effect disappeared in August (Table 2). Likewise, the C/N ratio was significantly higher in *V. myrtillus* than in *V. uliginosum*, and elevated CO_2 increased the C/N ratio in both species (Table 2).

Starch and sugar concentration was higher in *V. uliginosum* than in *V. myrtillus*, except for starch in June and sugar in August. Starch concentration was significantly higher at elevated CO_2 in both species in July and August. There was no CO_2 response in sugar concentration in either species for the June and July sampling, but a significantly increased sugar concentration at elevated CO_2 in August. At start of the vegetation period, lignin concentration tended to be higher in *V. myrtillus* plants compared *to V. uliginosum.* In August, this difference between plant species disappeared. Both species, however, showed a marginally significant reduction in lignin concentration at elevated compared to ambient CO_2 in August (Table 2).

Grasshopper performance

Grasshopper mortality did not differ significantly among treatments, but tended to be highest on *V. myrtillus* plants growing under elevated CO_2. Total grasshopper mortality was unexpectedly high, given that we started with instar 3 nymphs. After 25 days we lost half of all animals (Fig. 1).

Table 2 Quality of *Vaccinium* leaves fed on by *M. alpina* nymphs in ambient and elevated CO_2 (mean ± SE, $n = 5–10$). Sampling dates: 27 June, 28 July, 24 August. *SLA* Specific leaf area

Host plant	CO_2	June	July	August
Water (% of fresh mass)				
Vaccinium uliginosum	Ambient	70.2 ± 0.7	44.9 ± 1.4	51.0 ± 1.3
	Elevated	69.0 ± 1.6	45.0 ± 2.3	53.1 ± 0.8
Vaccinium myrtillus	Ambient	67.1 ± 0.8	41.9 ± 3.3	52.4 ± 0.9
	Elevated	64.9 ± 1.8	43.3 ± 1.9	54.9 ± 1.0
ANOVA	Host plant	$F_{1,18} = 6.76$*	$F_{1,7} = 0.46$	$F_{1,17} = 4.31$*
	CO_2	$F_{1,18} = 1.84$	$F_{1,9} = 0.06$	$F_{1,19} = 4.43$*
	CO_2 × host plant	$F_{1,18} = 0.15$	$F_{1,7} = 0.53$	$F_{1,17} = 0.17$
SLA (cm^2 g^{-1})				
V. uliginosum	Ambient	206 ± 5	128 ± 5	141 ± 4
	Elevated	199 ± 7	126 ± 3	147 ± 6
V. myrtillus	Ambient	238 ± 6	158 ± 7	180 ± 9
	Elevated	233 ± 12	149 ± 6	178 ± 6
ANOVA	Host plant	$F_{1,18} = 24.7$***	$F_{1,7} = 27.5$***	$F_{1,17} = 29.7$***
	CO_2	$F_{1,18} = 0.46$	$F_{1,9} = 0.46$	$F_{1,19} = 0.08$
	CO_2 × host plant	$F_{1,18} = 0.04$	$F_{1,7} = 0.01$	$F_{1,17} = 0.85$
C/N ratio				
V. uliginosum	Ambient	14.9 ± 0.7	17.1 ± 0.5	19.6 ± 0.5
	Elevated	18.6 ± 0.6	22.2 ± 1.4	23.0 ± 0.6
V. myrtillus	Ambient	18.4 ± 0.4	22.4 ± 0.8	23.4 ± 0.9
	Elevated	22.2 ± 1.3	27.0 ± 2.7	26.7 ± 1.1
ANOVA	Host plant	$F_{1,8} = 28.0$***	$F_{1,6} = 25.0$***	$F_{1,11} = 58.8$***
	CO_2	$F_{1,17} = 12.2$**	$F_{1,10} = 17.8$***	$F_{1,17} = 4.7$*
	CO_2 × host plant	$F_{1,8} = 0.00$	$F_{1,6} = 0.05$	$F_{1,11} = 1.04$
Nitrogen (% of dry mass)				
V. uliginosum	Ambient	3.06 ± 0.06	2.69 ± 0.08	2.23 ± 0.1
	Elevated	2.43 ± 0.08	2.02 ± 0.12	2.19 ± 0.12
V. myrtillus	Ambient	2.43 ± 0.06	2.00 ± 0.07	1.96 ± 0.06
	Elevated	2.00 ± 0.12	1.72 ± 0.15	1.75 ± 0.07
ANOVA	Host plant	$F_{1,8} = 36.3$***	$F_{1,6} = 32.3$***	$F_{1,11} = 9.92$**
	CO_2	$F_{1,17} = 14.8$***	$F_{1,10} = 17.5$**	$F_{1,17} = 1.24$
	CO_2 × host plant	$F_{1,8'} = 2.38$	$F_{1,6} = 3.1$	$F_{1,11} = 1.14$
Starch (% of dry mass)				
V. uliginosum	Ambient	5.5 ± 0.6	6.2 ± 0.7	4.9 ± 0.2
	Elevated	7.5 ± 0.7	8.4 ± 0.6	6.6 ± 0.5
V. myrtillus	Ambient	5.1 ± 0.5	4.2 ± 1.1	2.9 ± 0.7
	Elevated	6.0 ± 0.9	6.7 ± 0.8	4.3 ± 0.3
ANOVA	Host plant	$F_{1,18} = 1.71$	$F_{1,6} = 6.34$*	$F_{1,6} = 8.74$*
	CO_2	$F_{1,18} = 2.41$	$F_{1,10} = 8.27$**	$F_{1,16} = 8.47$**
	CO_2 × host plant	$F_{1,18} = 0.77$	$F_{1,6} = 0.3$	$F_{1,6} = 1.88$
Sugar (% of dry mass)				
V. uliginosum	Ambient	7.0 ± 0.5	7.6 ± 0.3	5.6 ± 0.2
	Elevated	7.5 ± 0.7	8.4 ± 0.6	6.6 ± 0.5
V. myrtillus	Ambient	6.3 ± 0.4	7.3 ± 0.4	5.6 ± 0.7
	Elevated	5.7 ± 0.6	6.4 ± 0.7	7.6 ± 0.6
ANOVA	Host plant	$F_{1,18} = 6.76$*	$F_{1,6} = 7.6$*	$F_{1,6} = 0.86$
	CO_2	$F_{1,18} = 0.00$	$F_{1,10} = 0.01$	$F_{1,16} = 5.33$*
	CO_2 × host plant	$F_{1,18} = 0.46$	$F_{1,6} = 1.81$	$F_{1,6} = 0.65$
Lignin (% of dry mass)				
V. uliginosum	Ambient	7.0 ± 0.6	–	8.1 ± 0.5
	Elevated	7.2 ± 0.2	–	7.3 ± 0.5
V. myrtillus	Ambient	9.5 ± 0.7	–	9.4 ± 0.9
	Elevated	8.2 ± 0.2	–	7.7 ± 0.4
ANOVA	Host plant	$F_{1,4} = 5.25$	–	$F_{1,1} = 0.04$
	CO_2	$F_{1,11} = 1.72$	–	$F_{1,12} = 3.78$
	CO_2 × host plant	$F_{1,4} = 0.81$	–	$F_{1,1} = 4.51$

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

We did not find a significant influence of host plant species or elevated CO_2 on RGR over the entire period of development (instar 3–5) (Fig. 2a, Table 3). However, there were clear host plant and CO_2 effects during specific stages of nymphal development (Fig. 2b, c; Table 3). During the initial stage, *M. alpina* individuals feeding on *V. uliginosum* showed an overall higher RGR than those feeding on *V. myrtillus*, and CO_2 had a significant negative effect on RGR (Table 3). The negative CO_2 effect was particularly strong when nymphs were feeding on *V. uliginosum* (Fig. 2b; Table 3). This plant species-specific difference in RGR disappeared at later stages of nymphal development and RGR was even higher, though not significant, in grasshoppers feeding on both high-CO_2 exposed *Vaccinium* species (Fig. 2c). Across all treatment combinations, simple linear regression analyses revealed positive correlations of RGR with nitrogen concentration ($r^2 = 0.66$, $F_{1,21} = 38.5$, $P < 0.0001$), water content

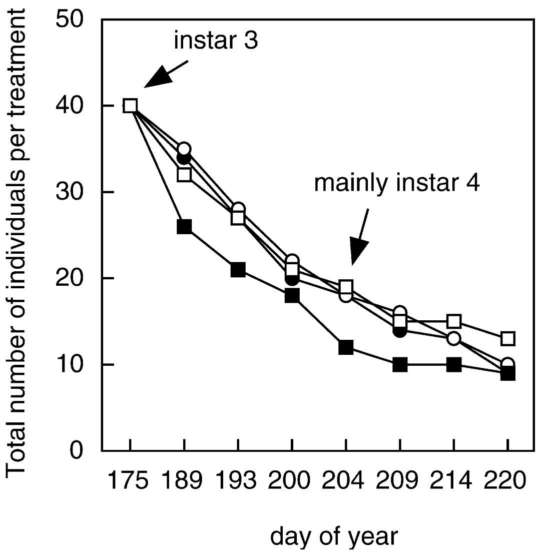

Fig. 1 Mortality during nymphal development. *Open circles: Vaccinium uliginosum* at ambient CO_2, *filled circles: V. uliginosum* at elevated CO_2, *open squares: Vaccinium myrtillus* at ambient CO_2, *filled squares: V. myrtillus* at elevated CO_2

($r^2 = 0.22$, $F_{1,30} = 8.6$, $P < 0.01$) and marginally negative correlations with lignin ($r^2 = 0.20$, $F_{1,12} = 3.0$, $P < 0.10$) from instar 3 to 4. At later developmental stages (instar 4–5) only starch showed a significant (positive) correlation with RGR ($r^2 = 0.35$, $F_{1,11} = 5.8$, $P = 0.03$).

Relative consumption rates tended to be higher in grasshoppers feeding on *V. uliginosum* (0.28 ± 0.04 mg mg^{-1} day^{-1}) than those feeding on *V. myrtillus* (0.17 ± 0.02 mg mg^{-1} day^{-1}, Table 3) Although there was no overall significant CO_2 effect on RCR (Table 3), grasshoppers feeding on *V. uliginosum* showed an average increase of 44% in RCR under elevated CO_2 (0.36 ± 0.04 mg mg^{-1} day^{-1} compared to 0.25 ± 0.04 mg mg^{-1} day^{-1} at ambient CO_2), whereas this increase was not observed in grasshoppers feeding on *V. myrtillus* under elevated CO_2 (0.16 ± 0.05 mg mg^{-1} day^{-1} compared to 0.18 ± 0.02 mg mg^{-1} day^{-1} at ambient CO_2). Pooled across all treatments, grasshoppers consumed an average of 21.8 mg plant biomass (dry mass) per individual and per day, which is in good accordance with other studies using more accurate estimates of RCR (Köhler and Schäller 1981).

Final adult biomass and development time

A total of 22 from an initial 160 individuals molted in the adult stage. Males were significantly lighter than females (males: 122 ± 5 mg, females: 144 ± 12 mg, Table 3). Final biomass was not significantly different among individuals feeding on different host species, but was marginally significantly reduced by elevated CO_2 (Table 3). However, this trend was sex-specific. Newly eclosed females showed a lower final mass at elevated CO_2 than at ambient CO_2 (−18%). In contrast, the newly eclosed males attained a higher biomass when feeding on elevated CO_2-grown plants (+14%), irrespective of the host plant species. These results of the sex-specific CO_2 response need to be interpreted with caution, because proper statistical analyses were not possible due to the loss of too many replicates. Compared to newly eclosed adults collected in the field, both males and females attained a somewhat lower final biomass under the experimental conditions (females −28%, males −19%).

In our trial, *M. alpina* needed on average 65 ± 4 days from instar 3 to the final molt. Development time was significantly prolonged by elevated CO_2 (Fig. 3, Table 3), and was shortest in grasshoppers feeding on *V. uliginosum* in control plots. There was no difference in development time between males and females of *M. alpina* (Fig. 3, Table 3).

Reproduction

Reproduction was poor in females developing in our experimental plots from stage 3 instar. Only females raised on ambient CO_2-grown *V. uliginosum* produced egg

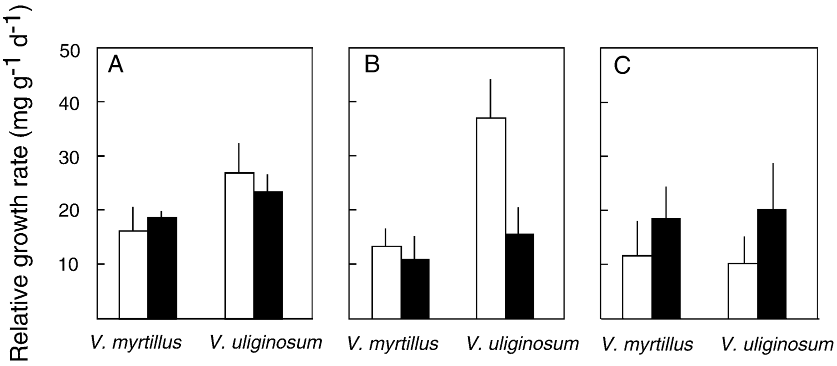

Fig. 2 Relative growth rates (RGR; mean ± SE) of grasshopper nymphs **a** over the entire period of development from 22 June 2002 to 12 August 2002 (instar 3 to instar 5), **b** during the initial phase (instar 3 to instar 4) from 22 June 2002 to 23 July 2002, and **c** during the second phase (instar 4 to instar 5) of development from

Table 3 Statistical analyses of grasshopper performance and reproduction using a split-plot model. *RGR* Relative growth rate, *RCR* relative consumption rate

Parameter	Factor	*F*	*P*
Grasshopper nypmhs			
RGR (instar 3–5)	Host plant	$F_{1,6}=3.3$	0.12
	CO_2	$F_{1,12}=0.0$	0.96
	CO_2 × host plant	$F_{1,6}=2.5$	0.17
RGR (instar 4)	Host plant	$\mathrm{F}_{1,12}=5.9$	0.03*
	CO_2	$\mathrm{F}_{1,16}=4.6$	<0.05*
	CO_2 × host plant	$\mathrm{F}_{1,12}=4.5$	<0.05*
RGR (instar 5)	Host plant	$F_{1,6}=1.1$	0.33
	CO_2	$F_{1,12}=2.3$	0.16
	CO_2 × host plant	$F_{1,6}=0.4$	0.56
RCR	Host plant	$F_{1,4}=4.7$	0.10
	CO_2	$F_{1,9}=0.2$	0.34
	CO_2 × host plant	$F_{1,4}=5.7$	0.08
Grasshopper imagos			
Final weight	Initial weight	$F_{1,9}=0.1$	0.77
	Host plant	$F_{1,4}=1.7$	0.27
	CO_2	$F_{1,9}=3.8$	0.08
	Sex	$F_{1,4}=7.9$	<0.05*
	Sex × host plant	$F_{1,4}=1.5$	0.29
	CO_2 × host plant	$F_{1,4}=0.0$	0.84
Development time	Initial weight	$F_{1,9}=4.6$	0.06
	Host plant	$F_{1,4}=2.7$	0.18
	CO_2	$F_{1,9}=14.4$	0.004**
	Sex	$F_{1,4}=0.0$	0.96
	Sex × host plant	$F_{1,4}=0.6$	0.49
	CO_2 × host plant	$F_{1,4}=3.5$	0.14
Adult reproduction			
Egg pod mass	Host plant	$F_{1,1}=1.6$	0.42
	CO_2	$F_{1,8}=7.7$	0.02*
Mass of secretion	Host plant	$F_{1,1}=213.2$	0.04*
	CO_2	$F_{1,8}=10.6$	0.01*
Average egg mass	Host plant	$F_{1,1}=2.0$	0.39
	CO_2	$F_{1,8}=2.7$	0.14
Number of eggs per pod	Host plant	$F_{1,1}=0.1$	0.80
	CO_2	$F_{1,8}=1.0$	0.36

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

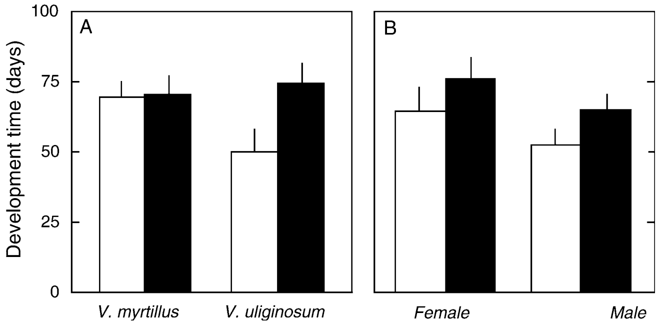

Fig. 3 Development time (mean ± SE) of *Miramella* individuals on two different food plant species (**a**) and separated for males and females (**b**). *Open bars* Ambient CO_2, *filled bars* elevated CO_2 plots

pods, with an average of 6.5 eggs, whereas no egg pods were deposited in any other treatment combinations.

Females of *M. alpina* collected in the field as freshly emerged adults and feeding in experimental plots only as adults produced an average of 0.6 egg pods per individual across all treatments. Of the 28 females, 57% did not produce any egg pods, 32% produced one egg pod, and 11% two egg pods. There were no significant effects of either CO_2 or host plant species on total egg pod production.

Mean egg pod mass, produced by individuals feeding on CO_2-exposed plants was significantly lower compared to that produced by individuals feeding on control plants (Fig. 4a, Table 3). This difference was due mainly to significantly lower mass of secretion, (Fig. 4b) and, to a lesser extent, to a reduced egg mass (Fig. 4c), with no significant CO_2 effect on egg numbers (Fig. 4d). Females having the choice among different plant species (mixed diet) showed essentially the same reproductive parameters as females feeding on ambient CO_2-grown *Vaccinium* (data not shown).

Food choice experiment

Given a choice of five different plant species, *G. punctata* was preferably consumed by males of *M. alpina*, followed by *V. uliginosum* and *V. myrtillus* ($F_{2,78}=2.7$, $P=0.07$, Fig. 5). In contrast, females feeding almost three times as much as males ($F_{1,78}=22.6$, $P<0.0001$) preferred *V. uliginosum* followed by *G. punctata* and *V. myrtillus*. Interestingly, we found a marginally significant CO_2 × plant × sex interaction ($\mathrm{F}_{2,78}=2.4$, $P=0.09$). Males consumed similar amounts of the two *Vaccinium* species and 46% more of *Gentiana* compared to *Vaccinium* when the leaves were produced at ambient CO_2. However, there was a shift towards more *G. punctata* and less *V. myrtillus* consumption by males when the leaves were grown at elevated CO_2. In contrast, females consumed similar amounts of *Gentiana* and *V. uliginosum* from ambient CO_2 plots. *Gentiana* consumption decreased by 42% in elevated CO_2-grown leaves. Less *Gentiana* consumption by females was accompanied by more *Vaccinium* consumption. *V. vitis idaea* was not consumed at all.

Discussion

Grasshopper development

In line with our initial hypothesis, we observed significant changes in leaf chemistry in both *Vaccinium* species in response to a CO_2-enriched atmosphere at treeline. Although the two dwarf shrub species differed significantly in most leaf quality traits, the CO_2 effect was essentially the same in both species. In general, nitrogen concentration decreased and starch and sugar concentrations increased at elevated CO_2. Insects faced with a CO_2-enriched atmosphere, which is commonly associated with lower food nitrogen concentrations, often exhibit slower growth rates (Traw et al. 1996; Williams et al. 1997; Hättenschwiler and Schafellner 1999). Nitrogen is known to be an important factor for herbivore development (Strong et al. 1984), including grasshoppers (McGinnis and Kasting 1965; Bernays and Chapman 1978). In a previous CO_2 experiment with grasshoppers, it was reported that lower nitrogen concentration at elevated CO_2 had a negative effect on relative growth of fifth instar nymphs of *Melanoplus* sp. (Johnson and Lincoln 1990). In our experiment, RGR of early instar nymphs of *M. alpina* was positively correlated with nitrogen and water, whereas higher lignin concentrations resulted in a lower RGR. These correlations diminished in later instars suggesting that CO_2-induced leaf quality changes were not as important for late instars as for early instars. Thus, it is relevant to consider the age (instar) of grasshopper nymphs in any evaluation of insect responses to elevated CO_2. Nutritive demands maybe differ depending on nymphal age, or grasshoppers may incorporate ingested food of a lower quality into body mass in older stages more efficiently. The importance of post-ingestive mechanisms was recently shown in a study with *Melanoplus* grasshoppers feeding on C_3 and C_4 grasses growing at ambient or elevated CO_2 (Barbehenn et al. 2004). Although the nutritional quality of C_3 grasses declined to a greater extent than that of C_4 grasses in elevated CO_2, no differences in grasshopper growth rates or consumption rates were found.

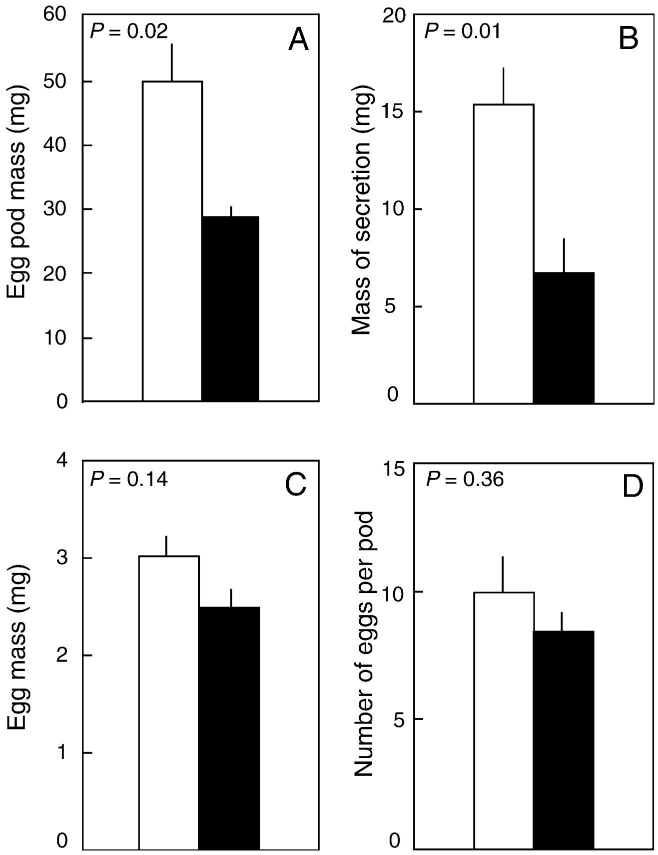

Fig. 4 Reproduction parameters of *M. alpina* females (mean ± SE). **a** Pod mass [mg dry mass dm)], **b** secretion weight (mg dm), **c** egg weight (mg dm), **d** number of eggs per pod. *Open bars* Ambient CO_2, *filled bars* elevated CO_2 plots

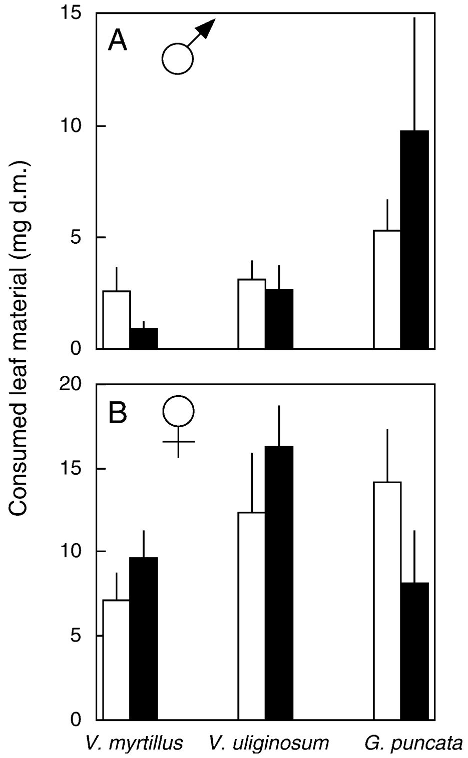

Fig. 5 Total leaf material consumed (mean ± SE) by female (**a**) and male (**b**) *M. alpina* after 24 h of feeding. *Open bars* Leaf material from ambient CO_2 plots, *filled bars* leaf material from elevated CO_2 plots

In our case, it is plausible to argue that lower nitrogen concentration in elevated CO_2-grown plants impaired the growth of early instar grasshoppers. Given that grasshoppers meet their requirements for water mainly through plant material uptake, the lower leaf water content at elevated CO_2 in June could also have been limiting for early instar growth, especially because June 2002 was rather dry. In line with this reasoning, Schäller and Köhler (1981) reported a clear preference for grasses with higher water content (76–78%) in two Mid-European acridoid species. Apparently, nitrogen concentration did not seem as important for grasshopper growth in later instars. Moreover, the negative CO_2 effect on leaf nitrogen concentration became less pronounced in late summer, and the initially lower water content in high CO_2-grown leaves actually turned into a higher water content later in the season, which could help explain the higher RGR at elevated CO_2 in older nymphs. Additionally, we found a strong positive correlation of RGR with starch from instar 4 to 5. Starch plays an important role in grasshopper nutrition because carbohydrates are known as phagostimulants and they are easy to digest (Bernays and Simpson 1990). Indeed, grasshoppers exhibited a higher RCR on high CO_2-grown, starch-rich *V. uliginosum* compared to control plants, and thus might have been able to compensate for lower nitrogen concentrations in later developmental stages.

We did not find any references to verify whether the relatively high grasshopper mortality observed in our experiment is common for *M. alpina* or not. Generally, there are high generation dependent variations in acridoid grasshopper mortality, ranging from 2 to 98% during nymphal development (Ingrisch and Köhler 1999), suggesting that the observed mortality in our experiment might have been a normal phenomenon for *M. alpina* populations. Although we tried to avoid including unhealthy animals in our experiment, we frequently observed grasshoppers infested by pathogenic fungi and parasitic mites in the close neighborhood of our study site (Asshoff and Köhler 2003). Perhaps the unusually wet August in 2002 (Table 1) also negatively influenced survival.

Surviving female grasshoppers showed a higher final biomass than males, irrespective of CO_2 treatment. This size dimorphism is typical for many acridoid grasshopper species. Female grasshoppers, however, achieved a lower final weight in elevated CO_2 plots than in ambient CO_2 plots, whereas the reverse was observed for the males. This result is in agreement with a previous report on the sex-specific CO_2 effect on final pupal weight of the lepidopteran *Lymantria dispar* (Traw et al. 1996). We assume that this result is attributable to a trade-off in female grasshoppers to allow egg production as early as possible (especially in a high elevation ecotone with a short vegetation period), and therefore they eclose earlier in the adult stage at the expense of lower final biomass (Joern and Behmer 1997). The prolonged development time of females in elevated CO_2 plots apparently was not enough to compensate for slower RGR to attain final weights similar to those of females in ambient CO_2 plots.

Reproduction

The much lower reproductive success in females developing under experimental conditions from their instar 3 stage compared to females exposed to experimental conditions only in their adult stage, is evidence for a significant influence of nymphal development on total grasshopper egg production. However, exposure to high CO_2-grown plants during the adult stage only, still had very important consequences for grasshopper reproduction. Two weeks after the start of the experiment with freshly molted adults, all 28 female grasshoppers were alive, and mating was observed frequently. After 3 weeks there were still 18 females equally distributed across the treatments. This time span should normally be sufficient to produce at least one egg pod. Under optimal greenhouse conditions, females of the genus *Miramella* lay on average three egg pods (Asshoff and Köhler 2003). In our field experiment, *Miramella* laid only one egg pod and exceptionally a second, much smaller, egg pod. Our findings of similar egg numbers produced by female grasshoppers in both CO_2 treatments are in agreement with an earlier study of butterfly reproduction under elevated CO_2 (Goverde and Erhardt 2002). The latter study found no significant CO_2 effect on the total number of eggs produced by *Coenonympha pamphilus* (Lepidoptera). In contrast, *Operophtera brumata* (Lepidoptera) produced more eggs in elevated CO_2 than in ambient CO_2 (Buse et al. 1998). Despite similar egg numbers, eggs in our experiment were lighter at elevated CO_2 compared to ambient CO_2 suggesting that they had lower yolk content. Brooks and Whittaker (1998) also found that eggs of *Gastrophysa viridula* (Coleoptera) were 15% lighter under elevated CO_2 conditions, in contrast to Buse et al. (1998) who found no significant CO_2 effect on egg mass. It is noteworthy that the strongest effect we observed was a decrease in secretion mass production under elevated CO_2. Consequently, eggs are less protected against unsuitable environmental conditions and mechanical damage, which might reduce hatching success in the following year. In addition, the prolonged nymphal development time at elevated CO_2 retards the first egg deposition until later in the season, which could also lead to a smaller total egg production or lower hatching success in the next season. It is generally assumed that only eggs deposited early in the summer have the chance to fully develop by the next year. In some recent studies investigating the biology of the genus *Miramella* it was found that only up to 50% of all eggs actually developed by the next growing season (Köhler et al. 1999; Asshoff and Köhler 2003). Both less egg deposition and less protection of the remaining eggs might negatively influence the life cycle and population dynamics of *M. alpina*.

Food choice

M. alpina females and males showed a different food plant preference, which was additionally influenced by CO_2 concentration. Diet selection for specific nutrients can vary between female and male grasshoppers. For example, females of two acridoid species displayed a higher preference for diets with a high proline content compared to males (Behmer and Joern 1994). Furthermore, it is known that elevated CO_2 can alter herbivore food plant preferences (Peters et al. 2000; Goverde and Erhardt 2002). In our experiment, a preference for *G. punctata* increased in males of *M. alpina*, whereas it decreased in females at elevated CO_2, leading to a substantially higher proportion of *G. punctata* consumption at elevated CO_2 in males than females. A shift in food plant species preference may have the greatest impact in response to high CO_2-induced leaf quality changes in generalist insect herbivores (Hättenschwiler and Schafellner 2004). In our field experiment where we focused on the two supposedly most suitable host species of *M. alpina*, the grasshoppers could not choose freely among different alternative food plant species, which might have influenced their response to elevated CO_2. For example, caterpillar growth of the butterfly *Coenonympha pamphilus* was not significantly affected by elevated CO_2 in a calcareous grassland (Goverde et al. 2003). This lack of effect was interpreted as being due to the possibility of insects being able to maintain an optimal nutritional status in the field by altering the proportions of different plant species consumed. A second reason discussed was the harsher climatic conditions in the field compared to the greenhouse, which is most often used for the assessment of herbivore responses to elevated CO_2, which could limit growth to such an extent that even reduced leaf nitrogen concentration will not limit growth any further. The grasshoppers in our experiment showed a somewhat lower adult biomass and a longer development time than those inferred from grasshoppers observed in the field. This might indicate that a single food plant is not optimal for *M. alpina*, but it is not at all clear to what extent polyphagous grasshoppers really depend on a mixed diet (Joern 1979). In greenhouse experiments it was observed that a single food plant species is as suitable as a mixture of different species for *M. alpina* (Hägele and Rowell-Rahier 1999).

In summary, we have demonstrated that elevated CO_2 reduces leaf quality in two dominant dwarf shrub species of the alpine treeline ecotone, and that this has consequences for grasshopper performance. Growth of early instar nymphs was particularly strongly affected by elevated CO_2, but adult reproduction was also impaired. The observed plant species-specific CO_2 effects on grasshopper performance suggest changes in feeding behavior and host plant-grasshopper interactions with the continuing rise in atmospheric CO_2 concentrations, which in turn has important implications for the structure and function of the treeline ecotone.

Acknowledgements We thank Tanya Handa for discussion and field support, Thomas Zumbrunn for field assistance, Luca Egli and Alain Studer for constructing and maintenance of the FACE setup and technical support, Oliver Bignucolo for chemical analyses, Günter Köhler and Klaus Reinhardt for grasshopper discussions, Dieter Spinnler for statistical advice and Christian Körner for helpful comments on the manuscript. The comments of two anonymous reviewers greatly improved an earlier draft of this manuscript. This research was funded through the Swiss National Science Foundation grant 31-061428.00.

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5 Tracing arthropod movement in a deciduous forest canopy using stable isotopes

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In: SCHMIDL, J. and A. FLOREN (Ed.): ***Canopy Arthropod Research in Central Europe*. *Basic and applied studies from the high frontier,*** **in press.**

Abstract

Using the Swiss canopy crane we investigated arthropod movement patterns in the canopy of a species-rich temperate forest. Since late September 2000, 14 trees of six different tree species are exposed to elevated CO_2 using the web-FACE method (a special method of Free Air CO_2-Enrichment) and simulating future atmospheric CO_2 concentrations during the growing season. The CO_2-enrichment gas has a specific $\delta^{13}C$ signature, which can be traced in leaf tissue and in higher trophic levels (e.g. herbivores). Using this $\delta^{13}C$ signal we investigated the mobility of arthropods between the CO_2-enriched and the control area. In the studied phytophagous groups we found significant correlations between $\delta^{13}C$ of leaf tissue with $\delta^{13}C$ of aphids, lepidopteran caterpillars, bush-cricket nymphs, true bugs (nymphs) and leafhoppers (nymphs and adults) suggesting that these groups display little spatial shifts between the CO_2-enriched area and the control area. In contrast, these correlations vanished in fully winged adults of bush-crickets and true bugs, suggesting a higher degree of mobility in the canopy. Omniphagous earwigs revealed a marginally significant correlation with $\delta^{13}C$ of body tissue and $\delta^{13}C$ of leaf tissue, whereas no correlations were found for predators (zoophagous), likely due to a wide prey spectrum, which dilutes the $\delta^{13}C$ signature.

Zusammenfassung

In der vorliegenden Arbeit untersuchten wir die Mobilität verschiedener Arthropoden in Kronendach eines typischen europäischen Mischwaldes. Wir nutzten hierzu den Swiss Canopy Crane (SCC) bei Hofstetten (Solothurn, Schweiz), der einen leichten Zugang in die Kronenregion ermöglicht. Seit Ende September werden die Kronen von 14 ausgewachsenen Bäumen (6 Baumarten) einer erhöhten atmosphärischen CO_2-Konzentration ausgesetzt. Das benutzte CO_2 Gas besitzt eine spezifische $\delta^{13}C$ Signatur, die in Blätter und höhere trophische Ebenen (z.B. Herbivore) eingebaut als Tracer verwendet werden kann. Wir bestimmten den $\delta^{13}C$ Wert verschiedener Arthropodengruppen als Maß für deren Mobilität im Kronendach. Von den untersuchten Arten hatten Laubheuschrecken (Larven), Wanzen (Larven), Schmetterlingsraupen, Blattläuse und Zikaden (Larven und Adulte) einen geringeren Aktionsradius, als die adulten, geflügelten Laubheuschrecken und Wanzen. Bei ominphagen Gruppen (Spinnen, Ohrwürmer und Ameisen) fand sich kein oder nur ein geringer Zusammenh ang zwischen dem $\delta^{13}C$ Wert der Blätter und der darauf gefundenen Individuen.. Dies kann in erster Linie durch das breit gefächerte

Nahrungsspektrum dieser Gruppen erklärt werden, durch welches das δ^{13}C Signal stark beeinflusst wurde.

Introduction

Stable carbon isotopes have become an indispensible tool for tracing the fate of carbon in ecosystems. Among the earliest attempts to assess the feeding behaviour and mobility of animals was the one by Tieszen et al. (1979) in Africa, who documented for grazers the intake of forage from habitats dominated by C3 or C4 grasses. Links between breeding and wintering grounds of birds have been established by analysing δ^{13}C of different populations (c.f. Rubenstein and Hobson 2004). Food-web studies, particulary the assignment of different animal groups to trophic levels have also sucsessfully adopted isotope signals (Ponsard and Arditi 2000, Eggers and Jones 2000).

Until now, this technique has rarely been applied to arthropods in forest canopies (e.g. Büthgen et al. 2003), although they play a key role in ecosystem functioning (Ozanne et al. 2003). Previous studies concerning arboreal arthropods, dealt with changes in seasonal abundances of arthropod communities in canopies (e.g. Wagner 2001) or with the spatial distributions of certain insect taxa (e.g. Simon and Linsenmair 2001).

Arthropod diversity was assessed in temperate European forest canopies, by Schubert (1998), Floren and Schmidl (1999), Kraus and Floren (2002), Arndt (2004), and the spatial distribution of canopy herbivores was studied (e.g. leaf galls) by Kamplicher and Teschner (2002). The influence of elevated atmospheric CO_2 on insect guilds was explored by Altermatt (2003). However, the mobility of different insect taxa in the canopy has never been investigated. Here we capitalize on a large forest isotope tracer experiment which permitted us to study insect movement patterns from and to a canopy area which receives CO_2-enrichement and thus produces δ^{13}C depleted food.

The experiment was conducted at the Swiss Canopy Crane (SCC) site, where long-term responses of temperate decidous forest trees to atmospheric CO_2-enrichment are explored (e.g. Cech et al. 2003, Leuzinger et al. 2005, Zotz et al. 2005). For arthropods of little mobility there should be a close correlation between the isotope signatures of arthropods and leaves they feed on. For highly mobile insects, we expect that δ^{13}C of arthropods does not correlate with host plant tissue values. High mobility comprises the possibility to switch between different trees and different nutrient quality, which can affect life history traits. Insects, which are only little mobile either during their nymphal stages or as adults, are forced to feed on one specific tree (or a limited area in the canopy), whereas mobile insects can change trees and search for alternative food resources.

In this study we quantified the net outcome of mobility for different insect groups in a temperate forest canopy. We differentiated insects by developmental stages and explored whether wingless insects (or nymphs) are able to move between trees. We hypothesize, that (1) due to different feeding types (phytophagous, zoophagous or omniphagous) there are differences in correlations between $\delta^{13}C$ of insect and host plant leaf tissue and that (2) insects which are capable to fly feed on a more diverse set of trees and thus exert higher movement activity in the canopy reflected in no or poor correlations between $\delta^{13}C$ of leaves and insects.

Material and Methods

Study site (Swiss Canopy Crane SCC)

The experimental facilities are located in a forest close to Hofstetten, 15 km south of Basel, Switzerland (47° 28 ′ N. 7 ° 30 ′ E, 550 m a.s.l.). Tree species composition is dominated by approximately 80 - 100 years old beeches (*Fagus sylvatica* [L.] and oaks (*Quercus petraea* [L.]) with heights between 30 to 36 m. Companion species are *Carpinus betulus* (L.), *Tilia platyphyllos* (L.), *Acer campestre* (L.), *Prunus avium* (L.) and some conifers (*Larix decidua* [Mill.], *Picea abies* [L.], *Pinus sylvestris* [L.] and *Abies alba* [Mill.]). A total of ca. 110 trees can be reached from the crane which covers a ground area of 2800 m^2 (Fig. 1, Pepin and Körner 2002, Körner and Zotz 2003). Since September 2000, 14 trees of six different tree species were exposed to elevated CO_2 using the web-FACE method (a special method of Free Air CO_2-Enrichment) for simulating future atmospheric CO_2 concentrations (Pepin and Körner 2002). This method uses 4 mm wide plastic tubes woven into the tree crowns (300-1000 m tubing per tree), which release pure CO_2 through laser-punched holes. The CO_2 release is controlled by a computer, connected with a 25-channel infrared gas analyzer (IRGA), sampling air from triplet end tubes (total of 75 sampling points). Within the upper crown of high CO_2 trees the daily mean concentration of CO_2 in the atmosphere is between 526 and 566 $\mu mol\ mol^{-1}$, compared to control trees at current ambient CO_2 (concentration of ca. 375 $\mu mol\ mol^{-1}$). Three individuals of *Fagus* and four individuals of *Quercus*, and *Carpinus*, and one individual each of *Tilia*, *Acer*, and *Prunus* are growing in elevated CO_2. Using a gondola a 45 m high construction crane provides access to every position in the forest canopy. Due to fossil fuel origin, the CO_2 used to enrich the canopy carries a strong stable carbon isotope label (δ^{13}C of ca. -31‰ compared to CO_2 in ambient air of -8‰) and thus serves as a tracer. Comparing δ^{13}C values between samples collected in the CO_2-enriched and the control area allow identification of the origin of carbon. In addition, the differences in δ^{13}C between the two treatments are related to the amount of new C in a specific C-pool.

Insect sampling

On two sampling dates in 2003 (30 May and 19 August), the third year of CO_2-enrichment, a total of 25 trees of 4 different species were sampled [7 *Carpinus* (3 in elevated CO_2, 3 control trees), 9*Fagus* (3 in elevated CO_2, 6 control trees), 6 *Querucs* (3 in elevated CO_2, 3 control trees) and 3 *Tilia* (1 in elevated CO_2, 2 control trees)] (Fig. 1). Samples were collected on warm, sunny days without precipitation. Three randomly chosen branches per tree in the upper canopy were shaken, while holding a round fabric-frame (60 cm) underneath the branch. Arthropods were transferred into plastic tubes and their guts were emptied within 12 hours prior to freezing at -30°C. Aphids were sampled only in May. They were collected with tweezers and directly transferred into tin cups prior to oven drying. All insects were classified to their family or if possible to their genus or species and separated based on their developmental stage. After oven drying at 70°C insects were ground with a steel ball mill (Mixer Mill, Retsch MM 2000, Germany). Very small arthropods were measured as a whole to minimize the loss of biomass during grinding. Insect extremities and wings were not included. For δ^{13}C analysis 0.6 to 0.8 mg of dried powder was rapped in tin cups. The samples were then combusted in an elemental analyzer (EA-1110, Carlo Erba Thermoquest, Italy) and transferred via an open split interface (Conflo II, Finnigan Mat, Germany) to a mass spectrometer (Delta S, Thermo Finnigan Mat, Germany).

In total, 262 arthropods were collected and the δ^{13}C was determined for each individual (Table 1). For the mobility study we only selected the main groups and investigated 32 spiders (adults), 9 earwigs (nymphs), 29 ants (workers only), 13 lepidopteran caterpillars, 28 weevils (17 *Rhynchaenus fagi*, adults), 29 bush-crickets (*Meconema* sp., nymphs and adults), 64 true bugs (40 Miridae (data not shown), 24 Pentatomidae, nymphs and adults) and 34 cicadae (mainly *Iassus lanio*, nymphs and adults, Table 1).

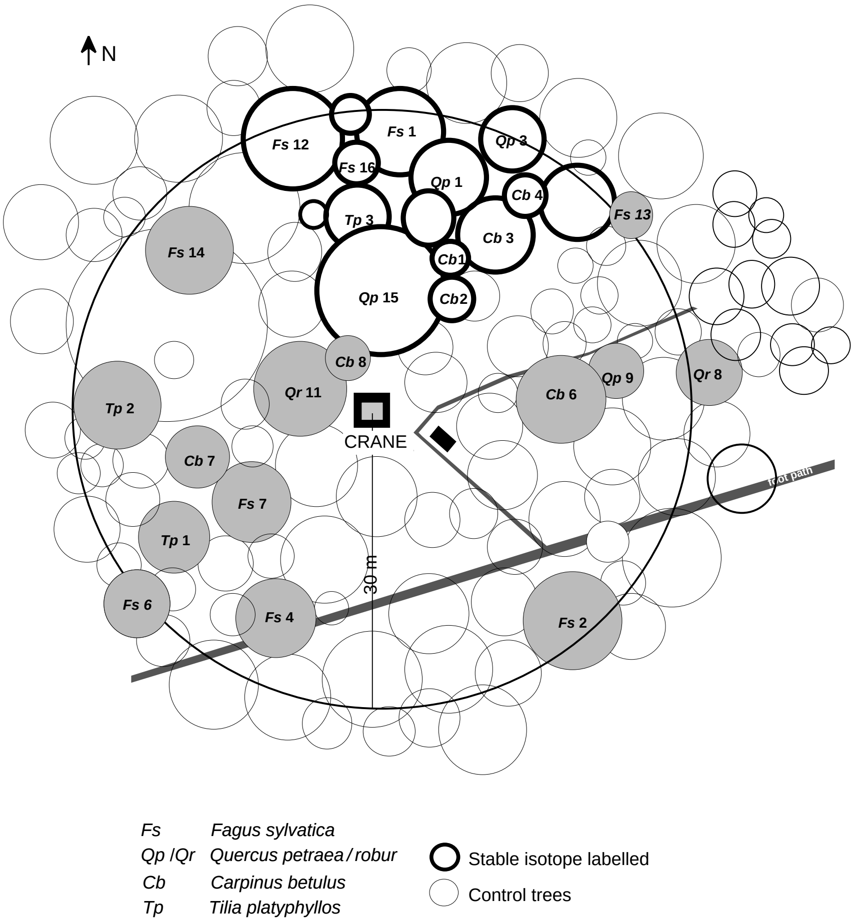

Fig.1: Map of the SCC site near Basel, Switzerland. Numbered trees were sampled.

Leaf sampling

In August 2003 five leaf discs per tree were collected randomly in the upper canopy and pooled prior to grinding. Samples were analyzed for δ^{13}C as described above.

In addition, leaves upon which aphids were found, were also collected.

Statistical analyses

Linear Regressions were used to correlate δ^{13}C values of insects with the δ^{13}C signal of leaves of the respective host tree. Leaf samples were available from August only. Consequently we plotted our δ^{13}C values of insects from either May or August against δ^{13}C of leaves collected in August. Since this was the third year of CO_2-enrichment, we expect a similar mix of old and new carbon in all species growing in elevated CO_2, however there was an unexpected high amount of new carbon in the *Tilia* tree (Keel et al., unpublished data). δ^{13}C of leaves is known to be higher in spring (ca. 1‰, Keel et al., unpublished data, Damesin and Lelarge 2003), most likely due to the accumulation of δ^{13}C-enriched starch.

During the growing season, $\delta^{13}C$ decreases due to the incorporation of photoassimilates with more negative $\delta^{13}C$ compared to starch.

Assuming the same trend for all tree species, the correlations between $\delta^{13}C$ of leaves and insects should be affected by this trend in a systematic way. Only the slopes of the regressions would have changed. A significant correlation between $\delta^{13}C$ of arthropod and $\delta^{13}C$ of host plant suggests that arthropods mobility is poor. Differences in $\delta^{13}C$ were analysed using ANOVA with the tracer treatment as fixed factor.

Results

$\delta^{13}C$ in leaf tissue and arthropods

Leaves of trees growing in elevated CO_2 revealed a significantly lower $\delta^{13}C$ compared to control trees based on the results of a two-way ANOVA with the CO_2 treatment and species as fixed factors. The difference between leaves of ambient and elevated CO_2 was highly significant [-27.3±0.2‰ (n=14) compared to -31.9±0.8‰, (n=11) $F_{1,17}=53.9$, $P<0.001$], whereas no singnificant difference between tree species $\delta^{13}C$ was found [*Carpinus*: -29.7±0.9‰ (n=7), *Fagus*: -28.4±0.8‰ (n=9), *Quercus*; -30.3±1.4‰ (n=6), *Tilia*: -29.5±2.7‰ (n=3), $F_{3,17}=2.1$, $P=0.14$], and no significant interaction between the both factors occurred ($F_{3,17}=2.4$, $P=0.10$). Differences reflect tree species-specific uptake of the $\delta^{13}C$ tracer, and in part different degrees of exposure to elevated CO_2, and hence, to the isotope signal [$\delta^{13}C$ (ambient CO_2) - $\delta^{13}C$ (elevated CO_2): *Carpinus*: 4.1‰, *Fagus*: 2.9‰, *Quercus*: 5.9‰, *Tilia*: 8.1‰. Comparing arthropod individuals found in the CO_2-enriched and the control area, we found significant differences in $\delta^{13}C$ for aphids (diff.: 3.8‰, $F_{1,18}=19.73$, $P<0.001$), lepidopteran caterpillars (diff.: 3.9‰, $F_{1,9}=25.31$, $P<0.001$), true bugs (nymphs, diff.: 3.0‰, $F_{1,3}=10.23$, $P<0.05$), leafhoppers (nymphs, diff.: 3.2‰, $F_{1,17}=14.22$, $P<0.01$ and adults, diff.: 2.4‰, $F_{1,3}=11.22$, $P<0.01$), bush-crickets (nymphs, diff.: 2.0‰, $F_{1,18}=34.43$, $P<0.001$), coleopterans (diff.: 1.8‰, $F_{1,15}=17.57$, $P<0.001$) and dermapterans (diff.: 1.6‰, $F_{1,7}=5.60$, $P<0.05$). No signficant differences were observed for spiders, true bugs (adults) and hymenopterans (ants). When examined for each CO_2 treatment separately $\delta^{13}C$ differed between insect taxa within both treatments. However, differences in $\delta^{13}C$ were more pronounced in the CO_2-enriched area (ambient: CO_2: $F_{11,97}=1.64$, $P<0.10$, elevated: CO_2: $F_{11,84}=9.76$, $P<0.001$). In groups where nymphs as well as adults were collected (bush-crickets, heteropterans and leafhoppers) $\delta^{13}C$ signatures were higher in adults compared to nymphs. Again, the difference was more pronounced in insects collected in the CO_2-enriched area compared to the control area.

Table 1: List of collected taxa and species in the Hofstetten forest canopy. * taken for isotope studies, L: nymphs, I: Imagines. Zoo: zoophag, phyto: phytophag, om: omniphag. Lepidoptera: caterpillars only. *C*: *Carpinus* trees, *F*: *Fagus* trees, *T*: *Tilia* trees, *Q*: *Quercus* trees

Order	Family	Genus/Species	Feeding guild	Host tress			
				C	*F*	*T*	*Q*
Araneida*	Araneidae		Chewer, zoo	x	x	x	x
Araneida *	Araneidae	*Araniella c.f cucurbitina*	Chewer, zoo				
Araneida *	Philodromidae		Chewer, zoo		x		
Araneida *	Salticidae		Chewer, zoo		x		
Araneida *	Tetragnathidae	*Tetragnatha*	Chewer, zoo		x		
Auchenorrhyncha*	Cercopidae	*Aphrophora alni*	Sucker, phyto	x			
Auchenorrhyncha*	Cicadellidae	*Oncopsis*	Sucker, phyto			x	
Auchenorrhyncha*	Cicadellidae	*Iassu lanio*	Sucker, phyto		x		x
Blattodea	Blattidae	*Ectobius sylvestris*	Chewer, phyto	x			x
Coeleoptera	(Cantharoidea)		Chewer, phyto	x	x		
Coeleoptera	Coccinellidae	*Coccinella*	Chewer, phyto	x	x		x
Coeleoptera	Curculionidae	*Curculio nucum*	Chewer, phyto	x	x		x
Coeleoptera*	Curculionidae	*Rhynchaenus fagi*	Chewer, phyto		x		
Coeleoptera	Elateridae		Chewer, phyto	x	x		
Coeleoptera	Scolytidae		Chewer, phyto				x
Dermaptera*	Forficulidae	*Chelidurella guentheri*	Chewer, om	x		x	x
Diptera				x			
Ensifera	Phaneropteridae	*Barbitstes serricauda*	Chewer, phyto	x		x	x
Ensifera	Phaneropteridae	*Leptophyes punctatissima*	Chewer, phyto				x
Ensifera*	Meconematidae	*Meconema meridionale*	Chewer, zoo	x	x		x
Ensifera*	Meconematidae	*Meconema thalassinum*	Chewer, zoo	x		x	x
Heteroptera*	Miridae	*Dryophilocori lavoquadrimaculatus*	Phyto/zoo				x
Heteroptera*	Miridae	*Psallus*	Phyto		x		
Heteroptera*	Miridae	*Rhabomiris striatellus*	Phyto (L)/zoo (I)		x		
Heteroptera*	Pentatomidae	*Dolycoris baccarum*	Phyto	x			
Heteroptera*	Pentatomidae	*Palomena prasina*	Phyto	x	x		x
Heteroptera*	Pentatomidae	*Palomena viridissima*	Phyto	x			
Heteroptera*	Pentatomidae	*Pentatoma rufipes*	Sucker, phyto		x	x	
Hymenoptera*	Formicidae	*Lasius fuliginosus*	Chewer, om	x	x	x	x
Lepidoptera*			Chewer, phyto		x	x	x
Planipennia	Chrysopidae		Chewer, zoo				x
Sternorrhyncha*	Drepanosiphidae	*Phyllaphis fagi*	Sucker, phyto		x		

Arthropod movement

In aphids (*Phyllaphis fagi*), lepidopteran caterpillars, and bush-cricket nymphs (*Meconema* sp.) $\delta^{13}C$ of leaves and insects correlated closely, whereas $\delta^{13}C$ of leaves correlated only marginally with coleopteran (*Rhynchaenus fagi*) and dermapteran species (*Chelidurella guentheri*) (Fig. 2, 3, Table 3).

The correlation between $\delta^{13}C$ of host plant leaf tissue and that of aphids was most pronounced, most likely due to the fact that they are (1) the least mobile group and (2) that leaves on which aphids were found were also the ones used for analysis. In contrast, we found no correlation for spiders (Araneida) and ants (Hymenoptera, Fig. 3, Table 3).

Regarding the insect taxa where nymphs and imagos were found, a $\delta^{13}C$ relationship with leaf tissue occurred in bush-crickets (nymphs), true bugs (nymphs) and leafhoppers (nymphs). The correlation between leafhoppers and leaf tissue remained in imagos, although it was somewhat weaker, whereas it vanished in adult heteropteran and bush-crickets (Fig. 3, Table 3).

Table 2: Results of linear regressions for $\delta^{13}C$ of insects and $\delta^{13}C$ of host plant leaves. L: nymphs, A: adults. *P* value indicates whether the slope of the regression is different from zero.

Insects	L/A	N	R^2 ($\delta^{13}C$)	*F* ($\delta^{13}C$)	*P* ($\delta^{13}C$)
Aphidae (*P. fagi*)	L	7	0.83	$F_{1,18}$ = 90.2	< 0.001***
Araneida	A	19	0.08	$F_{1,30}$ = 2.7	0.11
Auchenorrhyncha	L	10	0.62	$F_{1,19}$ = 30.9	< 0.001***
Auchenorrhyncha	A	9	0.40	$F_{1,11}$ = 7.2	0.02*
Coleoptera (*R. fagi*)	A	11	0.21	$F_{1,15}$ = 4.0	0.06
Dermaptera (*C. guentheri*)	L	2	0.42	$F_{1,7}$ = 5.0	0.06
Ensifera (*Meconema*)	L	8	0.47	$F_{1,17}$ = 14.9	< 0.01**
Ensifera (*Meconema*)	A	5	0.00	$F_{1,8}$ = 0.0	0.99
Heteroptera (*Pentatoma*)	L	2	0.81	$F_{1,3}$ = 18.3	0.02*
Heteroptera (*Pentatoma*)	A	13	0.00	$F_{1,14}$ = 0.1	0.78
Hymenoptera (*L. fuliginosus*)	A	16	0.02	$F_{1,15}$ = 0.3	0.58
Lepidoptera	L	7	0.61	$F_{1,9}$ = 14.2	< 0.01**

*<0.05, **<0.01, ***<0.001

Interestingly, when testing the relationship of arthropods' $\delta^{13}C$ and $\delta^{13}C$ of leaf tissue seperately either for the control area or the CO_2-enriched area we found, that in the latter there were still significant correlations, probably due to the higher variability of $\delta^{13}C$. Significant or marginally significant correlations in elevated CO_2 were found in bush cricket nymphs ($F_{1,10}$=3.37, *P*<0.1), leafhopper nymphs ($F_{1,7}$=5.23, *P*<0.06), ants ($F_{1,6}$=26.9, *P*<0.01) and aphids ($F_{1,11}$=42.7, *P*<0.001).

Discussion

Using $\delta^{13}C$ as a tracer, we found different mobility of arthropod taxa in the canopy of temperate trees. The $\delta^{13}C$ of host plant leaf tissue and insects correlated significantly in declining order for aphids, leafhoppers (nymphs and adults), bush-crickets (nymphs), lepidopteran caterpillars, and true bugs (nymphs).

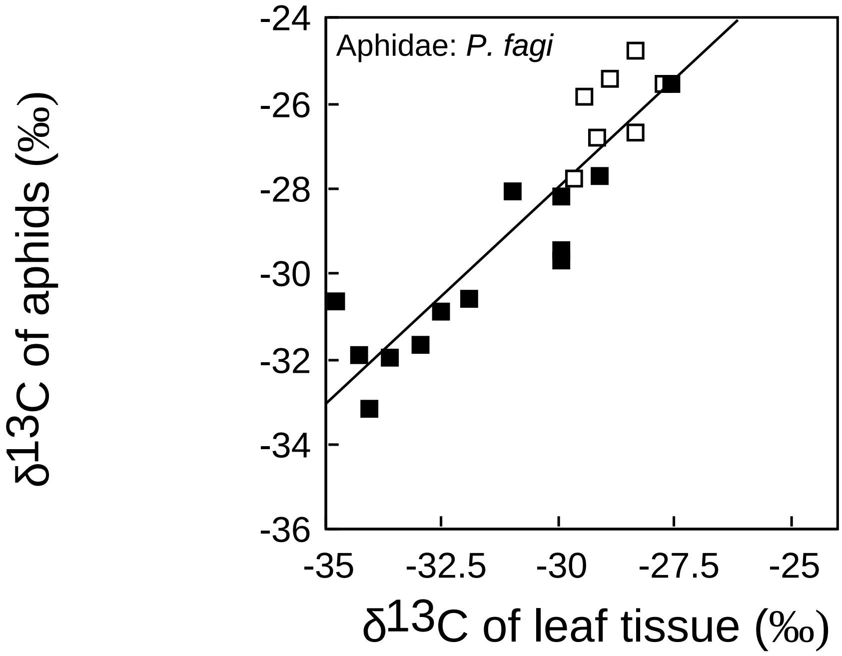

Fig.2: Linear Regressions of δ^{13}C content of aphids (*Phyllaphis fagi*) and δ^{13}C of host plant (leaf tissue). Black symbols: arthropods found in the CO$_2$-enriched area, white symbols: arthropods found in the control area.

The significance of these correlations was driven by the differences in δ^{13}C between leaves and arthropods collected in the CO$_2$-enriched area and the control area. Thus, we conclude that these arthropods remain in a specific area of the canopy and do not shift between CO$_2$-enriched and control trees. However, since different tree species within the same treatment differed little in leaf δ^{13}C, it is possible that insects moved from tree to tree within the same treatment area, as shown for leafhopper nymphs and bush-cricket nymphs. Apart from aphids, it is likely that all arthropods studied here moved to a certain extent, from one branch to another or even from one tree to another. However, in some groups there are shifts between CO$_2$-enriched and control area indicating that these arthropods moved between these areaa. A posteriori it is not surprising that the correlations were strongest in phytophagous insect groups because the δ^{13}C signature is reflected in δ^{13}C tissue of insects, in contrast to zoophagous spiders or omniphagous insects, consuming a wider range of food material with different δ^{13}C values.

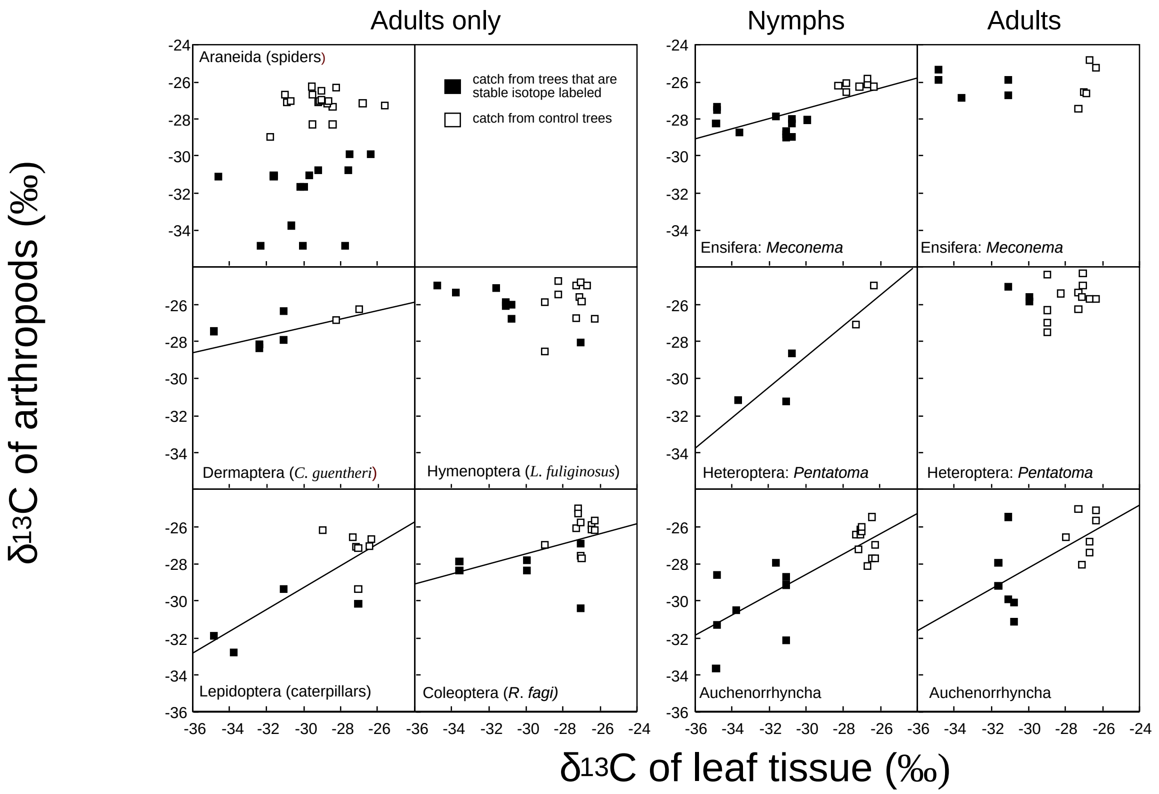

Fig.3: Linear Regressions of δ^{13}C (insect nymphs and imagos) and δ^{13}C of host plant (leaf tissue) for the repective groups (Statistical results are given in table 3, black symbols: arthropods found in the CO_2-enriched area, white symbols: arthropods found in the control area). The regressions for *Meconema* were already published in Asshoff and Amstutz (2004).

In the following section we discuss the examined groups in more detail. Of course, we cannot assign the results found for one species to the complete taxa. Different species have different life histories, which have to be considered when studying insect mobility in the canopy. For example, within the bush-crickets the phytophagous (e.g. *Barbitistes serricauda*) and zoophagous species *Meconema thallasinum* will differ in their mobility since *B. serricauda* is not capable of flying, in contrast to *M. thallasinum*, which is.

(1) Groups showing a low degree of mobility.

Aphidae (aphids): The strongest relationship between δ^{13}C of leaves and insects was found in unwinged nymphs of *Phyallphis fagi*. Since this species forms colonies on beech leaves, it is most likely that the nymphs remain on the same branch or even on the same leaf and therefore their entire biomass was synthesized at a specific site in the crown.

Auchenorryncha (leafhoppers): We found positive correlations for both leafhopper nymphs and adults. The three studied species here (*Oncopsis* sp., *Aphrophora alni*, *Iassus lanio*) are typically found on woody plants or in forests (Waloff 1973, Günthard 1987, Nickel 2003).

Theses species are able to fly, however, our results suggest that nymphs and adults do not move a lot. Similary Blüthgen et al (2003) found that $\delta^{13}C$ of homopterans (aphids and leafhoppers) correlated significantly with those of their host plant foliage.

Lepidoptera (butterfly caterpillars): Since a lot of caterpillars are known to be strongly host specific (Ebert 1993) and of course they cannot fly $\delta^{13}C$ of leaf tissue should directly be reflected in the $\delta^{13}C$ signature of the caterpillars as our results have shown.

(2) Groups showing a higher degree of mobility

Coleoptera (beetles): The weak correlation between $\delta^{13}C$ in *Rhynchaenus fagi* and $\delta^{13}C$ in host plant leaf tissue suggests that this beetle is mobile. In spring, nymphs of this species mine in beech leaves. By the end of July adults migrate to adjacent overwintering sites (e.g. spruce trees) or into forest litter. They do not feed until spring of the next year. Feeding and reproduction starts after bud break on beech leaves (Grimm 1973, Hiltbrunner and Flückiger 1992). However, it is somewhat surprising that this beech specialist does not show a more pronounced relationship with the $\delta^{13}C$ signature of leaves, despite its ability to move during certain parts of its live cycle. For these analyses we collected individuals from their overwintering habitats where they started feeding on unfolding *Fagus* leaves.

Araneida (spiders): We found no significant correlation with $\delta^{13}C$ of spiders and host plant leaf tissue. Although web-building spiders are sedentary (except when their offspring moves passively by wind (Southwood 1962), their prey belongs to the highly mobile category of canopy arthropods. Yet, most of the catch by spiders of the treatment zone seems to come from the treatment area, indicated by a clearly more negative $\delta^{13}C$ signature, than found in the control area. But the $\delta^{13}C$ signal was omly marginally significant (P=0.11, Fig. 3).

Dermaptera (earwigs): Instar 5 nymphs of the omniphagous species *Chelidurella guentheri* showed a marginally significant correlation with leaf tissue of the host plant. This species lives primarily, but not exclusively from life and dead plant matter. In addition, the nymphs consume dead and living insects and other organisms. Given the wide spectrum of food sources we expected a weak $\delta^{13}C$ signal in these insects and therefore a low correlation with $\delta^{13}C$ of leaves. The good correlation, thus comes as a surprise. As hypothesized earlier, earwigs predominantely move vertically (up and down one tree), rather than horizontally (between trees, Franke 1985, Asshoff and Amstutz 2004), which is in line with our data.

Hymenoptera (ants): The food spectrum of *Lasius fuliginosus* is wide (trophobiotic association with aphids and coccids but also zoophagous, Seifert 1996). Even if feeding on honeydew the nest may receive sugar from various trees. It is therefore not surprising that we did not find a clear correlation. Ants build their body from larval diets that incorporate a mixture of the entire colonies foreaging activity. Blüthgen et al. (2003) working with ants in the canopy and understorey found a correlation between δ^{13}C of ants and δ^{13}C of plant species from which they were collected. Similar to earwigs, ants may consume food from the canopy and from the forest floor thereby diluting the δ^{13}C signal. We assume, but cannot prove, that the movement pattern of ants is more vertical than horizontal, comparable to the studied earwigs. However, it is known, that individual ant workers can show a high fidelity of trees and even same leaves (Mody and Linsenmair 2003) and that is in turn in line with our significant correlation of δ^{13}C of ants found in the CO_2-enriched area with leaf tissue. However, the studies of Mody and Linsenmair (2003) and Blüthgen et al. (2003) were conducted in systems, differing strongly from our temperate forest system and therefore it is not clear whether results are comparable.

(3) Groups showing differences in mobility in different developmet stages

Ensifera (bush-crickets): Young *Meconema* (instar 2) are not very mobile in the canopy and stay on the tree where they hatched, as underlines by the high isotope correlations. This bush-cricket mainly feeds on aphids (U. Simon, pers. communication, Ingrisch and Köhler 1997). In adults of this zoophagous species, no correlation between δ^{13}C of body tissue and δ^{13}C of leaf tissue was found anymore, suggesting that imagos regularly change hosts, most likely by flying, once wings become fully developed (*M. thallasinum*) or by climbing and running (*M. meridionale*, Asshoff and Amstutz (2004).

Heteroptera (true bugs): Our correlations proved that the phytophagous heteropterans hardly move in their nymphal stage, whereas the opposite was observed in adults. Besides walking there are two other forms of locomotion such as (1) carriage in the upper air by wind and (2) low level flight, typically described as migration (Souherwood 1962). Winged adults most likely migrated in and out of the CO_2-enriched area and the control area.

A wide range of studies showed that rising atmospheric CO_2 concentrations impaired food quality for insect herbivores (Arnone et al. 1995, Lindroth 1996, Hättenschwiler and Schaffelner 2004, Asshoff and Hättenschwiler 2004). In some of these studies insects switched food plants under elevated CO_2 in search for food of higher quality, Immobile insects might suffer from CO_2-induced, species-specific changes in leaf quality, because they cannot escape from a given tree. However, this conclusion is perhaps relevant for experimental conditions with a step change in CO_2. The gradual increase of CO_2 concentration in the real world might permit adaptive changes also in relatively immobile insects. For example in long term species could change their host plant preferences (and also places for egg deposition) or might physiologically be capable do deal with a poorer nutrition quality. Resultant tree species-specific abundance of certain feeding guilds may differ from the current.

To test whether insects switch host trees due to poorer quality under elevated CO_2 it would be interesting to investigate whether species showing significant correlations with leaves in our study will be sustained in the CO_2-enriched area in the future. If not, this could be evidende for a higher degree of arthropod mobiltiy in order to ensure adequate food supply (by mixing different nutrition with different $\delta^{13}C$ values).

In summary, our results suggest that stable cabon isotopes are a valuable tool to study arthropod movement patterns in a forest canopy, especially for phytophagous insect groups. The most interesting result of our study is that juveniles of busk-crickets, true bugs and leafhoppers are rather stationary although they certainly have the possibility to distribute in the canopy.

Acknowledgements

We thank Erwin Amstutz for field support. Denise Wyniger, Roland Mühlethaler, Armin Coray, Nico Blüthgen and Christoph Germann helped with arthropod determination and provided literature. Olivier Bignucolo took the leaf samples. Stefan Scheu for stimulating discussions and for reading an earlier draft of the manuscript. Nico Blüthgen and an anoynmous reviewer provided constructive critizism. Susanna Pelaez-Riedl helped with Figs. 1 and 3.

6 Responses of deciduous forest trees to severe drought in Central Europe

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***Tree Physiology* 25: 641–650**

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Responses of deciduous forest trees to severe drought in Central Europe

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Received September 30, 2004; accepted January 15, 2005; published online April 1, 2005

Summary In 2003, Central Europe experienced the warmest summer on record combined with unusually low precipitation. We studied plant water relations and phenology in a 100-year-old mixed deciduous forest on a slope (no ground water table) near Basel using the Swiss Canopy Crane (SCC). The drought lasted from early June to mid September. We studied five deciduous tree species; half of the individuals were exposed to elevated CO_2 concentration ([CO_2]) (530 ppm) using a free-air, atmospheric CO_2-enrichment system. In late July, after the first eight weeks of drought, mean predawn leaf water potential about 30 m above ground was –0.9 MPa across all trees, dropping to a mean of –1.5 MPa in mid-August when the top 1 m of the soil profile had no plant accessible moisture. Mean stomatal conductance and rates of maximum net photosynthesis decreased considerably in mid-August across all species. However, daily peak values of sap flow remained surprisingly constant over the whole period in *Quercus petraea* (Matt.) Liebl., and decreased to only about half of the early summer maxima in *Fagus sylvatica* L. and *Carpinus betulus* L. (stomatal downregulation of flux). Although we detected no differences in most parameters between CO_2-treated and control trees, predawn leaf water potential tended to be less negative in trees exposed to elevated [CO_2]. Leaf longevity was greater in 2003 compared with the previous years, but the seasonal increase in stem basal area reached only about 75% of that in previous years. Our data suggest that the investigated tree species, particularly *Q. petraea*, did not experience severe water stress. However, an increased frequency of such exceptionally dry summers may have a more serious impact than a single event and would give *Q. petraea* a competitive advantage in the long run.

Keywords: drought, elevated CO_2 concentration, global climate change, sap flow, Swiss Canopy Crane, tree phenology, water relations, web-FACE.

Introduction

Drought represents a major constraint on plant growth and productivity in most terrestrial plant communities (Hinckley et al. 1979, Churkina and Running 1998). The record-breaking heat wave and low precipitation that Europe experienced in 2003 highlighted the potential consequences of drought events for temperate European forests. For desert and Mediterranean-type climates with hot dry summers, drought events are common and their impact on species assemblages and productivity has long been acknowledged (e.g., Mouillot et al. 2002). Fewer studies have addressed the role of severe drought in Europe, where such events have been reported in historic times but have been rare during the past century (Lloyd-Hughes and Saunders 2002), with the last severe drought in 1976. However, recent evidence suggests an increase in the frequency of extreme weather conditions in Europe related to global climate change (Schär et al. 2004). Given the economic importance of agriculture and forestry, and the potential impact of climatic change on these industries, the effects of drought have lately evoked interest beyond the scientific community.

Implications of temporal water shortage on temperate forest trees, e.g., with respect to leaf water status (Hinckley et al. 1981), stomatal conductance, photosynthesis (Epron and Dreyer 1993) and hydraulic conductivity (Bréda et al. 1993, Cochard et al. 1996), have been studied extensively. In the course of a drought, gradually decreasing stomatal conductance, predawn leaf water potential, assimilation and growth are commonly observed, accompanied by a stimulation of fine root growth. Tree organs (leaves, roots, stem) generally differ in their sensitivity to drought (Westgate and Boyer 1985, Bréda et al. 1993) and a whole-tree approach is needed rather than one restricted to the leaf level (Leuschner et al. 2001*a*). Furthermore, when investigating drought responses of plants in general, it is crucial to compare several species, as even closely related species may differ greatly in their drought responses (e.g., Gieger and Thomas 2002). Water relations also need to be studied together with growth because it is well known that cambial activity is much more sensitive to drought than leaf-level gas exchange (e.g., Macfarlane and Adams 1998). Species under investigation have predominantly been those of economic importance (e.g., *Picea abies* (L.) Karst., *Quercus petraea* (Matt.) Liebl., and *Fagus sylvatica* L.; Schwanz et al. 1996, Leuschner et al. 2001*a*). *Quercus petraea* was generally found to have a higher drought resistance than

F. sylvatica, its main competitor (Epron and Dreyer 1993, Backes and Leuschner 2000). However, *F. sylvatica* seems to outcompete *Q. petraea* over most of Western and Central Europe. Competitive superiority may thus depend rather on canopy architecture and leaf orientation (Leuschner et al. 2001*a*) or shade tolerance (Küppers and Schneider 1993) than on the ability to maintain growth and vitality under drought stress. The ultimate long-term reproductive success and hence species abundance and distribution is unlikely to depend on individual physiological processes that are impaired during a single drought period. Rather, it will be the duration and frequency of drought events over a number of years that will likely determine changes in community structure.

The combined effect of elevated CO_2 concentration ([CO_2]) and drought is of particular interest as their conjunction forms part of a likely scenario for summers in Europe in the near future (Schär et al. 2004). The multitude of studies on water relations in trees subjected to an elevated [CO_2] has been reviewed by Chaves and Pereira (1992), Saxe et al. (1998), Pospisilova and Čatský (1999), Körner (2000) and Medlyn et al. (2001). These reviews suggest that deciduous trees may be less affected by drought stress when exposed to an elevated [CO_2] and thus may extend their ranges into less favorable areas. Conifers, on the other hand, show little or no measurable stomatal response to an elevated [CO_2] (Ellsworth 1999, Körner 2000), and hence may benefit less from reduced canopy transpiration in response to elevated [CO_2]. In potted *Q. petraea*, Guehl et al. (1994) found greater biomass production during water shortage in elevated [CO_2]. Similarly, greater biomass production was found for cherry and oak seedlings during exposure to drought at elevated [CO_2] (Picon et al. 1996, Centritto et al. 1999). However, unlike their responses to controlled environments, plants in the field can respond to drought by exploring deeper soil horizons or by more intensive rooting in a given soil volume. For example, Chaves et al. (1995) found only a marginal reduction in water-use by *Q. ilex* exposed to drought. A previous study at the Swiss Canopy Crane (hereafter, SCC) site in Hofstetten, Switzerland, revealed a reduction in transpiration of only about 10% in response to drought as measured by sap flow, with *Q. petraea* and *F. sylvatica* showing the least reaction (Cech et al. 2003). These authors also found a reverse (positive) effect of elevated [CO_2] on sap flow under dry conditions. Clearly, more in situ studies are needed, in which tall trees experience simulated atmospheric change under natural conditions. Here we used free-air, atmospheric CO_2-enrichment technology (FACE, Pepin and Körner 2002) in combination with a canopy crane to study the combined effect of [CO_2] and drought on trees in a mature forest.

We took advantage of the unique combination of drought and an exceptional heat wave that occurred in Europe in the summer of 2003, the heat wave having a mean return period of more than 10,000 years (according to traditional climate models, Schär et al. 2004). Drought responses were explored at the SCC site in Hofstetten, Switzerland, where some of the experimental trees are subjected to an elevated [CO_2]. The aims of the study were twofold: (1) to compare drought responses in adult specimens of five common deciduous tree species using a multilevel approach including measurements of water potential, maximum net photosynthesis, leaf conductance, sap flow, growth and phenology; and (2) to investigate the influence that atmospheric CO_2-enrichment exerts on these parameters in situ.

Materials and methods

Site description and study species

The study site is located in a diverse mixed forest stand about 15 km south of Basel, Switzerland (47°28′ N, 7°30′ E, 550 m a.s.l.), with the SCC providing access to the canopy. The forest is 80–100 years old; tree height ranges from 30 to 35 m; tree density (diameter = 0.1 m) is 415 trees ha^{-1}; and stem basal area is 46 m^2 ha^{-1}. The leaf area index (LAI) of the canopy in the experimental area is about 5. The dominant stand species are *F. sylvatica* L. and *Q. petraea*. Deciduous broad-leaved *Carpinus betulus* L., *Tilia platyphyllos* Scop., *Acer campestre* L. and *Prunus avium* L., and three species of conifers (not included in this study), occur as companion species. *Quercus petraea, F. sylvatica, C. betulus, A. campestre* and *T. platyphyllos* were selected for observations, as abundant deciduous species typical of a Swiss lowland forest. Soils are of the rendzina type on calcareous bedrock (a silty loam with an accessible profile depth of about 30 cm and a pH of about 5.8 in the top 10 cm of the profile). The site is situated on a slope with no access to the ground water table and has an essentially rocky subsoil at 40 to 90 cm below the surface.

The typical humid temperate zone climate is characterized by mild winters and moderately warm summers. Mean January and July air temperatures are 2 and 19 °C, respectively. Long-term mean annual precipitation for the region is 990 mm, two thirds of which commonly falls during the growing season. The previous years (2001 and 2002) were average with respect to temperature and precipitation. In the study year (2003), the growing season for deciduous trees lasted from the beginning of April (bud break) to the beginning of November. Most measurements were started on June 1 and ended on September 11.

Canopy access and atmospheric CO_2 enrichment system

Access to the tree canopy was achieved by a 45-m free-standing tower crane equipped with a 30-m jib. Of the 64 trees in the crane area, 14 broad-leaved trees (four *F. sylvatica*, three *Q. petraea*, four *C. betulus*, one *T. platyphyllos*, one *A. campestre* and one *P. avium*, the last not used for this experiment) were selected for the elevated [CO_2] treatment in autumn of 2000 (all within a continuous plot). A similar number of control trees were located in the remaining crane area at a sufficient distance from the CO_2 release zone. Atmospheric CO_2 enrichment of the forest canopy was achieved by a free-air, pure CO_2 release system (Pepin and Körner 2002), which consisted of a web of 4-mm plastic tubes (about 0.5 km per tree) with laser-punched holes emitting pure CO_2 into the tree canopy (web-FACE). The rate at which CO_2 was injected into the tubing system and released around the tree crowns was set by computer-controlled magnetic valves to maintain a target [CO_2]

of 550 ppm as closely as possible (details in Pepin and Körner 2002). The system was set up in late September 2000 and operated in the three subsequent growing seasons (from budbreak until leaf fall) every day from dawn to dusk. The overall mean seasonal daytime [CO_2] within the canopy deviated slightly from the set point and was 520 ppm in 2001 and 530 ppm in 2002 and 2003.

Environmental data

Rain, throughfall precipitation, air humidity, potential evapotranspiration, temperature and soil humidity were monitored by weather stations located above the tree canopy at the top of the crane and in the understory. Tipping bucket rain gauges (RG1, Delta-T, Cambridge, U.K.) were used to monitor rain, and throughfall precipitation was quantified with 22 evenly spaced funnel-type rainwater collectors. Potential evapotranspiration was measured with two evaporometers (ET gauge, Spectrum Technologies, Plainfield, IL). Data were recorded as 10-min means with data loggers (DL3000, Delta-T). Soil moisture data were obtained at 15-cm depth from hourly measurements with theta-probes (ML2x, Delta-T) located within and immediately outside of the atmospheric CO_2-enrichment zone and connected to another data logger (DL2e, Delta-T). Additional soil water content across a 90-cm profile (the lower 50 cm representing eventual water in rock crevices) was monitored every 7–14 days during the 2003 growing season at four locations using time domain reflectometry (MP-917 and probes PRB-F, Environmental Sensor, Victoria, BC, Canada).

Leaf water potential measurements

Predawn leaf water potential was measured with a pressure chamber (SKPM 1400, Skye Instruments, Powys, U.K.) from the canopy crane gondola on 5 days from July to September (July 22, July 23, August 14, August 20 and September 12). The weather was clear on all five days with light availabilities up to 1600 mmol m^{-2} s^{-1}. On three occasions, daily courses of water potential were recorded. Samples were taken at similar heights above ground to avoid variability due to hydrostatic water potential and samples were wrapped with scotch tape immediately after cutting to prevent further transpiration. We sampled three to four individuals of *Q. petraea, F. sylvatica* and *C. betulus* and two of *A. campestre* and *T. platyphyllos*.

Sap flow measurements

Sap flux density within the xylem was measured by the constant heat-flow technique described by Granier (1985, 1987). Each sensor (UP, Kolkwitz, Germany) consists of two 20-mm-long probes (2 mm in diameter), each equipped with a copper-constantan thermocouple and wrapped with heating wire. The upper probe was inserted radially into the sapwood at breast height into bore holes lined with thin-walled aluminum tubing. The bore holes were separated vertically by about 15 cm. The upper probe was heated at a constant power of 200 mW, while the lower reference probe remained unheated. During conditions of zero sapflow, e.g., at night or during prolonged rain events, the temperature difference, ΔT, between the probes reaches a maximum (about 9–15 K). Sap flow during the day causes a decrease in ΔT by cooling the upper probe. Sap flux density within the xylem (J_S) measured in m^3 H_2O m^{-2} was calculated from an empirical relationship validated for several species by Granier (1985), and recently revalidated by Clearwater et al. (1999). We tested for azimuthal variability in sap flux densities, which revealed differences of up to a factor of three between six sensors in *Q. petraea,* whereas almost no differences were found between sensors in *F. sylvatica.* We also compared sap flux densities among trees and among species. Maximum sap flux density was between 30 and 90 J_S (10^{-6} m^3 H_2O m^{-2} s^{-1}) for *Q. petraea*, between 80 to 140 J_S for *F. sylvatica* and about 60 to 100 J_S in *C. betulus*. Given these large differences in absolute sap flow rates and the difficulty of calibrating and converting sap flow signals of mature trees with uncertain sapwood widths into absolute flow rates, we considered relative values only in the present study.

Measurements of J_S were performed from June 21 to October 1, 2003, on three dominant tree species (*Q. petraea*, *F. sylvatica* and *C. betulus*, $n = 2$, only one tree per species at the beginning of the season) using three to six sensors per tree (equally spaced around the stem). Sensors were protected against rain and external thermal influences by aluminum covers filled with polyester wool. Readings were taken at 30-s intervals and recorded as 10-min means using a multi-channel data logger (DL2e, Delta-T). The signals per tree were averaged over all sensors per stem.

Leaf gas exchange measurements

Instantaneous rates of CO_2/H_2O gas exchange were measured in situ in the first half of the day in early (mid-June), mid- (August) and late summer (early September 2003) on mature leaves fully exposed to the sun in the outer canopy of 25 trees (13 trees exposed to elevated [CO_2] and 12 controls) with a portable gas exchange system (Li-6400, Li-Cor, Lincoln, NE). From each tree, eight (June), four (August) and four (September) leaves from different branches were selected for gas exchange measurements, which were conducted at saturating photosynthetic photon flux (PPF) (1200 µmol m^{-2} s^{-1}; from an LED light source; Li-Cor 6400-02). Trees grown in an elevated [CO_2] were measured at about 530 ppm, control trees at about 360 ppm. Chamber temperature (June: 27.1 ± 1.5 °C; August 30.1 ± 0.9 °C; September: 20.9 ± 0.8 °C; mean ± SD) and relative humidity (June: 64 ± 9%; August 37 ± 2%; September: 60 ± 4%) tracked ambient conditions. A data set was recorded once the net rate of photosynthesis and stomatal conductance remained constant. Individual measurements lasted less than 5 min.

Growth and phenology data

Each month, from 2001 to 2003, we measured the breast-height diameter of 22 trees randomly distributed within the study site with fixed "Permanent Tree Girth-Tape" (D1-L, UMS GmbH, Munich, Germany; accuracy of measurement 0.1 mm). Trees measured were as follows: *Q. petraea*: $n = 6$, 3 ambient, 3 elevated [CO_2]; *F. sylvatica*: $n = 6$, 3 ambient, 3 elevated [CO_2]; *C. betulus*: $n = 6$, 3 ambient, 3 elevated [CO_2];

T. platyphyllos: $n = 2$, 1 ambient, 1 elevated [CO_2]; and *A. campestre*: $n = 2$, 1 ambient, 1 elevated [CO_2]. Leaf longevity, defined by the time span from 75% bud break to 75% leaf fall was documented in 2002 and 2003, based on observations on a total of 24 individuals of the dominant tree species (*C. betulus*: $n = 8$; *F. sylvatica*: $n = 6$; *Q. petraea*: $n = 6$; *T. platyphyllos*: $n = 2$; and *A. campestre*: $n = 2$; half of the individuals of each species being subjected to elevated [CO_2]).

Data analysis

Leaf water potential, photosynthesis parameters and sap flow data were evaluated by repeated-measures analysis of variance (ANOVA). Datasets that were not normally distributed (according to the Kolmogorov-Smirnov test for normality) were log-transformed before analysis. Tukey's HSD post hoc test for unequal sample size was used to detect differences between CO_2 treatments. Data analysis was performed with the software package STATISTICA Version 5.0 (Statsoft, Tulsa, OK). Error terms represent standard errors unless otherwise stated.

Results

Environmental data

At the SCC site, total accumulated rain during the study as defined above was less than 50% of the 10-year mean from 1989 to 1999, and spring precipitation was also well below the mean (Figure 1). Moreover, throughfall precipitation amounted to approximately half of the above-canopy precipitation of 188 mm, while potential evapotranspiration was 559 mm, i.e., exceeding throughfall precipitation almost sixfold during the same period. Mean monthly temperatures exceeded the long-term mean (1989–1999) dramatically (e.g., + 6.8 °C for June). Soil water content at 15-cm depth dropped from around 30 Vol.% in early June to 8 Vol.% within the first five weeks of drought and remained low with no plant accessible moisture throughout the rest of the study (Figure 1). Because of the stony subsoil at the study site, soil water between 15- and 90-cm depth as derived from TDR was low and spatially variable (6 to 18 Vol.%). However, irrespective of the absolute values, the signals remained mostly constant during the drought, indicating that none of the remaining moisture was taken up by trees from the monitored soil horizons. There was no significant difference in soil water between the elevated [CO_2]-treated area and the control area in the explored soil horizon (constant minimum readings; data not shown).

Rate of net photosynthesis and leaf conductance

Both net photosynthesis (A) and leaf conductance (g) decreased significantly, by 60 to 80%, from June to August in all species (Figures 2A and 2B). Intraspecific variation was large in both parameters, but nonsignificant in August when all species were strongly affected. Only *Q. petraea* (and to some extent *T. platyphyllos*) resumed g and A values in September comparable with those of June (Figures 2A and 2B), whereas the other species showed only partial recovery. Elevated [CO_2] had no significant effect on g. In contrast, elevated-[CO_2]-treated trees (all species pooled) assimilated more (12.7 ± 0.9 μmol CO_2 m^{-2} s^{-1}) than trees growing in ambient [CO_2] (8.3 ± 0.8 μmol CO_2 m^{-2} s^{-1}; repeated-measures ANOVA, $P < 0.002$, $F_{1,9} = 18.7$).

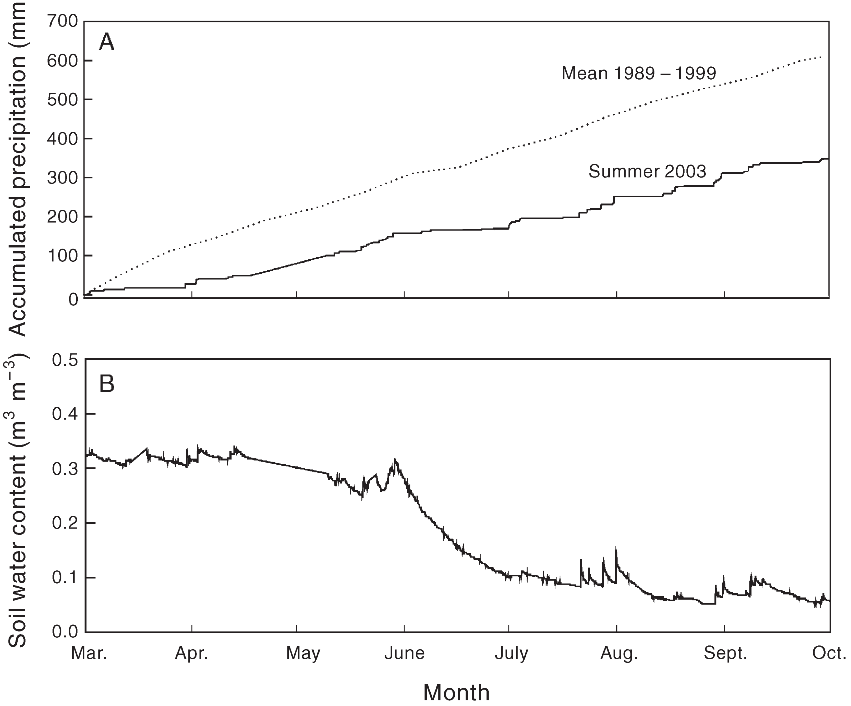

Figure 1. Accumulated seasonal rainfall (mean of 1989 to 1999, dotted line) compared with rainfall during the summer drought of 2003 at the SCC site in Hofstetten, Switzerland (solid line). Total rainfall during the study period (June 1 to September 11) was less than half the 10-year mean (188 versus 415 mm). Subsequently, substantial rainfall caused soil water content to increase as shown in the upper panel (soil water content at a depth of 15 cm).

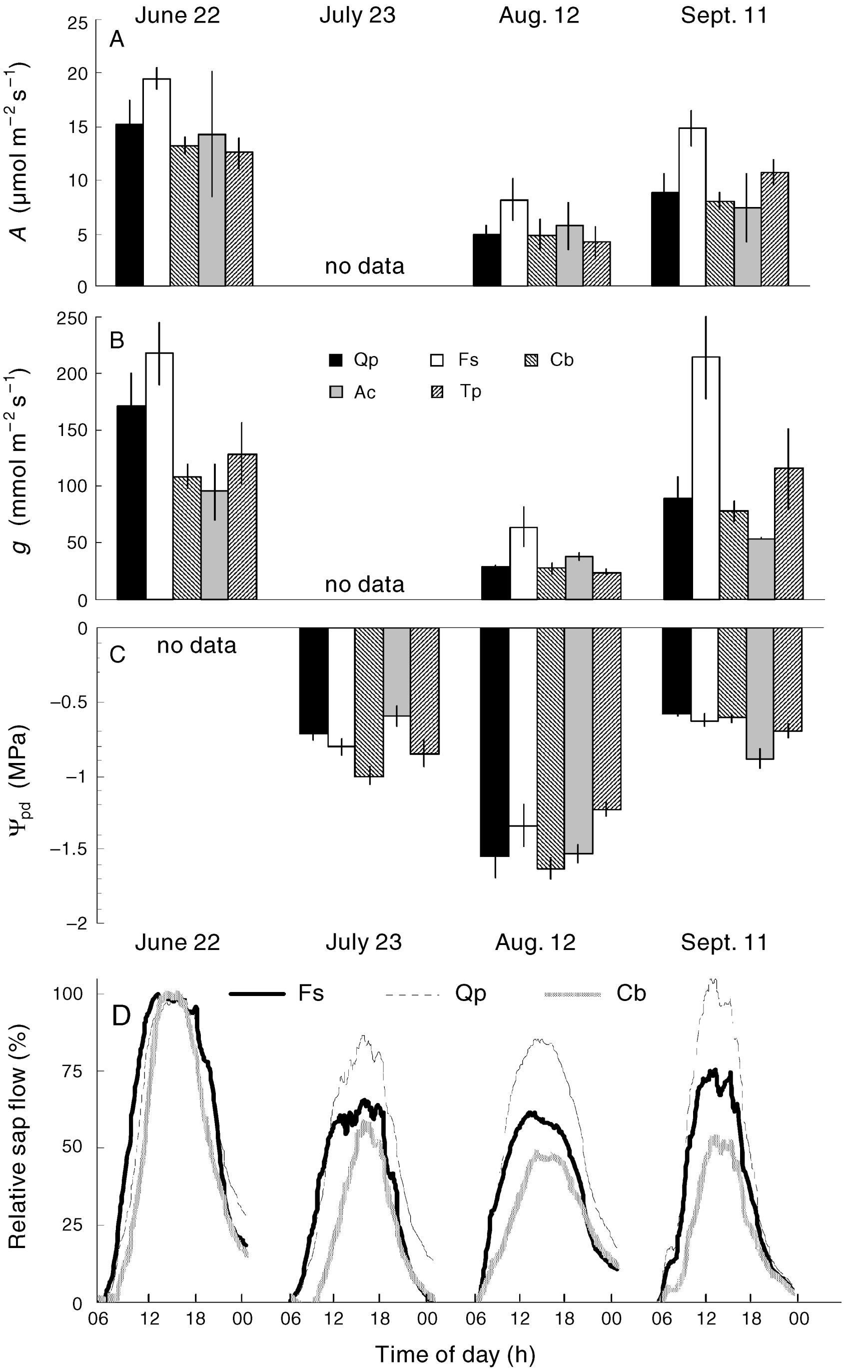

Figure 2. Net rate of maximum photosynthesis, *A* (A), stomatal conductance, *g* (B), predawn leaf water potential, Ψ_{pd} (C) and sap flow (relative to initial peak values; D) of three to five species (*Quercus petraea* (Qp), *Fagus sylvatica* (Fs), *Carpinus betulus* (Cb), *Acer campestre* (Ac) and *Tilia platyphyllos* (Tp), all in ambient [CO_2]) at the beginning (22 June), in the middle (23 July and 12 August) and towards the end (11 September) of the 2003 summer drought (means ± SE). Maximum standard errors for data in panel D are: 12.6 (*Q. petraea*), 18.0 (*F. sylvatica*) and 11.1 (*C. betulus*). All measurements were made on clear days and vapor pressure deficit reached 45, 18, 38 and 12 hPa on the four days shown.

Leaf water potential

At the end of the completely dry period between August 1 and August 14 (Figure 1), predawn leaf water potential (Ψ_{pd}) ranged from −1.7 MPa in *C. betulus* to −1.3 MPa in *Q. petraea* and −1.1 MPa in *T. platyphyllos* (Table 1 and Figure 2C). Over the course of the drought, Ψ_{pd} declined across all species (repeated-measures ANOVA, $P < 0.001$, $F_{4,24} = 82.2$) from a mean of about −0.8 MPa in mid-July to below −1.4 MPa in mid-August, reflecting severe soil desiccation. Mean Ψ_{pd} then recovered to about −0.9 MPa in the second half of August and about −0.7 MPa in mid-September. Predawn leaf water potential tended to be less negative in elevated [CO_2] (3-way ANOVA for all species, $P = 0.06$, $F_{1,6} = 5.3$), but there was no significant effect of [CO_2] on the diurnal minimum of leaf water potential ($P = 0.22$, $F_{1,6} = 1.8$). Trees started the day from different predawn leaf water potentials, but because of differences in daytime flux control, they arrived at similar minima by the afternoon.

Table 1. Leaf water potential (Ψ) of five tree species during the summer drought (2003) in Hofstetten, Switzerland. Shown are means (SE in parentheses) of predawn (Ψ_{pd}) and daily minimum (Ψ_{min}, early afternoon) leaf water potentials for each species. Measurements were on two to four individuals per species and treatment, except for *A. campestre* and *T. platyphyllos*. Abbreviations: A = trees grown under ambient [CO_2]; E = trees subjected to elevated [CO_2]. Different letters indicate significant differences between species (with CO_2 treatment and sampling date pooled, repeated-measures ANOVA, $P < 0.05$).

Ψ_{pd}	*F. sylvatica*^{a}		*Q. petraea*^{b}		*C. betulus*^{a}		*T. platyphyllos*^{c}		*A. campestre*^{a}		All species	
	A	E	A	E	A	E	A	E	A	E	A	E
July 22	−0.83 (0.03)	−0.66 (0.02)	−0.80 (0.03)	−0.79 (0.10)	−1.13 (0.07)	−1.00 (0.24)	−0.80	−0.63	−0.68	−0.56	−0.94 (0.09)	−0.80 (0.08)
July 23	−0.72 (0.06)	−0.70 (0.10)	−0.80 (0.02)	−0.82 (0.12)	−1.10 (0.07)	−0.90 (0.07)	−0.94	−0.76	−0.67	−0.54	−0.86 (0.06)	−0.78 (0.05)
Aug. 14	−1.44 (0.25)	−1.66 (0.14)	−1.42 (0.16)	−1.26 (0.26)	−1.71 (0.08)	−1.54 (0.10)	−1.07 (0.22)	−1.16	−1.54 (0.06)	−1.59	−1.50 (0.08)	−1.47 (0.09)
Aug. 20	−0.82 (0.11)	−0.86 (0.05)	−0.92 (0.03)	−0.89 (0.12)	−1.14 (0.08)	−0.91 (0.04)	−0.95 (0.05)	−0.80	−0.92 (0.03)	−1.01	−0.94 (0.04)	−0.88 (0.03)
Sept. 12	−0.57 (0.04)	−0.58 (0.02)	−0.67 (0.06)	−0.58 (0.05)	−0.61 (0.04)	−0.62 (0.04)	−0.72 (0.10)	−0.68	−0.93 (0.08)	−0.81	−0.68 (0.04)	−0.62 (0.02)

Ψ_{min}	*F. sylvatica*^{a}		*Q. petraea*^{a}		*C. betulus*^{b,c}		*T. platyphyllos*^{a,c}		*A. campestre*^{a,c}		All species	
	A	E	A	E	A	E	A	E	A	E	A	E
July 22	−1.77 (0.14)	−1.76 (0.05)	−2.18 (0.08)	−2.03 (0.06)	−2.27 (0.02)	−2.07 (0.09)	−1.53	−1.32	−2.00	−1.92	−1.95 (0.10)	−1.95 (0.09)
Aug. 20	−1.54 (0.20)	−1.52 (0.06)	−2.20 (0.09)	−2.03 (0.14)	−1.95 (0.13)	−1.88 (0.12)	−1.49 (0.10)	−1.35	−1.94 (0.13)	−1.75	−1.89 (0.09)	−1.82 (0.09)
Sept. 12	−1.21 (0.35)	−1.07 (0.08)	−1.55 (0.08)	−1.63 (0.09)	−1.40 (0.06)	−1.43 (0.16)	−1.29 (0.01)	−1.00	−1.32 (0.01)	−1.40	−1.35 (0.07)	−1.41 (0.10)

Sap flow

Relative sap flow became progressively lower as the drought advanced in *F. sylvatica* and *C. betulus*, although in *Q. petraea*, sap flux density decreased by only 15% on August 12 compared to June 22, versus 40 and 50% in *F. sylvatica* and *C. betulus*, respectively (Figure 2D). In mid-September, sap flux density in *Q. petraea* recovered fully, whereas in *F. sylvatica*, it recovered only slightly, and in *C. betulus*, sap flow continued to decrease. *Quercus petraea* and *C. betulus* reached maximum sap flow rates at higher vapor pressure deficits (around 8 to 9 hPa) than *F. sylvatica* (about 6 hPa), i.e., *F. sylvatica* started to control transpiration at a lower VPD, reducing transpiration by overcompensating stomatal regulation above a VPD of about 18 hPa (Figure 3). *Quercus petraea* showed the smallest decrease in transpiration during conditions of high VPD (flow stabilized by stomatal control), whereas *C. betulus* reduced sap flow considerably (about 50%) above a VPD of 30 hPa. After minor rain events during the drought, relative sap flow rates increased rapidly in *C. betulus* only, decreasing again shortly afterwards, which may explain, in part, the discrepancy between Figures 2D and 3.

Growth and phenology

In 2003, the seasonal increase in tree basal area was reduced by about 25% over all 22 trees monitored compared with the two previous years (2001 and 2002 versus 2003, $P = 0.06$) and basal area growth virtually ceased by early August (Figure 4). Surprisingly, we observed increasing stem diameters late in the year, which was most likely caused by wetting of the bark after the first extensive rainfalls. There were clear differences between species: *F. sylvatica* and *T. platyphyllos* were affected most, whereas *A. campestre, C. betulus* and *Q. petraea* did not differ as much in growth rates from the two previous years. In 2003, leaf longevity was prolonged by a mean of 22 days across all trees compared with the previous year (one-way ANOVA, $P < 0.001$). This difference was mainly due to delayed discoloration and leaf fall, whereas earlier bud break in spring 2003 accounted for only about 5 days of the increased leaf longevity. Although leaves looked more yellow than normal in some species (mainly *C. betulus*) at the peak of the drought in August, they became green again after the September rains and none of the monitored species showed signs of accelerated senescence or leaf fall. There was also no mid-season leaf shedding.

Discussion

Our study yielded three major findings: (1) *Q. petraea* maintained a consistently better water status during the drought than the other four species. Despite the high interspecific variability in drought response, no species showed signs of drought-induced leaf mortality; (2) across all trees, the summer drought of 2003 caused reduced basal stem area growth of all investigated species compared with growth in the two previous years; and (3) elevated [CO_2] resulted in less negative predawn leaf water potentials. However, we observed neither significantly lower stomatal conductance in trees exposed to elevated [CO_2] during the drought (though there was a small

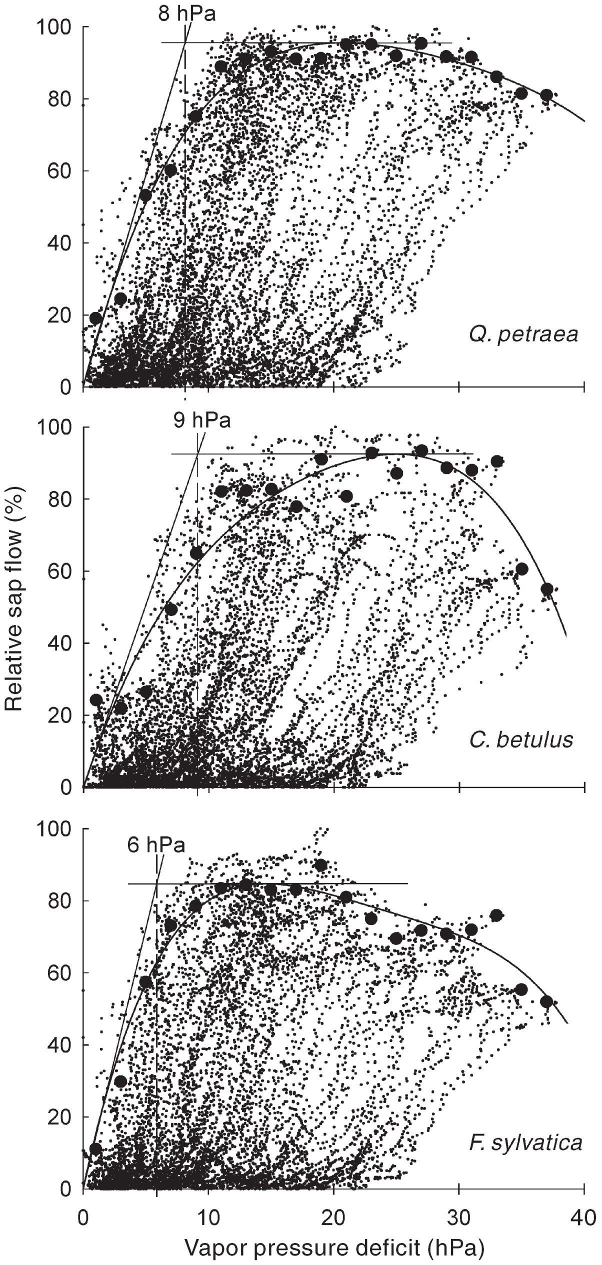

Figure 3. Vapor pressure deficit (VPD) plotted against relative sap flow for *Q. petraea*, *C. betulus* and *F. sylvatica*. Bold lines show best 4th order polynomial fit (least squares) for 95 percentiles of classes of 2 hPa VPD (bold dots). R^2_{adj} values are 0.97, 0.90 and 0.94 for *Q. petraea*, *C. betulus* and *F. sylvatica*, respectively, $P < 0.001$ for all three species, $n = 2$ (except for June and July $n = 1$).

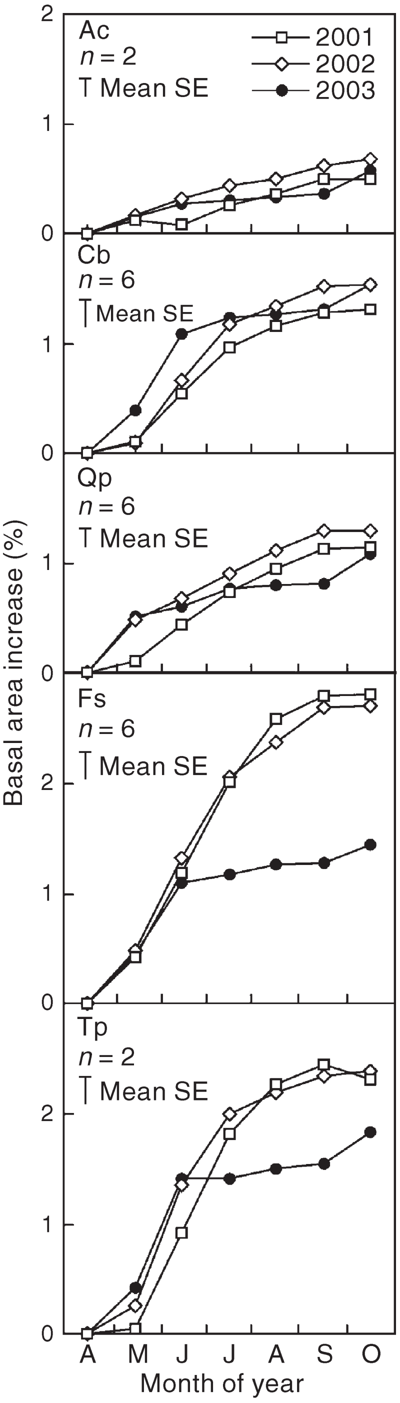

Figure 4. Stem basal area increase (%) in the drought year 2003 compared with the two previous years with mean precipitation (2001 and 2002) for the five species *Acer campestre* (Ac), *Carpinus betulus* (Cb), *Fagus sylvatica* (Fs), *Tilia platyphyllos* (Tp) and *Quercus petraea* (Qp). For clarity, the SE is averaged over the vegetation period (mean SE). Differences between trees grown under elevated CO_2 and ambient conditions were not significant, hence data were pooled for analysis.

trend in this direction) nor a significant [CO_2]-related increase in diameter growth across all species compared with controls.

Water relations during drought

The effect of severe drought as experienced in 2003 was aggravated by the heat wave which reduced the ratio of precipitation to evaporation further. Subtracting mean throughfall precipitation (about 100 mm) from potential evapotranspiration (559 mm) results in a negative water budget of about 460 mm during the study period, or a potential water loss of 4.5 mm per day. Even if actual evapotranspiration was substantially lower because of stomatal down-regulation, we estimate severe soil dessication down to at least 2.5 to 3 m, given the extremely rocky subsoil. In fact, many understory plants such as *Mercurialis perennis* L. wilted permanently. Canadell et al. (1996) reviewed the rooting depths of 21 temperate deciduous forest trees and arrived at 1.8 to 4.3 m, with a mean of 3.0 ± 0.2 m. *Quercus petraea* was found to have somewhat deeper rooting (max. 1.5 m) than *F. sylvatica* (1.1 m) (Leuschner et al. 2001*b*). Based on the estimates above, we expected much more severe signs of water shortage in the study trees. Surprisingly, this was not the case. This suggests that trees had access to deeper soil water through deep-reaching fine root systems, which may have been overlooked in excavation studies.

Overall, we found important differences in drought response among species, particularly between the co-dominant species *Q. petraea* and *F. sylvatica*. In essence, *Q. petraea* maintained surprisingly high transpiration rates (estimated by sap flow) although assimilation and stomatal conductance were depressed substantially (but were still twice as high as in *F. sylvatica* and *C. betulus* at the peak of the drought). There are two possible explanations for this: (1) both *F. sylvatica* and *Q. petraea* had access to deep water, but *F. sylvatica* did not tolerate such high flux rates and thus down-regulated transpiration by stomatal closure, or (2) *Q. petraea* had access to deep water that was inaccessible to *F. sylvatica*. We favor the first explanation, as we measured minimum leaf water potentials (Ψ_{min}) of –2.2 MPa in *Q. petraea* but values no lower than –1.8 MPa in *F. sylvatica* under the same conditions (Table 1).

According to Hacke and Sauter (1995), embolism occurs at leaf water potentials below –1.9 MPa in *F. sylvatica*, and Bréda et al. (1993) state that both beech and oak can be considered water stressed when leaf water potential drops below –2.0 MPa. Although there is one study reporting Ψ_{min} values below –3.0 MPa for both *F. sylvatica* and *Q. petraea* in Greece (Raftoyannis and Radoglou 2002), such low leaf water potentials have never been reported from Central or Western Europe. We argue that with a minimum predawn leaf water potential (Ψ_{pd}) of –1.66 MPa at the peak of drought, *F. sylvatica* trees were considerably water stressed, as such low Ψ_{pd} values have never been found previously in tall adult trees (Aranda et al. 2002, Peuke et al. 2002). However, *Q. petraea* showed higher Ψ_{pd} together with lower Ψ_{min} values, which suggests that this species suffered less from water shortage. The greater abundance of *F. sylvatica* across most of Central and Western European lowlands contrasts with the higher drought resistance of *Q. petraea*, indicating that drought has not been the dominant selective force so far. This issue has been amply discussed by Leuschner et al. (2001*a*, 2001*b*). These authors observed drought-stimulated fine root growth as well as generally high competitive ability in fine root growth in *F. sylvatica* but not in *Q. petraea.* The diffuse-porous *F. sylvatica* also seems to operate at lower sap flux densities than the ring porous *Q. petraea*, possibly because of its higher Huber-value (Lösch 2001). Nevertheless, we observed *F. sylvatica* trees down-regulating transpiration by stomatal closure at atmospheric vapor pressure deficits above about 10 hPa, thereby mitigating cavitation risk. Cavitation risk is higher in *F. sylvatica* than in *Q. pubescens*, an oak species closely related to the one studied here (Lösch 2001). Lower cavitation risk may thus explain the greater drought resistance of oak.

Carpinus betulus raised sap flow rates quickly after even minor rain events with little throughfall precipitation. This suggests that *C. betulus* features an effective fine root system in the shallowest soil horizon, allowing it to rapidly use small quantities of water that become available during otherwise dry periods (Figure 3). *Acer campestre* did not differ in *A* and *g* from the more drought-sensitive species *F. sylvatica* and *C. betulus*, whereas *T. platyphyllos* seemed to recover particularly well in September reaching pre-drought *A* and *g* values. Thus, *T. platyphyllos* may be a relatively drought-tolerant species, not previously recognized as such, whereas *A. campestre* seems to be more susceptible to drought.

The surprisingly low sap flow rates in all species at high VPD (e.g., almost zero flow at 20 hPa VPD; Figure 3) were a consequence of many extraordinarily hot nights (flow approaches 0) in August when predawn temperatures remained well above 20 °C and relative humidity was as low as 30%.

Growth and phenology

Intraspecific differences in physiological responses to drought matched patterns of basal stem area increase quite well. As expected, *Q. petraea* showed the least difference in growth reduction compared with the two previous years, whereas in *F. sylvatica*, water shortage had an unambiguous effect on basal area increase (Figure 4). *Carpinus betulus* showed rapid basal area growth during the spring when soil water availability was still high, but ceased growth almost completely during the drought. For *A. campestre* and *T. platyphyllos,* sample sizes are too low to allow interpretation of growth responses. Drought has generally been associated with reduced leaf longevity in deciduous species (Jonasson et al. 1997), depending on length and severity of the drought. In contrast, increases in leaf longevity as in our particular case have rarely been reported (but see Casper et al. 2001). The increase in stem basal area in October following the first autumn rains (Figure 4) is unlikely to have been caused by late season growth. The difference in thickness of fresh and air-dried bark samples (compare Zweifel et al. 2000, Asshoff et al., unpublished data) indicates that the apparent increase in basal area can be fully explained by bark swelling after rain.

Drought–CO_2 interactions

Elevated CO_2 in combination with drought caused less negative predawn leaf water potentials. As predawn leaf water potential is the sum of the (constant) hydrostatic potential plus the soil matrix potential, this indicates water savings of trees exposed to a high [CO_2] during drought. However, trees exposed to an elevated [CO_2] showed no measurable reduction in stomatal conductance during the drought, but small reductions may have escaped our assessment. A study by S.G. Keel et al. (Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, Villigen, Switzerland, personal communication), which was conducted at the same site, indicated that there is such a difference, but the mean reduction across species is small (about 10–15%) compared with the variation in the data.

We conclude that a drought as severe as that in Europe during 2003 exerts no damage on the tested tree species. The visual impression during this summer suggests that this applies to most lowland forests in this area, where obvious drought damage was restricted to hill tops, ridges or rock escarpments. However, we identified a pronounced differentiation in the impact of the drought on water consumption, leaf photosynthesis and growth across the studied species. Adult *Q. petraea*, it appears, copes best with drought events, but it is too early to predict a possible shift in species composition in mixed forests of the kinds studied.

Acknowledgments

We thank Olivier Bignucolo and Erwin Amstutz for crane operations, the Swiss Federal Office of the Environment (BUWAL) and the University of Basel. Financial support came from the Swiss National Science Foundation (Grant 3100–059769.99 to C. Körner and project P 3.2 of J. Fuhrer).

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7 Faster carbon turnover and transient growth stimulation in hundred-year-old forest trees exposed to elevated CO_2

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Abstract

Whether rising atmospheric CO_2 concentrations will cause forests to grow faster, and perhaps, store more carbon, is still an open question. Using a new CO_2 enrichment technology in combination with a canopy crane we found an immediate enhancement of carbon flux through 30- to 35-m tall temperate forest trees when exposed to elevated CO_2. However, except for one tree species in two out of four study years, no stimulation in stem growth was observed. There was no photosynthetic adjustment, CO_2 responses of leaf chemistry were smaller than commonly reported for younger trees, and tree species differed significantly in their sensitivity to CO_2. Although growing well, these mature trees did not accrete more biomass carbon in trunks in response to elevated CO_2 after 4 years. CO_2 fertilization effects on forest trees appear to depend on species, tree age and/or growth conditions, and seem to enhance fluxes rather than pools of carbon.

Dynamics of tree growth

The basis of this analysis is tree ring chronology that accounts for pre-treatment growth in every tree. This mitigates some of the obvious statistical constraints when the test objects in an experiment like this are tall trees. We sampled the outer 3cm in 39 stems of *Fagus*, *Quercus*, *Carpinus*, and *Tilia*, using a specially designed 4mm core-puncher in 2004. *Acer* and *Prunus* showed too little growth during these years, hence had to be dismissed from this analysis. Since the punching position hardly represents mean radial growth of a given stem (growth rings are not uniform), we used girth tapes, which integrate thickness growth over the whole stem during the 4 treatment years. The relative (tree rings) and absolute increment (girth tapes) correlate well (but rarely 1:1) and this correlation permits a reconstruction of pre-treatment absolute growth rates, assuming that the core versus girth-tape ratio has not changed over the recent past. All readings during the 4-year treatment period were standardized by the mean growth (expressed as % basal area increment) during five pre-treatment years (1996-2000).

The resultant tree basal area responses (Fig. 3) indicate a sharp and significant stimulation of growth in *Fagus* in the first year (+ 92%, $p=0.026$) with no effect in the other taxa at any time during the 4-year study. The initial response in *Fagus* disappeared in 2002, but recovered in 2003 ($p = 0.028$) during a centennial heat wave and associated drought, although overall growth was significantly reduced during this year. *Fagus* showed no effect in 2004 and the 2001-2004 cumulative signal was only marginally significant at $p = 0.07$.

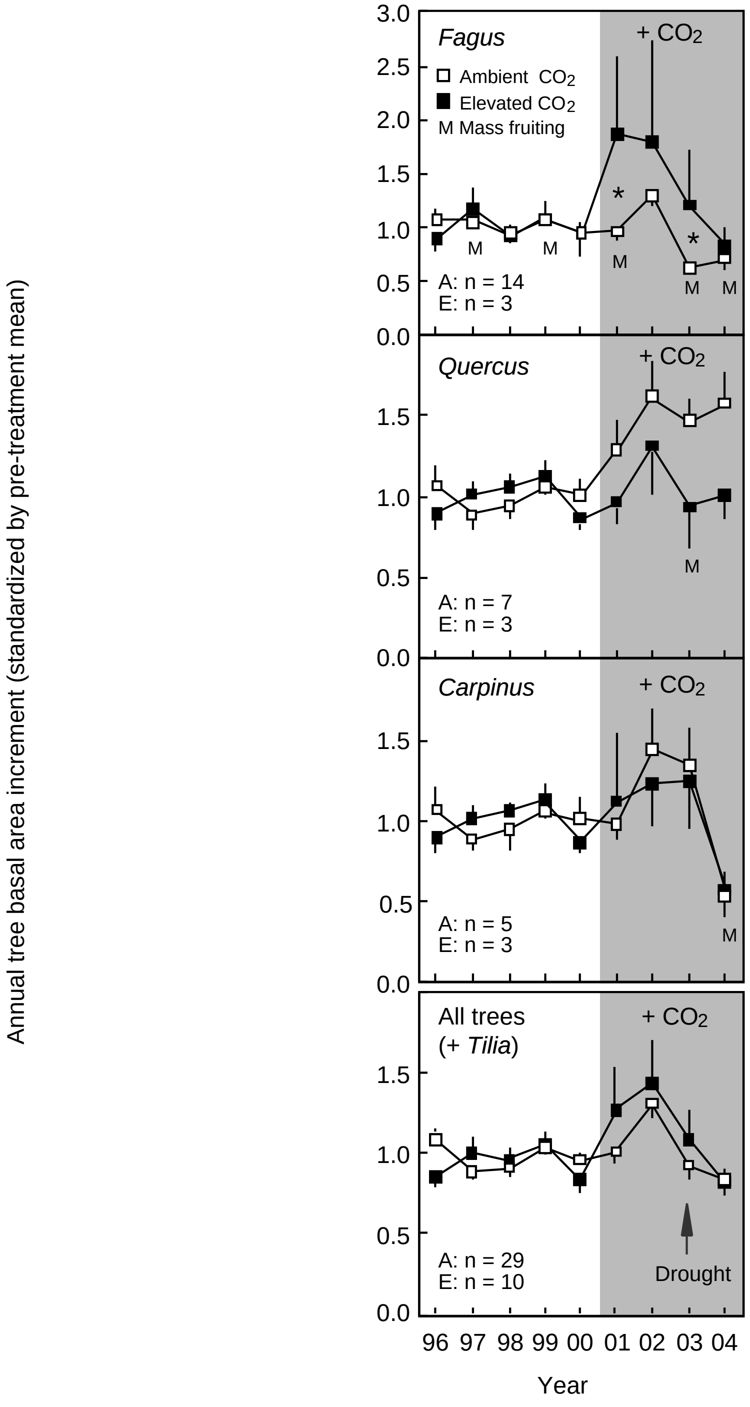

Fig. 3 Combined tree ring and girth tape data for basal area (BA) growth of trees, standardized by the mean BA increment for 1996-2000 for each tree (pre-treatment growth). The two asterisks in *Fagus* indicate years of a significant stimulation (p=0.026 in 2001 and p= 0.028 in 2003). 2003 was a year with a severe summer drought. All other year-by-year comparisons were not significant. No significant CO_2 x year interaction was observed in any species. The bottom diagram is for all trees that showed measurable growth, 10 in elevated CO_2 vs. 29 controls.

We performed a second analysis to test the responses of trees, which showed a measurable growth signal irrespective of species (all standardized by pre-treatment growth, 10 in elevated CO_2, 29 in ambient CO_2). This analysis yielded no significant basal area effect, because the *Fagus* signal was diluted by other species, and hence the cumulative CO_2 signal for all trees in 2004 was zero (p=0.79; p = 0.15 in 2001).

Original tree rings, standardized by the maximum radial increment per tree (=100%) yielded chronologies almost perfectly in phase for the elevated and ambient tree groups per species, but never produced any significant CO_2 effects (data not shown). However, these chronologies illustrate that rated by pre-treatment growth dynamics, the years 2001 and 2002 were indeed very good growth years (peak growth in all trees, including control trees) *versus* 2003 and 2004 (the latter with after-effects of the 2003 drought), exemplifying two very unfavourable years. The 4 treatment seasons thus included a pair of best and worst years compared to the pre-treatment trends in growth. Both *Fagus* and *Quercus* had masting years (mass fruiting), but these did not correlate in any meaningful way with radial growth or NSC concentrations (see reproduction). We attribute this to the very high and presumably saturated concentrations of mobile carbon compounds already found in these trees under current ambient CO_2 concentrations (Hoch et al. 2003). Apical growth was studied by tagging and revisiting leading shoots in the top canopy, but yielded no consistent trends within or across species and years.

The recovery of CO_2 stimulation in *Fagus* during the 2003 drought was associated with measurable improvements in water relations (Leuzinger et al. 2005). *Fagus* shows no stomatal response to elevated CO_2 and may have profited from its responsive, moisture saving neighbor species (unpubl. data). The data suggest no lasting growth stimulations by CO_2-enrichment in these mature trees after four years. This conclusion awaits a series of further study years to become robust. Other forest FACE experiments with younger trees have either shown a continuously growth stimulation (*Pinus taeda*, Hamilton et al. 2002) or a transitory response, very similar to the one shown here for *Fagus* (*Liquidambar styraciflua*, Norby et al. 2004). A lack of a response in stem growth or leaf litter production as found here does, however, not preclude a faster rate of below-ground (root) production, as was shown for the Oak Ridge FACE (Matamala et al. 2003), but these are transitory C-pools, which, however, could translate into more recalcitrant forms of carbon in soil humus.

8 Atmospheric CO_2 enrichment of alpine treeline conifers

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***New Phytologist* 156: 363-375**

New Phytologist

Research

Atmospheric CO_2 enrichment of alpine treeline conifers

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Received: *25 April 2002*
Accepted: *31 July 2002*

Summary

- Experimental CO_2 enrichment of mature *Larix decidua* and *Pinus uncinata* trees and their understory vegetation was used to test the carbon limitation hypothesis of treeline formation at the alpine treeline in Switzerland.
- Forty plots (each 1.1 m^2) were established; half of them were exposed to elevated (566 ppm) atmospheric CO_2 using a free air CO_2 enrichment (FACE) system releasing pure CO_2, and the other half were treated as controls at current ambient [CO_2].
- Reliable and adequate CO_2 control was achieved, with 63% and 90% of 1-min averages having a [CO_2] within ±10% and ±20% of the target value, respectively, which is comparable to previous FACE systems. Both tree species showed higher net photosynthesis, lower stomatal conductance, and increased accumulation of nonstructural carbohydrates in response to CO_2 in the first year of treatment. Quite unexpectedly, shoot length increment increased significantly at elevated CO_2 (up to 23%) compared with controls in both species.
- The pure CO_2 release technology proved suitable for CO_2 enrichment of native trees on this remote mountain slope. Our results suggest an improved C balance and growth of treeline trees in response to elevated CO_2. However, it is unclear whether this initial growth stimulation will persist in the longer term.

Key words: carbon limitation hypothesis, elevated CO_2, free air CO_2 enrichment (FACE), gas exchange, *Larix decidua*, *Pinus uncinata*, shoot growth, treeline ecotone.

© *New Phytologist* (2002) **156**: 363–375

Introduction

The occurrence and causal explanations of the alpine treelines around the world have been discussed intensively for over a century (Brockmann-Jerosch, 1919; Griggs, 1946; Wardle, 1971; Tranquillini, 1979; Grace, 1989; Körner, 1998; Sveinbjörnsson, 2000). Among the many hypotheses related to stress, disturbance, reproduction, nutrient limitation, growth limitation, and carbon limitation, the latter two have provided the most plausible suites of explanations for causes of treeline formation from a global perspective (Körner, 1998).

The carbon limitation hypothesis states that low temperatures and short vegetation periods (in extratropical areas) increasingly prevent a positive carbon balance of the tree life form burdened with a large proportion of nonproductive tissues with increasing altitude or latitude. Climatic control of CO_2 exchange can be seen as the immediate cause of a reduced carbon balance and growth at treeline. However, different studies have illustrated that photosynthetic carbon uptake in treeline trees is relatively insensitive to temperature (Pisek & Winkler, 1958; Slatyer & Ferrar, 1977; Häsler, 1982; Goldstein *et al.*, 1994). Pisek & Winkler (1958) in addition found only very small altitudinal differences in photosynthetic capacity or respiratory carbon losses at the leaf level. In a wider sense, the carbon limitation hypothesis also accounts for tissue damages and losses that can considerably reduce net carbon acquisition in the long term and can significantly impair tree growth in the hazardous treeline ecotone (Sveinbjörnsson, 2000). In support of the carbon limitation hypothesis, higher needle mortality and slower rates of shoot elongation were observed in white spruce trees at treeline compared to trees growing at lower altitudes in Alaska (Sveinbjörnsson, 2000). A recent study across an altitudinal gradient within the treeline ecotone (2080–2230 m asl) of the Alps, showed large variability in total tree biomass (up to

10-fold differences among individuals), but no correlation with altitude in equal-aged trees of three conifer species (Bernoulli & Körner, 1999), rather questioning the hypothesis of a carbon shortage in treeline trees. The alternative growth limitation hypothesis (Körner, 1998), assuming a sink rather than a source limitation of tree growth at treeline, states that tissue formation is temperature limited. If low temperatures restricted carbon investments more than C-acquisition, then an increasing accumulation of nonstructural carbohydrates with increasing altitude might be expected as it was indicated for *Pinus cembra* in the Central Alps (Hoch *et al.*, 2002).

The continuing rise in atmospheric CO_2 concentration directly improves carbon balance and stimulates growth in many tree species (Ceulemans & Mousseau, 1994; Curtis & Wang, 1998; Norby *et al.*, 1999) irrespective of any possible indirect effects of greenhouse warming on plant performance. It has been argued that trees at high altitudes may be particularly sensitive to CO_2 enrichment, because they live in an atmosphere of lower partial pressure of CO_2 (LaMarche *et al.*, 1984). In line with this reasoning, gas exchange studies of herbaceous plant species (Körner & Diemer, 1987) and global comparisons of stable carbon isotope data (Körner *et al.*, 1991), both gave clear evidence for a greater efficiency of CO_2 uptake at any given CO_2 partial pressure in plants from high altitudes, suggesting potentially greater sensitivity to CO_2 enrichment as compared to plants from lower altitudes. Several dendrochronological studies reported increasing tree ring widths in upper montane conifers over the last 150 yr (La Marche *et al.*, 1984; Graybill & Idso, 1993; Nicolussi *et al.*, 1995; Rolland *et al.*, 1998; Paulsen *et al.*, 2000), and rising CO_2 concentration was discussed as a possible explanation. Nonetheless, it remains difficult to tease apart all the potentially influential and interacting factors, such as changes in forest management, nitrogen deposition and recently increased temperatures (Kienast & Luxmoore, 1988). For example, the data set of La Marche *et al.* (1984) was found to be confounded with rainfall history (Graumlich, 1991).

The *in situ* exposure of treeline trees to a higher atmospheric CO_2 concentration may be the most promising approach to test whether or not the growth of these trees is stimulated by elevated [CO_2]. Such an experiment would also provide a direct test of the carbon limitation hypothesis of treeline formation.

Using the technique of pure CO_2 enrichment (Miglietta *et al.*, 2001; Okada *et al.*, 2001) we designed and constructed a free-air CO_2 enrichment (FACE) system for the exposure of trees and their understory vegetation in the alpine treeline ecotone at Stillberg, Davos in Switzerland (2180 m asl). The main hypotheses to be tested in this ongoing project are tree growth at treeline is limited by carbon assimilates, and the CO_2 response differs among species with likely consequences for biodiversity and structure of treeline ecotones. Here we report on the performance of the custom-made CO_2 supply and regulation system and the responses of the studied tree species to elevated CO_2 after the first growing season.

Materials and Methods

Study site and plant material

The experimental area is located within the long term research site at Stillberg, Davos in the Central Alps maintained by the Swiss Federal Institute for Snow and Avalanche Research (SLF), Davos as part of the Swiss Federal Research Institute WSL, Birmensdorf. The NE-exposed Stillberg research site was established in the late 1950s with the aim to explore afforestation techniques and the interaction with avalanche dynamics in the treeline ecotone. A large experiment was started in 1975 when a total of 92 000 individuals of high elevation provenances of three treeline species (*Pinus uncinata* Ramond, *Pinus cembra* L., *Larix decidua* L.) were planted across an area of 5 ha spanning an altitudinal range of 2080 and 2230 m asl. The design of the plantation allows the recognition of each individual seedling. Microclimate, snow dynamics, vegetation composition, gas exchange, tree growth and survival has been measured extensively over the last three decades (Kuoch & Amiet, 1970; Schönenberger, 1975; Häsler, 1982; Turner *et al.*, 1982; Schönenberger & Frey, 1988; Senn & Schönenberger, 2001). These established trees of the same age (*P. uncinata*: 29-yr-old, *L. decidua*: 27-yr-old at the start of our experiment) provide a unique experimental setup for the study of CO_2 effects on treeline trees.

An area of approximately 2500 m^2 at 2180 m asl, that is, at the upper end of the long-term research site, was selected for our study. This particular area was chosen because it is at or maybe slightly above the actual natural treeline (uppermost native adult tree at 2180 m asl), it is characterized by a rather homogenous microrelief, slope (25–30°), exposure (NE) and understory species composition (see below), and power and CO_2 supply can be made available at a relatively close distance.

The long-term average annual precipitation at the study site is 1050 mm with a mean maximum snow depth of 1.46 m, and the average temperature is −5.8°C in January and 9.4°C in July (Schönenberger & Frey, 1988). The growing season starts approximately on 15 June with bud break of larch and ends 25 September with needle senescence of larch (i.e. *c.* 110 days). Temperature, wind speed, precipitation and snow depth during the experimental year are shown in Table 1. The soil is classified as a Ranker (U.S. system: Lithic Haplumbrept) with a 10-cm-deep organic top soil underlain by siliceous bedrock (Paragneis, Schönenberger & Frey, 1988).

Except for the trees planted in 1975, there are no other trees within the study area. Survival of the planted trees has varied considerably such that within the area, *L. decidua* became the most abundant tree species. There are fewer *P. uncinata*

Table 1 Temperature, wind speed, precipitation and snow depth during the experimental year 2001 (meteorological station of the Stillberg research site at 2090 m asl, A. Streule)

Climatic parameter	Entire year	April	May	June	July	Aug	Sept	Oct
T air mean (°C)	2.3	–1.8	6.9	6.7	10.5	11.8	3.1	7.4
T air min (°C)	–19.2	–13.0	–2.7	–5.0	0.7	1.9	–4.1	–0.3
T air max (°C)	21.7	14.6	17.2	19.9	21.5	21.7	13.2	18.4
T soil mean (°C)	2.9	0.2	1.8	5.9	9.0	9.6	4.1	3.6
T soil min (°C)	– 0.3	0.2	0.2	1.8	5.8	6.4	2.3	1.0
T soil max (°C)	12.3	0.2	9.7	10.6	11.7	12.3	7.5	6.1
Wind mean (m s^{-1})	1.4	1.8	1.8	1.6	1.7	1.5	1.3	1.1
Precipitation (mm)	1520	109	61	279	216	187	158	70
Snow depth (m)	1.69*	1.45	0.51	0.02	0	0	0.07	0

All values are based on hourly means. Air temperature was measured 2 m above soil surface (4 m during winter), soil temperature at 0.1 m below the ground, and wind speed at 10 m above the ground. Sums of precipitation and monthly means of snow depth are shown (* maximum snow depth as it was measured in 2001).

individuals and only three individuals of *P. cembra*. This tree species composition is characteristic for the entire Stillberg research site and is explained by considerably higher mortalities in *P. uncinata* and especially in *P. cembra* due to fungal attacks (Senn & Schönenberger, 2001). The trees are not taller than approx. 1.5 m and are widely spaced, forming an open canopy with dense understory vegetation composed of 33 different plant species. The understory is dominated by the dwarf shrubs *Vaccinium myrtillus*, *Vaccinium uliginosum*, and *Empetrum hermaphroditum*. *Vaccinium vitis-idaea*, *Loiseleuria procumbens*, and *Rhododendron ferrugineum* are also present, but are less abundant. *Gentiana punctata*, *Homogyne alpina*, and *Melampyrum pratense* are the most common herbaceous species.

Experimental design

A total of 40 trees, 20 of each of the two species *L. decidua* and *P. uncinata*, together with their understory vegetation were selected for the experiment in early June 2001. The trees had to meet the following criteria for selection: intact terminal leading shoot; no signs of serious herbivory and/or diseases; total height between 0.8 and 1.5 m; and no more than one close (> 80 cm in distance) neighboring tree.

Forty hexagonal plots covering a surface area of 1.1 m^2 each and with one tree in the center were established, beginning with snowmelt on 12 June 2001. The comparatively small, single-tree plot size was chosen because trees are typically widely spaced with limited interactions among individuals in the treeline ecotone. Furthermore, statistical and technical considerations both clearly favored single-tree plots. Due to the much lower abundance of pine trees than larch trees, single-tree plots enabled us to include sufficient and equal numbers of individuals of both species which would not have been possible with larger and consequently fewer plots. Given the steep and uneven terrain of the study site, it would have become also increasingly difficult to set up technical constructions and maintain a homogenous CO_2 concentration with larger plot sizes. A hexagonal stainless steel frame, held in a horizontal position by three wooden posts, was used to mount a ring of 24 vertical plastic tubes (15 cm apart from each other), individually cut according to plot-specific variations in microrelief, surrounding each plot (Fig. 1). The commercially available plastic tubes, designed originally for conventional drip irrigation systems (Drip Store Inc., Escondido, CA, USA), have an inner diameter of 4.3 mm and laser-drilled holes of uniform diameter (0.5 mm, one every 15 cm on one side). Through these holes, oriented to the center of the plot, CO_2 jets were injected. To keep the tubes straight and rigid, a stainless steel rod of 3 mm in diameter was inserted into each tube and the sealed end of the tube was inserted 2–4 cm into the soil. These relatively light constructions around each plot would make it easy to adjust plot height and width in accordance with tree growth, if needed during the course of the experiment.

Half of all plots were randomly assigned to an elevated [CO_2] atmosphere (growing season average of 566 ppm, that is, roughly twice the preindustrial concentration) and the other half served as control plots at a current ambient [CO_2] (*c.* 367 ppm), resulting in a replication of 10 plots per CO_2 treatment and per tree species. At the altitude of our research site, the treatment CO_2 concentrations correspond to a partial pressure of CO_2 of 45 Pa (elevated) and 29 Pa (ambient). Initial tests of CO_2 enrichment started on 16 June 2001 and CO_2 exposure of all 20 plots began on 26 June, just before budbreak of the trees, and continued until 20 September 2001, when larch needles started to turn yellow.

Carbon dioxide consumption during the first month of the experiment was considerably higher than initially calculated due primarily to strong winds at the experimental area. Although this did not markedly affect the performance of the CO_2 regulation system and the maintenance of the target

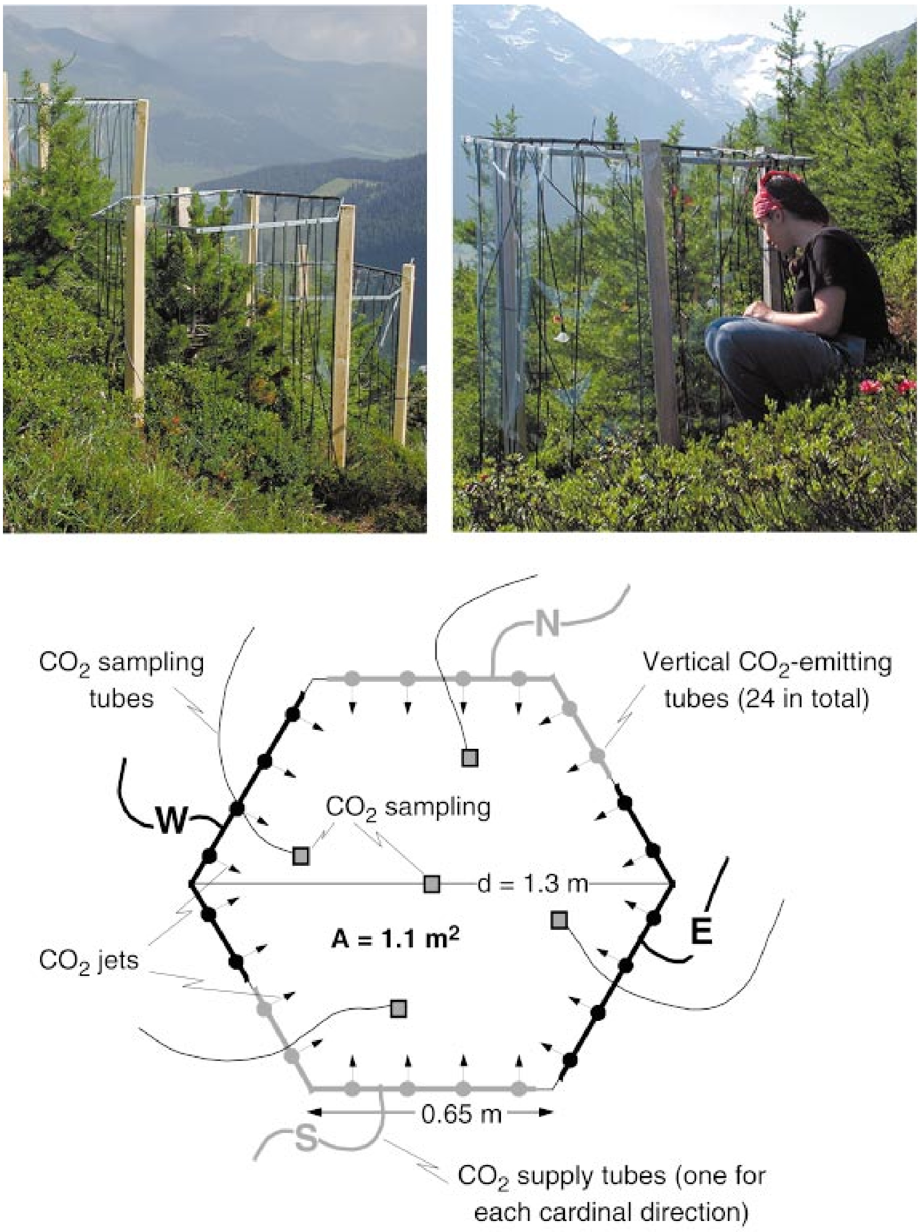

Fig. 1 Experimental plots at the research site Stillberg, Davos, Switzerland, 2180 m asl. The construction for support of CO$_2$ emitting tubes surrounding a tree in the center of each plot and its understory vegetation is shown in the two pictures on top. Tubes for CO$_2$ emission extend from the top (slightly above the tree top) all the way down to the soil surface to ensure complete CO$_2$ exposure of all the vegetation within the plot (control plots look the same). A schematic view from the top of an experimental plot is shown below. The hexagonal metal frame on top of the plot holding all tubing, arrangement of CO$_2$ supply and CO$_2$ emitting tubes along each cardinal direction, and the position of CO$_2$ sampling lines within the plot are shown.

[CO$_2$], budgetary considerations forced us to construct wind shields facing the main wind direction (N) in order to reduce costs. The 40 wind shields (treatment and control plots) were made of transparent 0.75 mm thick polyethylene film (Melinex 400, Hifi Industrial Film Ltd, Al Hoorn, NL, USA) that is neutral to light transmission in the visible spectrum as well as to UV. These screens were attached directly to the horizontal steel frame and the vertical CO$_2$ emitting tubes and covered one third of the total plot circumference from the top to *c.* 0.2 m above the soil surface. Thus, the understory vegetation was not screened and free access for pollinators and ground-living insects were guaranteed from all sides of the plots.

Pure CO$_2$ enrichment system

The relatively new technology of free-air CO$_2$ enrichment (FACE) using pure CO$_2$ injection was chosen as the

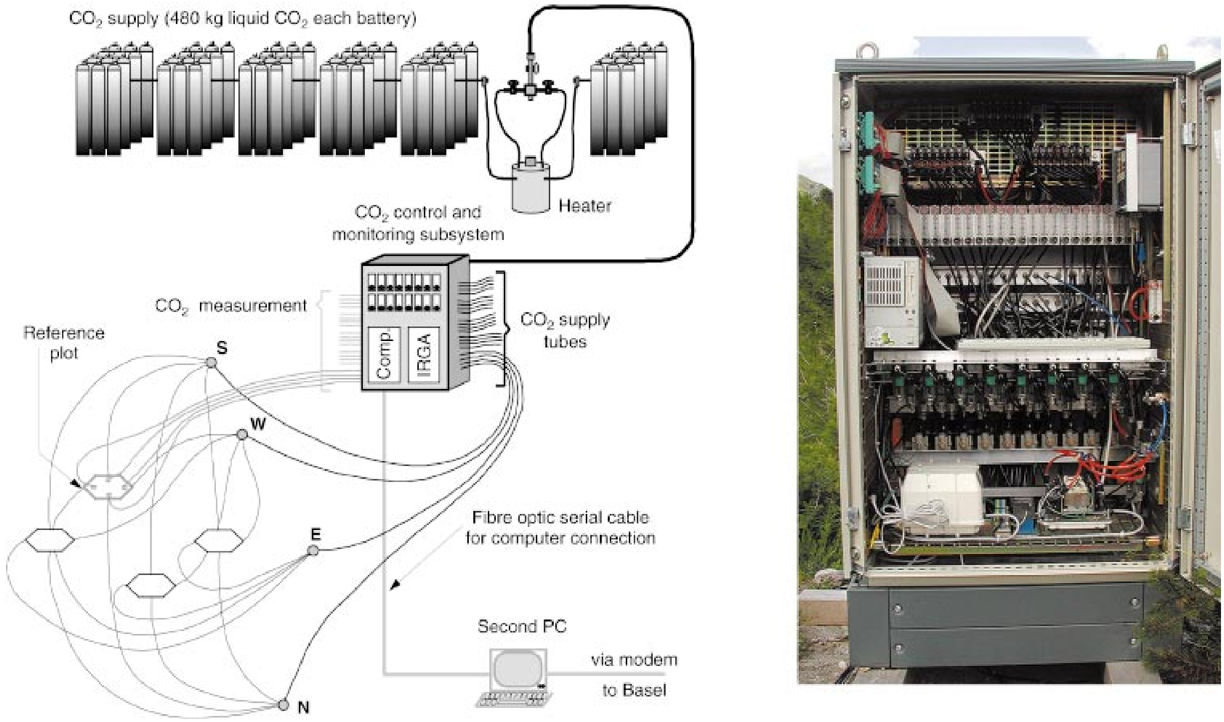

Fig. 2 Experimental setup of the supply, control, monitoring and field enrichment of CO_2. The control and monitoring system in the field contained the infrared gas analyzer (IRGA) for CO_2 measurement, the industrial control PC and the solenoid valves for CO_2 flow control (shown in detail on the right). The field-based control computer was connected to a second PC within the Stillberg research facility (Hüttiboden) via fibre optic cable connection. Four enriched plots are supplied with CO_2 according to actual CO_2 concentrations measured within a reference plot (a total of five reference plots for 20 enriched plots). Single bold letters indicate cardinal directions measured for and supplied with CO_2 individually.

technically and logistically most suitable method for CO_2 enrichment in the uneven terrain and remote location of this alpine treeline ecotone. The most apparent difference of the 'pure CO_2 release' technology compared to the traditional FACE technology is the omission of blowers and the provision of premixed CO_2-enriched air of a particular set point [CO_2]. Free-air CO_2 enrichment with pure CO_2 injection is successfully being used in at least three ongoing long-term and large-scale experiments with rice (Okada *et al.*, 2001), a poplar plantation (Miglietta *et al.*, 2001), and a mature deciduous forest (Pepin & Körner, 2002). These studies reported highly reliable CO_2 control and a similar performance of the pure CO_2 system compared to the traditional FACE system, with the additional advantage of comparatively low construction and maintenance costs. An effective mixing of CO_2 with the bulk air is achieved because CO_2 is released under high pressure (5 bar) producing a rapidly distributing CO_2 jet (Miglietta *et al.*, 2001) and because of the large number of small laser-drilled holes along the plot edges (in our case an average of 215 CO_2 emitting holes per 1.4 m^3 volume of air).

Six 'batteries' of 12 single bottles of totally 480 kg liquid CO_2 (i.e. 2880 kg CO_2 in sum, with a total freight weight of 9 tons) were stored on a helicopter platform adjacent to the research station at 2230 m asl. Because a hiking trail is the only access to the research site, the 'CO_2-batteries' had to be brought in by helicopter (Rotex AG, Liechtenstein) from the nearest road at 1600 m asl biweekly. Carbon dioxide is delivered to an electric heat-exchanger, vaporized and supplied to an array of two-way normally closed solenoid valves (EVT317, SMC Pneumatik, Engelsbach, Germany). These solenoid valves are part of the custom-made CO_2 control and measurement system in the center of the experimental area about 100 m in distance from the helicopter platform where the CO_2 batteries are stored (Fig. 2). Four CO_2 supply lines were used per plot allowing independent CO_2 injection rates for each cardinal direction. The injection valves were actuated separately by the control program run on an industrial PC placed in the field (Fig. 2) via 24-volt DC solenoids. A pulse-width modulation routine adjusted the duration of the pulse (max. 2 s) used to drive the injection solenoids as a function of measured [CO_2] in the tree canopy in each cardinal direction (CO_2 sampling approximately 0.25 m from the center of the plot and 50 cm above the ground, Fig. 1). The target [CO_2] was set at 550 ppm.

Control signals and data logging were implemented using a custom control program run on the industrial PC in the field. This CO_2 control system in the field was located in the center of the research area and contained the CO_2 release and monitoring systems, an infrared gas analyzer (IRGA, LI-800, Li-Cor Inc. Lincoln, NE, USA), two vacuum pumps, and digital boards driving the solenoid valves (Fig. 2). The field-based system was connected to a second computer located in the research station about 160 m from the research area by a fibre optic serial cable (Fig. 2). This second PC was used for data storage, viewing and processing, for modifying the control program and for communication with the Botanical Institute in Basel via modem.

Temporal and spatial variability of concentrations within experimental plots was determined using a customized 24-port sequential sampler in connection to the IRGA. Twenty sampling lines were used to monitor [CO_2] in each cardinal direction of five reference plots and to drive the injection solenoids. The reference plots were carefully selected to represent another three CO_2-enriched plots that were supplied with CO_2 by the same 1 cm I.D. polyurethane tubing (connected by manifolds to one common solenoid valve per cardinal direction, Fig. 2). The remaining four sampling lines were regularly moved within and among plots during the first three weeks of the experiment in order to fine-tune the system performance. Afterwards they were installed in the center of four plots to continuously measure [CO_2].

Air from CO_2-enriched plots was continuously drawn by a vacuum pump (YP-70VC, ASF Thomas, Wuppertal, Germany) through all the sampling lines (6 mm I.D. polyurethane tubing). Sampled air was then pumped sequentially from each port through a manifold of 24 three-way solenoid valves at a flow rate of 1 l min^{-1} (YP-40VC, ASF Thomas, Wuppertal, Germany) and routed through the gas analyzer. Each channel was monitored during 15 s, allowing sufficient time to purge the measurement system. After each measurement cycle an additional measurement of a calibration gas (391 ppm CO_2) was taken to verify the stability of the IRGA. A given sampling line was scanned approximately once every 6 min. Carbon dioxide readings from the IRGA were monitored at 1-s intervals, and only the last reading was recorded.

Plant sampling and data analysis

Length of the current-year leading shoot and of five mid-canopy lateral shoots was measured monthly in all CO_2-enriched and control trees of both species beginning in July and ending in late September 2001. Total length attained by the same shoots at the end of the previous year (2000) was additionally measured and treated as a covariable in the statistical tests of treatment effects on shoot length increment. Numbers of current-year needles per unit shoot length, projected area and needle dry mass (oven dried at 80°C) were determined in one fully mature, lateral shoot of each individual tree harvested on 24 August 2001 between 18:00 and 20:00 h local time.

The same needle material from the harvest described above was ground and used for chemical analyses. Nitrogen and carbon concentrations (% of dry mass) were determined with a CHN-analyzer (Model 900, LECO Instruments, St. Joseph, Michigan, USA). Nonstructural carbohydrates (NSC = starch, sucrose, glucose and fructose) were analyzed using an enzymatic starch digestion and a spectrophotometric glucose test after invertase and isomerase addition (Körner & Miglietta, 1994).

Gas exchange of intact current-year shoots (the same shoots that were harvested thereafter, see above) of all trees was measured during three consecutive days in late August (22nd to 24th) 2001 between 9.30 and 15.30 h. Gas exchange was measured at treatment CO_2 concentrations and saturating light levels (natural full sunlight > 1200 μmol photons m^{-2} s^{-1}) using the Li-Cor-6400 photosynthesis system (Li-Cor Inc., Lincoln, Nebraska, USA) and the conifer chamber (LI-6400–05). Total shoot length enclosed in the gas exchange chamber ranged between 35 and 60 mm in larch and between 20 and 40 mm in pine, with a total of 111–383 individual needles with a projected leaf area of 14.3–45.7 cm^2 in larch and 53–136 needles with a projected leaf area of 30.0–54.6 cm^2 in pine. For all measurements the internal Li-Cor-6400 CO_2 control was used to achieve treatment CO_2 concentrations. Conifer cuvette temperature (21.1 ± 0.4°C) and air humidity (77.3 ± 6.3% RH) were kept constant. Leaf temperature during measurements ranged between 20°C and 24°C.

The overall model for data analyses was a 2 × 2 model I analysis of variance to test for differences between species and CO_2 treatments with a replication of $n = 10$ plots. Multivariate repeated measures analysis of covariance was used to test for the effects of species, CO_2, and shoot length attained during the last year's growing season (covariable) on shoot length increment over time (three dates in 2001). To meet the requirement of normal distribution, percentage data (e.g. N concentration) were transformed with arcsine [square root (y)] before analyses.

Results

CO_2 control in the treeline FACE system

The FACE system using pure CO_2 injection was operational during daytime from 26 June to 20 September 2001, that is, 95% of the growing season. Records of CO_2 concentrations within high [CO_2] exposed plots throughout the growing season demonstrated that the system could adequately maintain CO_2 concentrations close to the target concentration of 550 ppm. We measured an average daytime CO_2 concentration of 566 ppm (partial pressure of 45 Pa) in the elevated CO_2 plots over the entire growing season compared to an ambient [CO_2] of 367 ppm (partial pressure of 29 Pa). Representative diurnal curves of instantaneously measured [CO_2] within the high [CO_2] exposed area of the five reference plots showed comparatively small fluctuations around the target concentration (Fig. 3). The diurnal averages of [CO_2] ranged from 550 to 575 ppm with relatively few individual measurements below 450 ppm or over 800 ppm

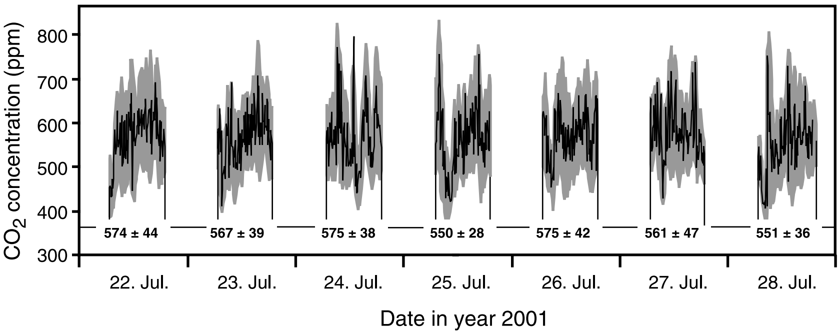

Fig. 3 Diurnal course of CO$_2$ concentrations (enriched for 12 h from 7 am to 7 pm) measured within five CO$_2$-enriched plots over a representative mid summer week in July 2001 (measurements are recorded every six minutes). The mean of five reference plots (black hairline) ± the standard deviation (grey area) are shown. Bold numbers at the bottom of the graph indicate mean daytime CO$_2$ concentrations (± SD).

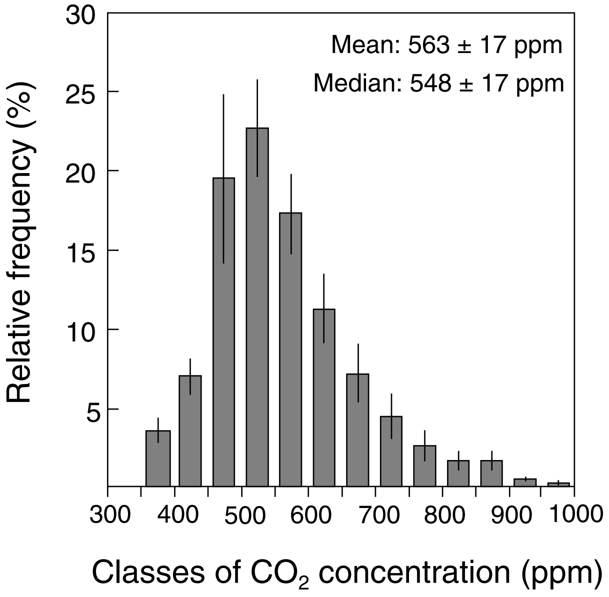

Fig. 4 Frequency distribution of daytime CO$_2$ concentrations (1 s readings) measured within the five reference plots during a representative week in July (22.–28.7. 2001). Bars represent mean values ± SE of 5 plots.

(Fig. 3). The frequency distribution of [CO$_2$] during that same period from 22 to 28 July 2001 showed more than 70% of all readings within the range of 450 and 650 ppm and a median [CO$_2$] of 548 ppm (Fig. 4). Carbon dioxide concentrations lower than 450 ppm were recorded in 10% of all 1-s IRGA readings, and less than 4.5% of all 1-s IRGA readings were above 800 ppm (Fig. 4). Short-term excursions of concentrations exceeding 1000 ppm were rarely measured (< 0.1%). Over the entire growing season, 11.1% of all readings were below 450 ppm, and 5.5% were higher than 800 ppm (mean across the five reference plots). Sixty-nine percent of these readings were within the range of 450 and 650 ppm. Based on these measurements, the CO$_2$ regulation system is estimated to control CO$_2$ levels within ±10% of the target concentration for 42% of the exposure time, and within ±20% for 74% of the total exposure time. This estimate is based on instantaneous 1-s readings of [CO$_2$] taken every 15 s (grab samples). From four such 1-s readings consecutively measured at each cardinal direction within a given plot, we calculated 'one-minute averages'. These 1-min averages of [CO$_2$] were for *c*. 63% of the total exposure time within ±10%, and for *c*. 90% of the total exposure time within ±20% of the target concentration.

Carbon dioxide sampling lines in the center of the plots served to further explore within and among plot variation in [CO$_2$]. The characterization of the variation among plots was particularly important in assessing the suitability of CO$_2$ regulation for four plots based on CO$_2$ measurements at the four peripheral positions within just one of these plots (reference plot). Representative diurnal curves of [CO$_2$] indicated that similar CO$_2$ atmospheres could be maintained among plots (Fig. 5). Although the time course of [CO$_2$] differed somewhat among plots, mean [CO$_2$] varied little and daytime minimum and maximum values were comparable (Fig. 5). Not surprisingly, there was some gradient of [CO$_2$] from the periphery

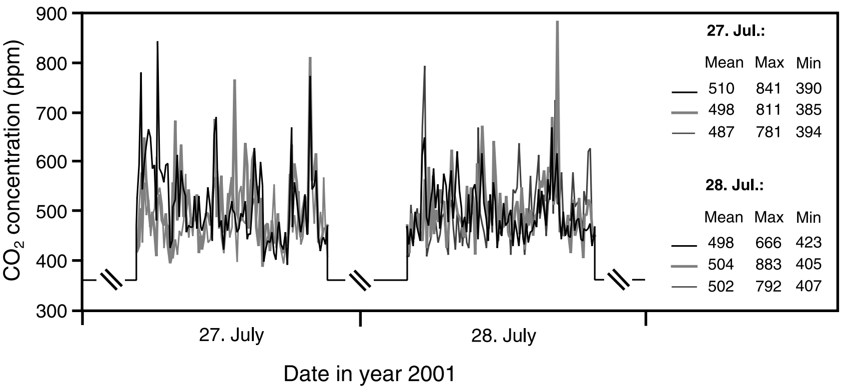

Fig. 5 Diurnal course of CO$_2$ concentrations measured in the center of three CO$_2$-enriched plots over two days in July 2001. The solid black line shows the CO$_2$ concentrations measured in the center of the reference plot used to control and regulate the CO$_2$ concentrations for the other plots. The grey and dashed lines represent CO$_2$ concentrations for two additional plots regulated according to measurements made in the reference plot. Numbers within the figure indicate the mean, maximum and minimum CO$_2$ concentrations for all three plots.

Table 2 Density, mass, gas exchange and quality of fully developed current-year needles in the two studied tree species grown at either ambient (Amb.) or elevated (Elev.) atmospheric CO_2 concentrations

	Larix decidua			*Pinus uncinata*		
	Amb. CO_2	Elev. CO_2	Δ percentage	Amb. CO_2	Elev. CO_2	Δ percentage
Needle density (# cm^{-1} shoot)	54 ± 4	50 ± 6	−7%	36 ± 3	35 ± 2	−3%
Needle mass (100 g of needles)	0.123 ± 0.009	0.133 ± 0.006	+8%	1.086 ± 0.091	1.235 ± 0.068	+14%
SLA (cm^{-2} g^{-1})	92.2 ± 2.9	89.3 ± 2.7	−3%	37.7 ± 1.7	33.9 ± 1.0	−10%
$A_{\text{max (area)}}$ (μmol m^{-2} s^{-1})	10.1 ± 0.3	14.7 ± 0.6	+46%	7.1 ± 0.2	11.1 ± 0.4	+56%
$A_{\text{max (mass)}}$ (μmol g^{-1} s^{-1})	0.094 ± 0.005	0.127 ± 0.006	+35%	0.027 ± 0.001	0.037 ± 0.001	+37%
g_s (mmol m^{-2} s^{-1})	215 ± 15	169 ± 14	−21%	139 ± 6	117 ± 9	−16%
Nitrogen (% of dry mass)	2.30 ± 0.07	2.11 ± 0.09	−8%	1.21 ± 0.03	1.16 ± 0.02	−4%
NSC (% of dry mass)	18.3 ± 0.5	21.4 ± 1.0	+17%	10.4 ± 0.8	14.3 ± 0.7	+38%
Sugar (% of dry mass)	12.1 ± 0.4	13.1 ± 0.4	+8%	5.1 ± 0.3	5.7 ± 0.1	+12%
Starch (% of dry mass)	6.2 ± 0.6	8.3 ± 0.9	+34%	5.3 ± 0.6	8.6 ± 0.6	+62%

Means ± SE of $n = 10$ trees at the end of August 2001 are shown along with the relative difference between ambient and elevated CO_2.

towards the center of the plots. Mean CO_2 concentrations in the center were roughly 50 ppm lower than those measured at the periphery used for CO_2 control and regulation. Increasing wind speed resulted in somewhat higher spatial and temporal variation in [CO_2] within plots, but had no detectable influence on the daytime mean [CO_2]. Maintaining the target CO_2 value during very windy conditions, however, markedly increased the overall CO_2 consumption which was the reason for the construction of transparent wind shields facing the main wind direction (N). The relative insensitivity of the CO_2 regulation system to changes in wind speed regardless whether the small sized wind shields were present or not, may be explained by the low stature of the experimental trees, and more importantly, by the comparatively small plot size.

To maintain [CO_2] close to our target of 550 ppm within a total of 20 CO_2 enriched plots of a total volume of $20 \times 1.1\ m^2 \times 1.3$ m (average height of CO_2 emitting tubes) which is 28. 6 m^3, we used an average of about 20 kg CO_2 per hour. This corresponds to a total CO_2 consumption of 18.5 tons per growing season (*c.* 105 days).

Responses of treeline conifers to elevated [CO_2]

Light-saturated net photosynthesis (A_{max}) of fully mature current-year shoots developed under treatment conditions was significantly higher in larch than in pine trees (Tables 2 and 3). Both species showed a significantly increased photosynthetic CO_2 uptake of current-year needles when grown in a CO_2 enriched atmosphere (Tables 2 and 3). In addition, we observed a highly significant species × CO_2 interaction on A_{max} expressed per unit of needle dry mass (Table 3). This interaction term was significant because larch had a much higher absolute CO_2 response (+0.033 μmol g^{-1} s^{-1}) compared to pine (+0.010 μmol g^{-1} s^{-1}), even though the two species showed a similar relative CO_2 stimulation of $A_{\text{max (mass)}}$. Similar to A_{max}, stomatal conductance (g_s) was also considerably higher in larch than in pine (Tables 2 and 3). Elevated [CO_2] reduced g_s in both species, the difference between CO_2 treatments being more pronounced in larch than in pine (Table 2).

Nitrogen concentrations determined in the same needles used for gas exchange measurements were almost twice as high in larch compared to pine (Table 2). Elevated [CO_2] had a significantly negative effect on N concentration (Table 3), but the effect was comparatively small. Concentrations of nonstructural carbohydrates (NSC) were 50% to 75% higher in needles of larch compared to pine (Table 2). Higher NSC in larch was exclusively due to higher sugar concentrations whereas concentrations of starch were essentially the same in the two species. Concentrations of NSC were significantly higher in needles grown in a CO_2 enriched atmosphere compared to controls, and this effect resulted mainly from starch accumulation with sugar concentration less affected. NSC in

Table 3 Analysis of variance of needle parameters to test for differences between species, CO_2 concentrations and their interactions

Source of variance	Needle parameter	*F*	*P*	Needle parameter	*F*	*P*
Species	Needle density	16.87	0.0002	g_s	31.80	< 0.0001
CO_2		0.425	0.519		8.696	0.0056
Sp × CO_2		0.102	0.752		1.189	0.283
Species	Needle mass	335.5	< 0.0001	Nitrogen	348.0	< 0.0001
CO_2		1.970	0.170		4.356	0.044
Sp × CO_2		1.499	0.229		1.002	0.324
Species	SLA	615.8	< 0.0001	NSC	92.56	< 0.0001
CO_2		2.797	0.104		21.00	< 0.0001
Sp × CO_2		0.137	0.714		1.123	0.296
Species	A_{max} (area)	68.31	< 0.0001	Sugar	605.3	< 0.0001
CO_2		111.3	< 0.0001		8.287	0.0067
Sp × CO_2		0.670	0.419		0.023	0.881
Species	A_{max} (mass)	385.3	< 0.0001	Starch	0.255	0.617
CO_2		30.31	< 0.0001		15.13	0.0004
Sp × CO_2		8.664	0.0059		1.135	0.294

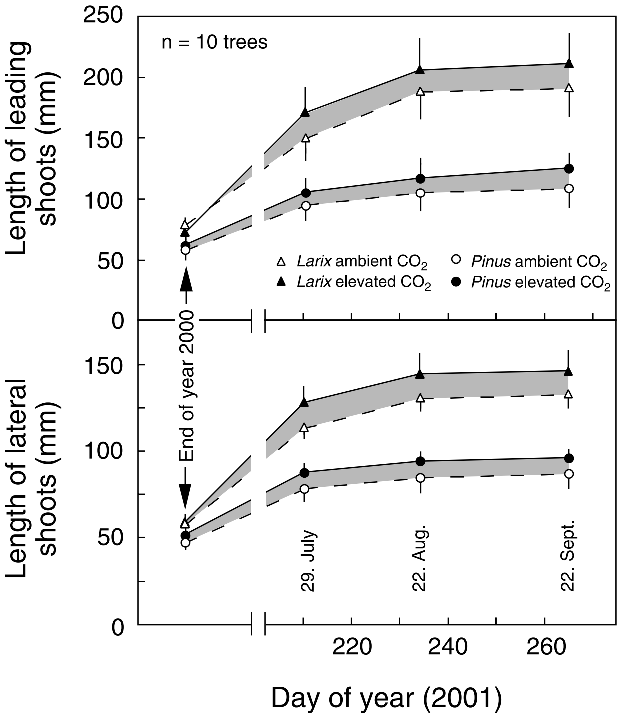

Fig. 6 Length increment of leading shoots (top) and lateral shoots (bottom) of *Larix decidua* (triangles) and *Pinus uncinata* (circles) grown at either current ambient CO_2 concentrations (open symbols) or at elevated CO_2 concentrations (closed symbols) during the first growing season of treatment conditions. Data points are mean ± SE of 10 measured trees (one leading shoot and five lateral shoots per tree were measured). Shaded areas highlight the differences between CO_2 treatments within each of the two species.

Table 4 Multivariate repeated measures analysis of covariance to test for effects of species and CO_2 on length growth in leading and lateral shoots during the 2001 growing season

	Leading shoots		Lateral shoots	
Source of variance	*F*	*P*	*F*	*P*
Shoot length in 2000	40.30	< 0.0001	48.71	< 0.0001
Species (Sp)	41.26	< 0.0001	49.02	< 0.0001
CO_2	5.799	0.022	4.649	0.038
Sp × CO_2	2.645	0.113	2.611	0.115
Day (d)	0.028	0.973	2.047	0.145
d × Sp	7.006	0.003	6.785	0.003
d × CO_2	0.379	0.687	0.404	0.671
d × Sp × CO_2	0.033	0.968	0.167	0.847

Shoot length of the previous year was included in the model as a covariable.

branches (pine only, including wood and bark) grown in elevated [CO_2] contained 10.1% NSC of their total dry mass compared to 8.8% in controls ($F_{1,18} = 3.228$, $P = 0.093$). Roughly two thirds of the overall NSC in branches were sugars that did not respond to elevated [CO_2]. By contrast, starch concentrations increased significantly from 2.9% at ambient [CO_2] to 3.7% at elevated [CO_2] ($F_{1,18} = 5.630$, $P < 0.05$).

Specific leaf area (SLA) and needle dry mass differed greatly between species (Table 2). The overall CO_2 effect on SLA and needle dry mass was not significant (Table 3). However, testing the CO_2 effect within species with separate one-way ANOVAs, pine showed a lower SLA ($F_{1,18} = 12.711$, $P < 0.01$) at elevated [CO_2] than at ambient [CO_2], but no difference in needle mass ($F_{1,18} = 1.560$, $P = 0.230$). Needle density per unit of shoot length was higher in larch than pine, but was not affected by CO_2 in either species (Table 2).

Current-year shoots were longer in larch than in pine (Fig. 6, Table 4) irrespective of CO_2 treatment. Length increment in leading and lateral shoots increased significantly in response to CO_2 enrichment in both species (Fig. 6, Table 4). At the end of the growing season 2001, current-year leading shoots were on average 25 mm longer (+23%) in larch and 11 mm longer in pine (+22%) when grown at elevated [CO_2]. The current-year increment of lateral shoots was on average 13 mm larger (+18%) in larch and 5 mm larger (+13%) in pine in a CO_2 enriched atmosphere compared to ambient [CO_2].

Discussion

Performance of the treeline FACE

The design and setup of an experimental system to expose native plants in the alpine treeline ecotone to an elevated atmospheric CO_2 concentration was challenging because of the difficult access (no roads creating CO_2 transport problems), and the steep and uneven terrain. These constraints limited the construction of large sized field installations and made the use of a traditional free-air CO_2 enrichment (FACE) system (Hendrey *et al.*, 1993, 1999) impractical. FACE rings typically have a diameter of > 20 m and use around 2 tons CO_2 per ring and per day. While considerably reducing CO_2 consumption, the use of tall open top chambers (OTCs) would have posed other problems, technical difficulties for field installation, large changes in microclimate to the frequent wind occurrence, and high radiation being the most obvious ones. Hence, we customized the pure CO_2-release technology (Miglietta *et al.*, 2001; Okada *et al.*, 2001; S. Roberts, pers. comm.) for the peculiar situation on a mountain slope. The CO_2 control system described here was able to maintain [CO_2] within ±20% of the target value for 90% of the exposure time (1-min means), similar to the > 91% and 90% in the pure CO_2 FACE systems described, respectively, by Miglietta *et al.* (2001) and Okada *et al.* (2001), and to the 92% in the 'traditionally designed' Duke forest FACE (Hendrey *et al.*, 1999). One minute average [CO_2] within ±10% of the target value were recorded for 63% of the exposure time in this study which is similar to the 60% reported for the rice FACE (Okada *et al.*, 2001), but somewhat lower than the 69% for the Duke forest FACE (Hendrey *et al.*, 1999) and the 75% for the POPFACE (Miglietta *et al.*, 2001). It should be emphasized that our 1-min averages actually derived from four 1-s measurements every 15 s, and therefore rather underestimate the proportion of exposure time within a certain limit of target compared to the estimates of other FACE systems based on averages of continuous readings.

Spatial distribution of [CO_2] was rather homogenous within the exposed volume of air. Continuous measurements at the periphery and in the center of the CO_2 enriched plots at mid-height of the tree canopies showed rarely differences exceeding *c.* 60 ppm CO_2. Extensive [CO_2] measurements during the initial phase of system setup and in a prototype system constructed in Basel before the actual experiment started, indicated decreasing [CO_2] from the bottom to the top of the plot (data not shown). This [CO_2] gradient was commonly less than 100 ppm with [CO_2] *c.* 50 ppm higher than the target at the bottom and *c.* 50 ppm lower than the target at the top of the plot, resulting in more than 80% of the total exposed volume being within ±10% of the target. Spatial variability of [CO_2] in our system was lower than in other pure CO_2 fumigation systems (Miglietta *et al.*, 2001; Okada *et al.*, 2001; Pepin & Körner, 2002). This is explained by the much smaller plot size chosen here and by the use of a vertical array of small diameter laser-drilled CO_2 emitting holes of a high density (an average of 215 holes per 1.4 m^3 volume of air). The four independently acting CO_2 control points within such a small volume of air, enabled us to achieve a highly fine-tuned CO_2 control and regulation. The spatially close arrangement of [CO_2] control points used in the CO_2 regulation algorithm was the reason why measurements of wind speed and direction were not needed for a reliable feedback algorithm, which may be the most evident difference to other FACE systems that need controlling for [CO_2] over an area of up to 650 times the size of our plots.

Short-term variability in [CO_2] and excursions of very high [CO_2] (> 1000 ppm) are likely to occur more frequently in any FACE using pure CO_2 injection than in FACE operated with premixed air using blowers (Pinter *et al.*, 2000). Such fluctuations in [CO_2] can affect physiological processes in plants (Cardon *et al.*, 1995). However, we recorded [CO_2] higher than 1000 ppm less than 0.1% of the time and [CO_2] deviating more than ±20% of the target value lasted seldomly longer than a few seconds. It takes at least one minute to induce changes in stomatal conductance in most plants and even longer in trees (Ellsworth *et al.*, 1995; Hendrey *et al.*, 1997; Saxe *et al.*, 1998). Therefore, it is unlikely that short-term variations in [CO_2] observed here had any significant effect on plant responses to elevated [CO_2].

Using pure CO_2 emitted through fine tubing with very small diameter laser-drilled holes under high pressure provided a useful alternative type of FACE system for the CO_2 enrichment of vegetation in the treeline ecotone. The fine tubing, originally designed for irrigation purposes and first used for atmospheric CO_2 enrichment by Steven Roberts from San Diego State University (pers. comm.) is flexible in its application and might be the only alternative for *in situ* CO_2 enrichment in some 'difficult' environments. It has even been modified for use in 35 m tall old-growth forest canopies (web-FACE, Pepin & Körner, 2002), until recently not believed to be possibly studied in CO_2 research.

Growth of alpine treeline conifers in a CO_2 enriched atmosphere

The physiological responses to elevated [CO_2] observed at the needle and branch level in the two tree species studied, are among the most consistent plant responses to atmospheric CO_2 enrichment (Ceulemans & Mousseau, 1994; Poorter *et al.*, 1997; Norby *et al.*, 1999; Körner, 2000). The mean enhancement in photosynthesis of 51% (on a needle area basis) in response to elevated [CO_2] measured in needles developed after the initiation of the CO_2 treatment, compares well with the mean stimulation of 66% calculated from a number of studies with trees growing in the field (Norby *et al.*, 1999), and even better if only conifers are considered (53%, Norby *et al.*, 1999). The CO_2 responses in stomatal conductance (g_s) reported in the literature are not consistent and range from no differences to comparatively large reductions (Curtis & Wang, 1998; Norby *et al.*, 1999). A frequently confirmed pattern, however, is that conifers show less and often not significant reductions in g_s than do deciduous tree species (Saxe *et al.*, 1998). In comparison to recent studies with different conifer species showing no or only moderate (up to −14%) CO_2 effects on g_s (Dixon *et al.*, 1995; Tissue *et al.*, 1997; Wang & Kellomäki, 1997; Ellsworth, 1999), we found slightly greater and significant responses to elevated [CO_2] in the present study. However, the CO_2 effect on g_s can change somewhat over the course of the season (Egli *et al.*, 1998) which was not assessed here. Moreover, larch as the more responsive of the two studied species functionally compares better with broadleaf deciduous species than with conifers.

The data compilation by Norby *et al.* (1999) showed an average decrease in leaf nitrogen concentration of 11% in conifers that is a little more than was found here. Lower leaf [N] in larch and pine trees observed here, was exclusively due to a dilution effect of higher nonstructural carbohydrate (NSC) concentrations under elevated CO_2. If expressed on either a leaf area basis or on NSC-free needle dry mass the CO_2 effect on needle [N] disappears (data not shown). These data suggest that CO_2 is unlikely to have had any effect on N allocation during that first year of exposure.

Both tree species showed immediate growth responses upon CO_2 enrichment in the first growing season. While such fast responses to increasing CO_2 are commonly found when starting with small seedlings, particularly when they are not limited by other resources (Ceulemans & Mousseau, 1994; Norby *et al.*, 1999), it was a rather unexpected result in our study with comparatively old trees, growing in the densely vegetated treeline ecotone. Trees competing for resources other than CO_2 and rooting in soils of naturally low fertility showed no or only moderate CO_2 induced growth stimulation in several previous studies (Norby *et al.*, 1992; Hättenschwiler & Körner, 1998; Spinnler *et al.*, 2002). Moreover, current-year shoot elongation in trees with a determinate shoot growth pattern, such as pine and larch, is believed to be largely determined by previous year's carbon balance and bud formation. For that reason, CO_2 effects on shoot growth – if any – were expected to occur in the second year of growth under treatment conditions at the earliest. The up to 23% increase in length growth of current-year shoots exposed to elevated CO_2, thus, is considered a particularly strong response to CO_2 enrichment in the trees studied here.

Higher rates of photosynthetic carbon assimilation, increased accumulation of nonstructural carbohydrates in leaves and branches, and increased shoot growth, all are strong evidence for a significantly improved carbon balance in larch and pine trees growing in a CO_2-enriched atmosphere at treeline. With regard to our first hypothesis (tree growth at treeline is carbon limited), these results suggest that tree growth at the upper alpine treeline might indeed be limited by carbon availability and that rising atmospheric [CO_2] can stimulate tree growth in the treeline ecotone. However, this is a preliminary conclusion based on first-year data and needs verifying in the coming years with an extended assessment of growth including measurements of stem diameter increment and root growth. A long-term perspective is generally important in field experiments due to the variability of climatic factors affecting the CO_2 response (Körner, 2000), and particularly in studies involving long-lived plants such as trees, because diminishing CO_2 responses over time are likely (Körner, 1995; Loehle, 1995; Hättenschwiler *et al.*, 1997; Oren *et al.*, 2001). The first-year data reported here form an important baseline for a multiyear evaluation of the responses of these trees to atmospheric CO_2 enrichment, as necessary for the testing of hypotheses about causes of treeline formation and possible consequences for treeline dynamics in response to global change.

There is little evidence in support of our second hypothesis (tree species differ in their response to CO_2) so far, because larch and pine trees were similarly affected by elevated CO_2. Nonetheless, the significantly greater CO_2 effect on photosynthesis per unit of needle dry mass in larch compared to pine may be indicative of future stronger responses of this deciduous conifer in the long run. Needless to say that this remains speculative because CO_2 effects on rates of photosynthesis and growth rarely correlate (Curtis *et al.*, 1996), and sustained CO_2 stimulation of leaf-level photosynthesis at times may have no detectable influence on growth in the same plants (Hättenschwiler & Körner, 1996; Egli *et al.*, 1998). On the other hand, minute not yet identified differences in the CO_2 responses between species might gain in importance over time, particularly if biotic interactions such as those with understory species, herbivores or mycorrhizae come into play.

Acknowledgements

We thank Steven Roberts for discussions and ideas for setting up the pure CO_2 fumigation system, Steeve Pepin for help in constructing a prototype, Franz Leuenberger and his team for their logistical support, Lukas Zimmermann for software development, Thomas Zumbrunn for the characterization of the understory vegetation and the photographs in Figs 1 and 2, Olivier Bignucolo for chemical analyses, Alain Studer for technical support, and Arnold Streule for providing meteorological data. This research was funded through the Swiss National Science Foundation grant 31–061428.00 to

S.H. and a doctoral scholarship (Fonds québecois de la recherche sur la nature et les technologies) to I.T.H.

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9 Other publications during my PhD (abstracts)

A. Orthopteren Nachweise (Dermaptera, Saltatoria, Blattoptera) und Mobilität im Kronendach eines mitteleuropäischen Mischwaldes - Beobachtungen von einem Kran

ASSHOFF R, AMSTUTZ E (2004) *Articulata* 19: 205-215.

Abstract The Orthopteran fauna of a representative Central European deciduous forest canopy is described using the Swiss Canopy Crane (SCC) at Hofstetten (canton Solothurn, Switzerland). A total of seven species (*Chelidurella guentheri, Ectobius sylvestris, Barbitistes serricauda, Leptophyes punctatissima, Meconema meridionale, Meconema thalassinum, Tetrix subulata*), nymphs as well as adults, were found on different tree species. Most Orthopteran species were observed on *Carpinus betulus*, *Quercus* sp. and *Tilia platyphyllos*, with significantly less abundance on *Fagus sylvatica*. The most abundant Orthoptera found was *M. thalassinum*, followed by *M. meridionale, C. guentheri,* and *B. serricauda*. We did not find a significant correlation between tree height and grasshopper abundance, suggesting that bush-crickets are not bound to a certain canopy area. A different distribution in the canopy of males and females of *Meconema* sp. during the vegetation period suggests that there is a sex-specific mobility. Stable isotope ($\delta^{13}C$) studies revealed that there is a highly significant correlation between *Meconema* L1/2-larvae with the leaf tissue on which the buch-cricket was found, suggesting that young larvae of *Meconema* and their prey are not very mobil. This correlation vanished when comparing leaf tissue with adult *Meconema* individuals. Hence, we conclude, that *Meconema* imagos have a certain horizontal mobility in a forest canopy while larvae stay on the trees where they hatched.

B. Zur Biologie der Alpinen Gebirgsschrecke *Miramella alpina* (Fruhstorfer, 1921) (Acrididae: Catantopidae).

ASSHOFF R, KÖHLER G (2003) *Jahresbericht der naturforschenden Gesellschaft Graubünden* 112: 5-20.

Abstract To the biology of the alpine grasshopper *Miramella alpina* (Kollar, 1833) (Acrididae: Catantopinae). - A population of *Miramella alpina* from Stillberg (2200 m above sea level) near Davos/ Graubünden was observed over two generations in the field and reared under greenhouse conditions. Some hitherto poorly known aspects of juvenile and adult development, reproduction, parasites, food-plants, and embryogenic development have been studied. After hatching in mid June, the juveniles develop over 40-50 days and reach the adult stage towards the end of July. The obligate five juvenile instars are well separated by the length of the postfemora. The adults can survive in the field over 2-3 months. Copulations could be observed from about one week after final molt throughout the whole season, with a maximum in mid August. A female produces about 3 egg-pods with a total of 30 eggs during one season in a subalpine habitat. Nymphs and adults were found to be infested by a fungus (Entomophaga), a nematode (*Mermis* sp.), and a mite of the genus *Eutrombidium*. About 40 food-plants of *M. alpina* are known, with a wide spectrum of secondary plant substances. Within the genus *Vaccinium* leaves of *V. uliginosum* were preferred to *V. myrtillus* leaves in early nymphal instars, but this preference diminishes in later instars. The post-diapause development of eggs was broadly scattered, with few eggs hatched.

C. *Tachycines asynamorus* Adelung, 1902, *Periplaneta australasiae* (Fabricius, 1775) und *Pycnoscelus surinamensis* (Linné, 1758) (Ensifera et Blattodea) im Botanischen Garten Basel.

ASSHOFF R, CORAY A (2003) *Mitteilungen der Entomologischen Gesellschaft Basel* 53: 42-55.

Abstract The occurrences of the Greenhouse camel-cricket (*Tachycines asynamorus*) and two cockroaches, the Australian cockroach (*Periplaneta australasiae*) and the Surinam cockroach (*Pycnoscelus surinamensis*) are reported from the Botanical Garden of Basel. These species, generally known as pests, are described in terms of their distribution and ecology. The record of *Pycnoscelus surinamensis* is the first one published for Switzerland.

D. Anfänge einer wissenschaftlichen Forstentomologie und ihrer Popularisierung behandelt an einem historischen Beispiel von 1797/98 - Die Nonne (*Lymantria monacha* L.)

ASSHOFF R (2003) *Centralblatt für das gesamte Forstwesen* 120: 101-116.

Abstract The beginning of forest entomology as a science is described based on a historical example from the late 18th century (1797/1798). In this discourse, in which several authors of this time period get a word (especially GEORG GOTTFRIED ZINKE), it becomes obvious that the focus in this era was primarily the protection and conservation of forests, substantially thought of as a source of raw material. In this case the nun moth (*Lymantria monacha* L.) was the appearing pest in areas of the southern part of central Germany. To protect the forests adequately, information was required about the biology of insect pests, which was rare at that time. With tremendous effort, the authors compiled this knowledge and a first discussion arose, admittedly without a consistent terminology. The state of the science at the time regarding the nun moth (e.g. caterpillar - and mothphenology, feeding plants) and how this was gained is discussed in this article.

10 Summary and conclusions

10.1 Mature trees in a CO_2 enriched world

Mature (app. 100 years old) temperate forest trees were exposed to an increased atmospheric CO_2 concentration for four growing seasons at the Swiss Canopy Crane site (SCC) near Basel (Switzerland) using web-FACE (Free air CO_2 enrichment) technology. For 11 trees (5 different species) in elevated CO_2 and 26 control trees growing at ambient CO_2 I documented seasonal phenology, measured basal area (BA) increment, main and lateral shoot growth, leaf area/shoot length ratio and calculated a branching index. At the end of 2001, one year after the onset of the experiment, I found a significant BA growth stimulation in *Fagus* growing in elevated CO_2. *Fagus* exhibited a second significant BA stimulation in 2003, when Europe experienced a centennial drought (no response in 2002 and 2004). None of the other species showed a stem growth response to CO_2 in any of the 4 years. Also when tested across all species, BA increment did not show a significant response to elevated CO_2 (neither when tested per year nor cumulative for four years). The inclusion of *Prunus* and *Tilia* did not change the picture. *Fagus* showed a significant higher lateral branching in elevated CO_2 in 2002 when shoots developed from buds that were formed during the first season of CO_2 enrichment (2001). The effect disappeared in 2003. In *Quercus*, there was a steady increase in leading shoot length in high CO_2 trees resulting in longer leading shoots after 4 years of CO_2 enrichement. Phenology (bud break, leaf fall, leaf duration) was highly species-specific and did not reveal a consistent effect of elevated CO_2. Our four-year data set reflects a highly dynamic and species-specific response of tree growth to a step change in CO_2 supply. In spite of some transient growth stimulation our results do not support the notion that mature forest trees will grow better in a CO_2 enriched world and thus act as carbon sinks.

10.2 Plant-insect interaction in a CO_2 enriched world

I studied two insect species feeding on in situ CO_2 enriched plants at treeline. *Miramella alpina* fed on two *Vaccinium* species and *Zeiraphera diniana* on *Larix decidua* shoots. In both plant species elevated CO_2 reduced nutritional quality, which affected the performance of the insects feeding on them. Relative growth rates of *Miramella* were lower in animals feeding on *V. myrtillus* compared to those feeding on *V. uliginosum* and grasshoppers growth was affected by elevated CO_2 depending on plant species and nymphal development stage. Relative growth rate of *Miramella* correlated with CO_2 induced changes in leaf water, nitrogen, and starch concentrations, depending on grasshoppers' instar. Elevated CO_2 resulted in reduced female adult weight; irrespective of plant species elevated CO_2 prolonged development time in animals feeding on *V. uliginosum* only, but did not cause a significant difference in nymphal mortality. Newly molted adults of *Miramella* produced lighter eggs and less secretion, (serving as egg protection) under elevated CO_2. When grasshoppers had a choice among four different plant species grown either under ambient or elevated CO_2, the consumption of *V. myrtillus* and *V. uliginosum* leaves increased under elevated CO_2 in females while it decreased in males compared to ambient CO_2-grown leaves. These findings suggest that rising atmospheric CO_2 distinctly affects leaf chemistry in two important dwarf shrub species at the alpine treeline, leading to changes in feeding behaviour, growth, and reproduction of the most important insect herbivore in this system.

The study of *Zeiraphera* (larch bud moth) revealed that larvae grew slower under elevated CO_2 compared to ambient CO_2 when trees had remained undefoliated in the previous year. If, however, trees had been defoliated, this response was reversed, with a faster growth of larch bud moth on high CO_2-exposed trees than on control trees. Pupal weight was not affected by either CO_2 or defoliation. Thus elevated CO_2 and defoliation had only minor effects of larch bud moth performance. Needle maturation over the course of the season incurres significant compositional changes. N concentration was on average 38% lower and lignin concentration 55% higher in early July than in mid June 2003. In conclusion, my results suggest that elevated CO_2 and defoliation induced changes in larch needle quality have only little impact on larch bud moth performance at the alpine treeline, and, in particular, indicate no reinforced negative effect of these two factors. However, the pronounced changes of needle quality during needle maturation might suggest that any shifts in tree phenology due to global change may be of greater importance for alpine larch bud moth populations than the direct impact of CO_2 on needle quality.

An increasing number of studies suggest that switching of host plants due to rising atmospheric CO_2 concentrations is probable (Goverde and Erhardt 2003, Hättenschwiller and Schafellner 2004, Agrell et al. 2005). However all the experimental work on effects of elevated CO_2 on insect herbivores compares insects in ambient CO_2 conditions and insects feeding on plants, which have been abruptly subjected to the chosen elevated CO_2 levels.

One fundamental point, which enables insects to switch host plants, is the degree of insect mobility. A priori it would be presumed that nymphs are less mobile than adults, but this was never experimentally tested. I showed that for example caterpillar or grasshopper nymphs do not switch between tree species or even trees during their larval stages. The poorer food quality becomes with rising CO_2 concentrations the higher might be the need to switch feeding plants in the future. However, the change in leaf chemistry as well as related changes in insect behaviour (e.g. switching host plants for feeding or egg deposition) will occur gradually. It is not known, whether adaptions of insect-plant relations will occur over the next decades (Whittaker 1999, 2003).

10.3 Conclusion

This work revealed insight into a number of biotic influences of elevated CO_2 under most realistic experimental conditions. Across all tests it became clear that atmospheric CO_2-enrichment exerts rather subtle influences all related to biodiversity.

The major conclusion of my growth analysis in a mature deciduous forest is that after four years these tall trees do not respond to elevated CO_2 with more stem growth. If there is a sustained response it would be small and much longer observation periods would be needed to identify such a response, and it is almost certain that such responses will be species specific as they were during the starting phase of this experiment. From what I see at the Swiss forest FACE site, a first approximation would be that there is no gain in carbon stocking in a CO_2 rich future in such trees. A smaller CO_2 responsiveness of larger, more complex test systems was already obvious as CO_2 research switched from seedlings (pots) to saplings (open top chambers), a trend now finding strong support by data from the tallest and oldest forest studied so far under free air CO_2 enrichment. My plant-animal interaction studies with plant material grown in situ under elevated CO_2 are in line with other studies, but do not permit a simple generalizing conclusion. I showed that food plant species matter and even the sex of the feeding animals played a role. Performance of both insect species is likely to become impaired by rising CO_2 concentration. However, the overall picture is highly complex.

Scientist are now beginning to study the effects of elevated CO_2 on plants and insects in "natural" (e.g. FACE experiments) systems although they have to deal with a lot more variability compared to earlier works in controlled conditions, which makes the analysis more difficult. So far, too little attention was put on species specifity although evidence is emerging that generalisation of effects found in plants, as well as in insects is not possible because they show distinct species specific reactions to elevated CO_2, as I have shown in my thesis.

11 Literature

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12 Curriculum vitae

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Schulische Ausbildung

1982 - 1984	Grundschule Kenn bei Trier
1984 - 1993	Besuch des Auguste-Viktoria-Gymnasiums in Trier; Abschluss mit dem Abitur am 18.06. 1993

Studium

1994 - 2001	Studium der Biologie, Philosophie und Erziehungswissenschaft (Lehramt Gymnasium) an der Friedrich-Schiller Universität Jena, der Universität Leipzig und der Universität Basel.
04/ 98 – 05/ 99	Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft (WSL), Birmensdorf (Sektion: Jahrringe und Standort, Entomologie)
03/01	Abschluss mit dem 1. Staatsexamen in Biologie und Philosophie für das Lehramt an Gymnasien
07/01 – 07/05	Doktorand am Botanischen Institut der Universität Basel
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Praktische Tätigkeiten

08/93 – 11/ 94	Zivildienst bei der Gesellschaft für Naturschutz und Ornithologie e.V. (GNOR) - Arbeitskreis Pfalz mit Sitz in Neustadt/Weinstraße
08/96 – 09/1996	Heuschreckenkartierung auf Werksvertragsbasis für die “Thüringer Landesanstalt für Umweltschutz” (TLU) mit Sitz in Jena
07/97, 08/98	Heuschrecken- und Schneckenkartierung auf Werksvertragsbasis für das Planungsbüro “Landschaftspflege- und Naturschutzservice” (LaNaServ), Rottdorf
07/00, 08/00	Arbeiten zu Reproduktionsparametern der Goldschrecke (*Euthystira brachyptera*) auf Werksvertragsbasis bei Dr. K. Reinhardt (Jena)
07/01, 02, 03, 04, 05	Lehrassistent des Pflanzenökologischen Praktikums des Botanischen Instituts der Uni Basel in Engelberg

Exkursionen

08/96	Teilnahme an der 11th International Dendroecological Field Week in Selma (Schweiz)
08/97	Teilnahme an der 12th International Dendroecological Field Week, Seminskyi Pereval, Altai Republik, (Russland)

Sonstige Aktivitäten

1995-2004	diverse Exkursionsleitungen (Faunistik), Klassenlagerbetreuung, Arbeiten auf Werksvertragsbasis

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