Tree and ecosystem responses to four years of in situ CO_2 enrichment at the Swiss treeline

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"Der Zauberberg"

-Thomas Mann

(when titling his book about a guy who journeyed to Davos for what was to be a three week visit but ended up being a seven-year stay full of self-realizations and, needless to say, "stories")

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1 General introduction

The treeline, that is "the abrupt transition in life-form dominance beyond which plants with massive single stems and tall crowns either can not be developed, become unaffordable or are disadvantageous" (Körner 2003a), has long been a subject of fascination to ecologists. The alpine treeline itself represents one of the best studied distributional boundaries as documented in classic monographs by Brockmann-Jerosch (1919), Däniker (1923) and Tranquilini (1979). However, as argued by Körner (1998) in his review (that captured my interest as I was in the end phases of writing my Master's thesis and brought me several years later to the University of Basel to pursue this doctoral degree), despite the avid and clear correlation of treeline position and mean growing season temperature (5.5 to 7.5 °C), a convincing mechanistic explanation is lacking that accounts for this phenomenon and withstands testing across the non-arid mountain regions of the world. Few attempts have focused on a general, unifying hypothesis that could operate on all mountains, rather, most have focused on modulating reasons of more regional significance (Körner 1998).

Two explanations that have received particular attention and have become the focus of several research projects at the Institute of Botany of the University of Basel in the last decade are the "carbon limitation" versus "growth limitation" hypotheses. The former suggests a source limitation, that due to low daytime temperatures at the treeline, trees are incapable of fixing sufficient carbon to meet the needs of their 'expensive' growth form. The latter suggests a sink limitation, that trees have sufficient carbon, but due to thermal limitation of meristematic activity with direct consequences for structural growth, they are not able to invest it. Several approaches have been used to address these hypotheses: (1) there has been an extensive assessment of the actual rooting zone growth temperatures in treeline forests worldwide between 1996 and 2003 at 46 sites (Körner & Paulsen 2004), (2) based on the understanding that trees accumulate non-structural carbon reserves when they are sink rather than source limited, a comparative study of non-structural carbon charging of treeline trees across a latitudinal gradient from Mexico, Switzerland to northern Sweden has been carried out (Hoch et al. 2002, Hoch & Körner 2003) and now within the context of this study, (3) an *in situ* CO_2 enrichment site of treeline trees with the intention to stimulate sources rather than sinks and directly test carbon limitation has been running since 2001 (Hättenschwiler et al. 2002).

Beyond the pure ecological curiosity that prompted the earlier documented research on treelines in the 20th century, the question of carbon limitation in treeline trees is of high relevance to ecologists in the 21st century, where global climate and atmospheric change and its direct and indirect effects on ecosystems has become a hot topic (Walker et al. 1999, Körner 2003b). The question of whether trees, that store close to 90% of carbon fixed as plant biomass, are sinks or sources at a global scale has received much attention in the entire controversy surrounding carbon sequestration (Norby et al. 1999, Körner et al. 2005, Asshoff et al. 2006) and in the search for a general ecological understanding of forest ecosystem function (Körner 2003c). Treeline growth records are of exceptional value as annual ring width is highly sensitive to temperature, correlating well with the instrumental climate record (Gindl et al. 2000, Paulsen et al. 2000, Frank & Esper 2002), and thus is highly useful to dendroclimatologists, for example, for the reconstruction of past climate patterns (Schweingruber 1996, Briffa et al. 2002). While recent increases in tree ring width of upper montane and treeline conifers have been suggested to reflect a warming trend (Nicolussi et al. 1995, Rolland et al. 1998, Paulsen et al. 2000, Büntgen et al. 2005), it has also been hypothesized that rising atmospheric CO_2 concentrations might be contributing to growth stimulation particularly at high altitudes (LaMarche 1984), although multiple other factors may create confounding interactions (Graumlich 1991, Nicolussi et al. 1995, Grace et al. 2002). Thus our experimental manipulation of CO_2 at the treeline has equally important implications for the key assumptions in dendroclimatological reconstructions.

1.1 Treeline trees in a CO_2 enriched world

The ever-increasing rise in atmospheric CO_2 concentration due to fossil fuel burning and land use change has resulted in current concentrations that are higher than ever in the last 25 million years and exceed more than double that of the glacial minimum concentrations of the last 650 000 years (Petit et al. 1999, Pearson & Palmer 2000, Siegenthaler et al. 2005). Since the basic resource for plants is CO_2, the nearly 30% increase of carbon availability within only 150 years is one of the most dramatic and continuing aspects of global change (Körner 2000, 2003b), much effort in recent decades has gone towards understanding how plants will respond to changes in atmospheric CO_2 (see reviews by Curtis & Wang 1998, Saxe et al. 1998, Ceulemans et al. 1999, Norby et al. 1999, Nowak et al. 2004, Ainsworth & Long 2005). Early studies consisted of glasshouse experiments, which then progressed to open top chamber field experiments and as methods developed further, the application of free air CO_2 enrichment (FACE) technology in giant rings (Hendrey et al. 1999), later with pure CO_2 injections systems (Miglietta et al. 2001, Okada et al. 2001), and eventually within the natural canopy matrix itself (web-FACE; Pepin & Körner 2002). The success of these various techniques gave the necessary confidence to establish the first-ever FACE site at the upper altitudinal treeline, despite the obvious hardships and challenges associated with gas delivery and distribution in the remote and harsh treeline ecotone at 2180 m asl (details in Hättenschwiler et al. 2002). Our site provided a method for a direct *in situ* test of carbon limitation of treeline trees, while equally addressing potential direct and indirect effects of CO_2 fertilization on the treeline ecosystem.

We established the Swiss treeline FACE site in June 2001, on Stillberg (Davos, Switzerland), coinciding with the beginning of my doctoral studies, in the sparsely forested upper end of a long-term afforestation experimental site of the Swiss Federal Institute for Snow and Avalanche Research. Trees of high elevation provenances of three treeline species, *Larix decidua* L., *Pinus cembra* L., and *Pinus uncinata* Ramond. were planted in 1975 (Schönenberger & Frey 1988). In 2001, the dominant survivor on the mountain was *L. decidua*, followed by *P. uncinata* and very few individuals of *P. cembra*; both latter pine species were subject to high mortality due to fungal attacks (Senn & Schönenberger 2001). We thus chose *L. decidua* and *P. uncinata* as our study species and constructed a FACE system in order to provided ca. 550 μmol mol^{-1} CO_2 during the photosynthetically active daylight hours for the snow-free growing seasons to half of the forty designated study trees and their dense understory vegetation.

Although the primary interest was to address the carbon limitation hypothesis as described above, the carbon balance of treeline trees can also be heavily impacted by biotic factors such as herbivory. Intense defoliation can occur as a response to periodic outbreaks of insects such as the larch bud moth, *Zeiraphera diniana* (Baltensweiler et al.

1977, Weber 1997), or the spruce bud worm, *Choristoneura* species (Morin et al. 1993, Weber and Schweingruber 1995) and as part of the general study, we were interested in seeing whether there would be a potential interaction of CO_2 enrichment and defoliation on the trees. Half of the trees were thus subject to a one-time 80% defoliation treatment simulating such an insect outbreak (as described in detail by Handa et al. 2005 in chapter 2).

1.2 This doctoral thesis

Within the framework of my dissertation, I have attempted to address the question of carbon balance of trees growing at their altitudinal limit by studying their response to CO_2 enrichment and defoliation at a variety of scales ranging from the leaf level, to the branch and stem level and finally, to the roots and below-ground components of the ecosystem.

Chapter 2 begins at the leaf level and examines the shoot increment growth responses to CO_2 enrichment (carbon source enhancement) and defoliation (carbon source removal) in light of a full set of needle physiological observations and the contrasting evergreen and deciduous life strategies of both conifers. Specifically, over the first three years of the experiment, it addresses the questions (1) are treeline trees carbon limited, (2) can biotic factors such as intense herbivory modify the observed growth response to elevated CO_2 and (3) are responses to both treatments and their interactions species specific?

Chapter 3 presents the tree ring increment record of all forty trees over four years and contrasts it to the shoot increment growth record in order to understand if (1) tree growth as evident in the stem record is stimulated in a future CO_2 enriched atmosphere, (2) how stem increment record in the presence and absence of elevated CO_2 might be modified by an extreme defoliation event and (3) if at the wood anatomy level, tracheid characteristics are influenced by elevated CO_2 according to, or independently of, any changes in stem diameter increment?

Chapter 4 expands the study, which in the previous chapters focused exclusively on above-ground responses, to the below-ground world and looks at the root dynamics of the trees and their understory plants in the ecosystem. Here, I ask (1) is there an increase in below-ground carbon allocation as evident in new root production, standing crop estimates and root litter decomposition in response to elevated CO_2, (2) how much and how rapidly is new C being incorporated into the root system and (3) is there an effect of elevated CO_2 on root quality as measured through starch and N content with potential implications for root longevity and root activity? As put bluntly by Pregitzer (2002), "the fine roots of perennial plants are a royal pain to study". Indeed, our experiment offered many hours of contemplative meditation while sorting roots and thereafter, and while I recognize multiple methodological challenges, I remain convinced that this dataset will be of substantial importance to the global change research community as there is a paucity of studies on root responses to elevated CO_2, particularly for mature systems in the field studied over multiple years.

Chapters 5, 6, 7 and 8 present contributions from the collective Swiss treeline FACE project in which I am co-author, either because data was collected by me as part of the broad scope of my dissertation or because of the central role that I played in project coordination and general data collection, particularly in the fourth and fifth field season. Chapter 5 consists primarily of a methodological paper describing our FACE site but presents also the first year shoot increment record of the trees that I documented. Chapters 6 and 7 focus on below-ground dynamics extending beyond the roots to the dissolved organic matter in the soils. The mobile form of soil organic matter plays a central role in soil C cycling and our data lends support for a potentially controversial 'priming effect' as a consequence of elevated CO_2 concentrations, i.e. an increased mobilisation of old dissolved organic carbon through stimulation of microbial activity. The final chapter 8 is a synthesis paper of the ecosystem level questions that we addressed collectively as a team over the last 5 years and is still in preparation as it draws on data from all five graduate students and several other project partners, thus only the abstract is shown. My long-term presence at the site resulted in five-year measurement records of (a) tree physiological properties (photosynthesis, stomatal conductance, specific leaf area), (b) the sequential incorporation of the stable isotope tracer $\delta^{13}C$ in the needle, dwarf shrub and root fractions of the system and (c) the shoot increment record of the study trees. These five year records will be of strong value to the synthesis paper in progress.

Formally, each chapter in which I am a lead author is structured as an independent contribution, with the references given at the end of the thesis.

2 A test of the treeline carbon limitation hypothesis by in situ CO_2 enrichment and defoliation

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A TEST OF THE TREELINE CARBON LIMITATION HYPOTHESIS BY IN SITU CO_2 ENRICHMENT AND DEFOLIATION

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Abstract. Historically, carbon limitation, through a shortage of photoassimilates has been argued to limit the growth of trees at the upper altitudinal treeline. In a three-year free-air CO_2 enrichment (FACE) experiment, two species of 30-year-old alpine conifers (*Larix decidua* and *Pinus uncinata*) were studied to test this hypothesis in situ in the Swiss Central Alps (2180 m above sea level). CO_2 enrichment was combined with foliage removal to test the effect of altered source–sink relationships on tree growth and leaf level responses. Elevated CO_2 enhanced photosynthesis and increased nonstructural carbohydrate (NSC) concentrations in the needles of both species. While the deciduous larch trees showed longer needles and a stimulation of shoot growth over all three seasons when grown in situ under elevated CO_2, pine trees showed no such responses. Irrespective of CO_2 concentration, defoliation in both species stimulated photosynthesis and increased stomatal conductance in remaining current-year needles in the treatment year and reduced leaf nitrogen concentration in the year following defoliation. Defoliated larch trees had fewer and shorter needles with reduced NSC concentrations in the year following defoliation and showed no stimulation in shoot elongation when exposed to elevated CO_2. In contrast, defoliation of evergreen pine trees had no effect on needle NSC concentrations, but stimulated shoot elongation when defoliated trees were exposed to elevated CO_2. After three years, our results suggest that deciduous larch is carbon limited at treeline, while evergreen pine is not. However, as indicated by the defoliation treatment, the carbon economy of these trees can clearly be modified by extreme events. The expected changes in growth of these treeline trees with improving carbon availability as atmospheric CO_2 continues to increase will thus depend on both the interplay between biotic and abiotic processes, and the species or tree functional types involved.

Key words: alpine; biodiversity; elevated CO_2; Larix decidua; Pinus uncinata; *shoot growth; source–sink balance; timberline.*

INTRODUCTION

The carbon source and sink balance of trees growing at the high elevation treeline has received increasing attention in the debate surrounding a potential mechanism for the general existence of treeline boundaries (Körner 1998, Sveinbjörnsson 2000, Li et al. 2002, Hoch and Körner 2003, Smith et al. 2003). Much of the earlier work (see references in Hättenschwiler et al. 2002) emphasized environmental constraints related to pronounced thermal seasonality of mid- and high-latitude regions (e.g., short growing seasons, harsh winter temperatures, frost desiccation, wind abrasion, and ice blasting), yet failed to explain the occurrence of treelines in low latitude regions. Prompted largely by the observation that the worldwide treeline position in non-arid regions correlates to a mean growing season temperature of ~5–7°C (Körner 1998), there have been increasing efforts to work toward a unifying mechanistic treeline theory adopting a global perspective (Wardle 1998, Jobbagy and Jackson 2000, Hoch and Körner 2003, Körner and Paulsen 2004).

Two of the emerging key hypotheses for treeline formation focus on potential thermal constraints to basic physiological processes connected to the carbon balance of the trees (Körner 1998): (1) the ''carbon limitation hypothesis,'' a source limitation through insufficient net carbon acquisition due to low daytime temperatures, and (2) the ''growth limitation hypothesis,'' or sink limitation through thermal limitation of meristematic activity with direct consequences for structural growth. To date, there has been little evidence in support of the carbon limitation hypothesis, although no direct manipulative test has been carried out. The photosynthetic carbon uptake in treeline trees does not appear to be highly sensitive to temperature (Häsler 1982, Goldstein et al. 1994). Furthermore, studies of the mobile carbon pool, measured as nonstructural carbohydrates and lipids, in trees across an altitudinal and latitudinal transect at treeline, have shown that the accumulated carbon reserves are not lower at high than at low elevations (Hoch and Körner 2003), and can be rapidly recovered following defoliation stress (Li et al. 2002), also casting doubt on carbon limitation.

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A direct test of the carbon limitation hypothesis is possible by documenting the carbon economy of treeline trees in response to a manipulative carbon source enhancement, such as in situ atmospheric CO_2 enrichment. The potential consequences of atmospheric CO_2 enrichment on forests have received much attention as the discussion continues regarding their role in carbon sequestration and rates of carbon cycling in response to the ongoing atmospheric changes (Saxe et al. 1998, Norby et al. 1999, Norby et al. 2001, Körner 2003*a*, *b*). Trees at high altitudes may be particularly sensitive to CO_2 enrichment since they grow in an atmosphere of lower partial pressure of CO_2 (La Marche et al. 1984). Concurrent with rising atmospheric CO_2 concentrations, tree ring widths of upper montane (La Marche et al. 1984, Graybill and Idso 1993, Nicolussi et al. 1995, Rolland et al. 1998, Paulsen et al. 2000) and arctic treeline conifers (MacDonald et al. 1998, Esper and Schweingruber 2004) have increased in the last 150 years, although other factors (e.g., forest management, nitrogen deposition, temperature, precipitation) have also changed, creating multiple interactions and possible explanations of the observed trend (Graumlich 1991, Grace et al. 2002).

The carbon balance of treeline trees can also be heavily impacted by biotic factors such as herbivory. Intense defoliation can occur as a response to periodic outbreaks of insects such as the larch bud moth, *Zeiraphera diniana* (Baltensweiler et al. 1977, Weber 1997), or the spruce bud worm, *Choristoneura* species (Morin et al. 1993, Weber and Schweingruber 1995). Defoliation reduces net carbon acquisition and can prompt recovery strategies like the rapid mobilization of carbohydrate stores (Li et al. 2002) which may lead to general overcompensatory growth (sensu McNaughton 1983, Maschinski and Whitham 1989, Vanderklein and Reich 1999, but see Aarssen and Irwin 1991) or a restricted local modular growth response to the damage (Tuomi 1988, Haukioja et al. 1990, Honkanen et al. 1994). Qualitative responses can result such as an amelioration of foliage quality for herbivores (Danell and Huss-Danell 1985, Haukioja et al. 1990) or the induction of plant defense compounds to deter herbivores (Herms and Mattson 1992, Honkanen et al. 1999). Most studies of atmospheric CO_2 enrichment have reported foliar chemical changes such as increased concentrations of nonstructural carbohydrates and reduced foliar N concentrations (Norby et al. 1999, Körner 2003*a*, *b*). These CO_2-mediated changes in forage quality (i.e., forage of generally lower nutritive value) can affect growth rates, population dynamics, and feeding behavior (compensatory consumption) of herbivores (Roth et al. 1998, Hättenschwiler and Schafellner 1999), which in turn might trigger feedbacks that further impact the carbon balance of the trees.

This study reports a carbon-source manipulation experiment of European larch and mountain pine at the alpine treeline in order to directly test the carbon limitation hypothesis of treeline formation and to assess how the carbon balance of these trees might change in a CO_2-enriched world. Over three years, we assessed the response of these two conifer species of contrasting growth strategies to a carbon-source enhancement (free-air CO_2 enrichment) and a carbon-source removal (80% defoliation in the second year) treatment in order to determine whether (1) these treeline species are carbon limited, (2) how biotic factors such as intense herbivory might modify the observed growth response to elevated CO_2, and (3) whether the responses to both manipulation treatments would show species specificity. Part of the first-year treatment response of the trees to elevated CO_2 has been previously documented together with the description of the experimental facility (Hättenschwiler et al. 2002). This report focuses on the interactive effects of CO_2 enrichment and defoliation during the second and third year.

METHODS

Study site description

The experiment was carried out at Stillberg, Davos (Central Alps, Switzerland), in a long-term research area established in the late 1950s to study afforestation techniques and avalanche stability by the Swiss Federal Institute of Snow and Avalanche Research (SLF, Davos, Switzerland). The slope faces the northeast and the soil (classified as Ranker/Lithic Haplumbrept) consists typically of a 10 cm deep organic top layer on siliceous (Paragneis) bedrock (Schönenberger and Frey 1988). Long-term mean annual precipitation is 1050 mm, mean maximum snow depth is 1.46 m, and mean January and July temperatures are −5.8° and 9.4°C, respectively (Schönenberger and Frey 1988).

In 1975, 92 000 individuals of high elevation provenances of three treeline species, *Larix decidua* L., *Pinus cembra* L., and *Pinus uncinata* Ramond were planted in a massive afforestation experiment across an area of 5 ha, spanning an altitudinal range of 2080–2230 m. The dominant survivor on the mountain has been *L. decidua*, followed by *P. uncinata* and few individuals of *P. cembra*; both of the latter pine species were subject to high mortality due to fungal attacks (Senn and Schönenberger 2001). In the upper end of the long-term research area, trees are not taller than ~1.5 m and form a sparse open canopy with dense understory vegetation composed dominantly of dwarf shrubs such as *Vaccinium myrtillus*, *Vaccinium uliginosum*, *Empetrum hermaphroditum*, and common herbaceous species such as *Gentiana punctata*, *Homogyne alpina*, and *Melampyrum pratense*. Within a relatively homogeneous 2500-m^2 area on a slope of 25–30° in the upper end of the research area (2180 m asl; representative of, or slightly above, the actual natural treeline), we established our free-air CO_2 enrichment (FACE) study site (Hättenschwiler et al. 2002).

Study species and experimental design

Larix decidua, the European larch, is a deciduous conifer with indeterminate shoot growth. It produces both short and long shoots and has the ability to activate the meristem of a short shoot to grow into a long shoot if an apical bud is damaged (Clausen and Kozlowski 1967). Canopy expansion occurs via long shoot production. In contrast, *Pinus uncinata*, the mountain pine, is an evergreen conifer with determinate shoot growth where much of the current-year growth is predetermined in buds developed the previous growing season. In early June 2001, 20 trees each of *L. decidua* (27 years old) and *P. uncinata* (29 years old) were selected based on the following criteria: one dominant stem, no serious signs of disease or herbivory, not more than one close (<80 cm) neighboring tree, ≥2 m distance to the next study tree, and a height of 0.8–1.5 m. In spring (2002), one pine (luckily a ''full control'' tree) suffered serious fungal attacks; it was omitted from the 2002 analyses and was replaced (June 2003) with a comparable tree.

Using a split-plot approach, the 40 trees were assigned to 10 groups of four neighboring trees, two each of larch and pine trees per group, in order to facilitate logistics of CO_2 distribution and regulation. Half of these groups were randomly assigned to an elevated CO_2 treatment, while remaining groups served as controls at ambient CO_2 concentration (~367 μL/L). The pure-CO_2 release technology in use at our FACE study site has been previously described in detail by Hättenschwiler et al. (2002). Forty plots (1.1 m^2) were erected around each tree by means of three wooden posts and a hexagonal stainless steel frame. As trees grew during the experiment, frames were lifted accordingly (most adjustments were made in spring 2003) such that the crown of the tree always remained in the center of the plot. From each frame, 24 laser-punched drip irrigation tubes (inner diameter 4.3 mm, 0.5 mm laser holes every 15 cm; Drip Store, Escondido, California, USA) were hung vertically around the ring 15 cm apart from each other, weighted with a 3-mm stainless steel rod to maintain rigidity and buried 2–4 cm into the soil. Each plot was fed by four supply tubes of pure CO_2 attached in the four cardinal directions in the inner circumference of the frame. Temporal and spatial variability of concentrations was regulated at the group level (four trees) by means of a monitoring system (Hättenschwiler et al. 2002). The CO_2 enrichment treatment was applied for three growing seasons (~15 June–15 September, 2001–2003) during daylight hours when weather conditions were suitable. Under highly suboptimal weather conditions, e.g., photon flux density (PFD) <100 $\mu mol \cdot m^{-}2 \cdot s^{-1}$, wind speeds >75 km/h, temperatures <5°C, snow, or sleet, we interrupted CO_2 release because of the enormous cost of helicopter gas transport. Technical failure (<5%) or weather conditions meant that trees received CO_2 enrichment for 75%, 81%, and 73% of the 2001, 2002, and 2003 growing seasons, respectively. Seasonal mean CO_2 concentrations (2001–2003; ± 1 SD) were 566 ± 42, 582 ± 35, and 579 ± 52 μL/L.

In late June of the second year of treatment (2002), following bud break, but prior to full needle extension, half of the trees in each group for every species and CO_2 treatment combinations were assigned to an 80% defoliation treatment (n = 5 per level of species, CO_2, and defoliation). Larches were defoliated by systematically cutting four out of every five short shoots above the meristem throughout the entire tree (Fig. 1). All new long shoots were left intact. Pines were defoliated by cutting 80% of the proximal needles away within each of four age classes from 1999–2002 on the entire tree with care not to damage the shoot (1–2 mm stubs remained). The distal 20% of the needles within each age class were left untouched (Fig. 1).

Tree observations, sampling, and statistical analysis

Detailed phenological observations relating to bud swelling, bud burst, and needle expansion were documented every 5–7 d in June of 2002 and 2003. Observations of needle senescence of larches were made every 10–14 d in September–October of 2002 and 2003. Needle elongation and specific leaf area were measured over a seasonal course (19 July, 28 July, and 14 August) in 2002 and on 30 July 2003. Five needle fascicles per tree (two and ~30 needles per fascicle in pine and larch, respectively) were harvested, placed in a plexiglass tray, imaged with a scanner and transparency unit light source (Epson Expression 1650, Epson America, Long Beach, California, USA), and analyzed with the WinSeedle software (Regent Instruments, Québec City, Québec, Canada) for total needle length, projected needle area, and mean number of needles per fascicle. Needles were then oven dried (80°C) and some of these samples (14 August 2002 and 30 July 2003), in addition to needle and wood samples from the gas-exchange related harvests (19 September 2002 and 3 August 2003), were subsequently ground to a homogenous powder for chemical analyses. Nonstructural carbohydrates (NSC), defined as the sum of starch and low-molecular-weight sugars (sucrose, glucose, and fructose), were quantified by using an enzymatic starch digestion and a spectrophotometric glucose test after invertase and isomerase addition (Hoch and Körner 2003). Total C and N concentration of the samples was measured with a CHN-analyzer (model 900, LECO Instruments, St. Joseph, Michigan, USA).

Gas exchange of intact current-year shoots was measured under light saturating conditions (natural sunlight between 09:30 and 15:30, >1200 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) using the LI-COR 6400 photosynthesis system (LI-COR, Lincoln, Nebraska, USA) with the conifer cuvette (LI-6400-05) during 1–19 September 2002 and 2–4 August 2003. Measurements were made later than desired and over a discontinuous period in 2002 due to overcast weather conditions through Au-

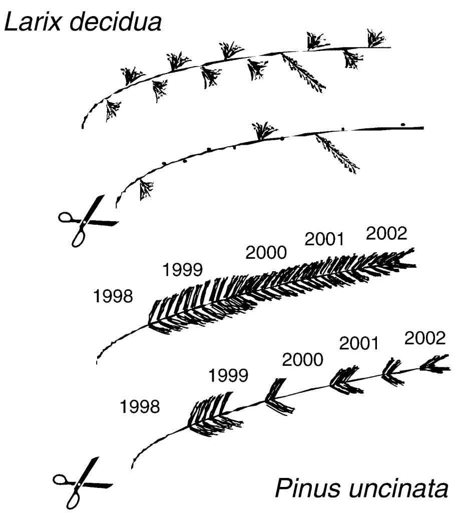

FIG. 1. Schematic representation of the 80% defoliation treatment applied to both tree species in late June 2002. Larch trees (top) were defoliated by cutting away four out of every five short shoots throughout the tree. Pine trees were defoliated by cutting away 80% of the proximal needles within each age class from 1999 to 2002.

gust–September. Nonetheless, all larches were measured prior to any sign of senescence. Temperature and relative humidity inside the cuvette were kept constant and leaf temperature ranged from 19 to 26°C. Every tree was measured at the treatment CO_2 concentration it was growing in, as well as the opposite treatment CO_2 concentration (using the internal Li-Cor 6400 CO_2 control) to test for possible photosynthetic downregulation. All measured shoots were subsequently harvested and total projected area of the needles in the conifer chamber was determined.

Elongation of the terminal current-year shoot of five marked mid-canopy lateral branches per tree, as well as the leading shoot of the tree, was measured biweekly (to the nearest mm) throughout all growing seasons of CO_2 enrichment. On each branch, the length of the shoot increment in the year prior to any CO_2 enrichment (2000) was measured.

Analysis of variance techniques were used to test for CO_2, defoliation, and species effects on all measured parameters using a full factorial split-plot model in each year. Tests were conducted separately by species (factors were CO_2 concentration and defoliation) when parameters were larch-specific (i.e., needle senescence, leaf longevity, number of needles per fascicle) and for all needle quality analyses in 2002, given different harvest dates for both species. Shoot length data were analyzed using a repeated measures analysis of covariance where shoot length increment of the year prior to the beginning of the experiment (2000) was included as a covariable to account for relative differences in trees. Total needle length and shoot length data were log transformed. Analyses were performed with R (version 1.6.1, R Development Core Team 2002; *available online*).[3]

RESULTS

Tree phenology, growth and development of needles

Phenological observations showed that full needle unfolding occurred between 17 and 27 June (Table 1): time of unfolding was similar in both species in 2002, but was 4 ± 3 d (mean ± 1 SE) later in larch than pine trees in 2003 (species; $F_{1,16} = 13.9$, $P < 0.01$). Larches grown under elevated CO_2 unfolded their needles 5 ± 1 d earlier in 2002 (CO_2; $F_{1,8} = 3.50$, $P < 0.1$) and 7 ± 4 d earlier in 2003 (not significant), after one and two respective seasons of CO_2 enrichment. Elevated CO_2 had no influence on needle senescence of larches in 2002, but defoliation in late June 2002 resulted in a slightly earlier needle senescence (5 ± 4 d) in a period spanning 8–31 October 2002 (Table 1; $F_{1,8} = 3.88$, $P < 0.1$). In 2003, needle senescence of larch spanned 7–27 October and showed no response to either treatment, although there was a tendency for a later senescence in trees exposed to elevated CO_2. Pines showed little variation across treatments (Table 1), with swelled buds unfolding completely by 20–24 June in both years.

Elongation of needles measured over a seasonal course in 2002 showed a significant stimulation in response to elevated CO_2 (Tables 2 and 3), predominantly in larches (+16%), but this CO_2 effect disappeared in 2003. There was no influence of defoliation on total needle length of remaining current-year needles in either season (Table 3), and neither larch nor pine produced a new flush of needles following the defoliation treatment. The number of needle per fascicle in larch was also unaffected by elevated CO_2 in either year (Tables 2 and 3). Defoliated larches, however, had fewer remaining needles per fascicle in the year of defoliation (2002; 6 ± 2 fewer needles) and 2003 (4 ± 2 fewer needles) than undefoliated larches (Table 3). Pines did not show increased needle length in response to elevated CO_2. However, defoliated pines growing under elevated CO_2 had slightly longer needles than defoliated ambient pines in both growing seasons, contrasting particularly to the larch response in 2003 (Table 2, significant CO_2 × defoliation × species; Table 3). The number of needles per fascicle in pine is a fixed trait unlike in larch where it is indeterminate.

Gas exchange

Light-saturated net photosynthesis ($A_{\max}$; subsequent highlighted results refer to $A_{\max}$ [mass] only) of fully mature current-year shoots was two- to three-fold higher in larch than in pine trees (Tables 2 and 3), and was significantly stimulated in both species under elevated CO_2 in 2002 and 2003 (Table 3). Larches showed higher photosynthetic CO_2 uptake (2002, +49%; 2003,

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[3] ⟨http://www.r-project.org⟩

TABLE 1. Summary of phenological observations in *Larix decidua* and *Pinus uncinata* under differing CO_2 concentrations and defoliation regimes in two consecutive growing seasons.

	Larix decidua						*Pinus uncinata*	
	Needles unfolded (day of year)		Senescence (day of year)		Leaf longevity (d)		Needles unfolded (day of year)	
Treatment	2002	2003	2002	2003	2002	2003	2002	2003
Ambient CO_2	173 ± 1	178 ± 2	288 ± 2	288 ± 3	115 ± 1	111 ± 4	175 ± 4	171 ± 2
Elevated CO_2	168 ± 1	171 ± 3	288 ± 4	291 ± 4	120 ± 3	119 ± 2	171 ± 0	171 ± 2
Ambient CO_2, defoliated	171 ± 1	176 ± 2	281 ± 2	289 ± 3	110 ± 4	113 ± 4	172 ± 1	173 ± 2
Elevated CO_2, defoliated	170 ± 1	178 ± 2	284 ± 2	290 ± 1	114 ± 2	112 ± 2	171 ± 0	172 ± 0

Note: All values shown are means ± 1 SE; $n = 5$ trees. The defoliation treatment was applied after 2002 needles had unfolded. Phenological categories refer to the status of >75% of the needles in a tree unfolding or (in the autumn) senescing to a golden yellow (larch only). Day 1 of the year is 1 January.

+36%) than pines (2002, +39%; 2003, +24%) when grown and measured in an elevated CO_2 atmosphere (Table 3). Defoliation of trees resulted in significantly higher photosynthesis in 2002 in larch (+7%) and pine (+52%), but no difference in 2003 in either species (Table 3). The relative photosynthetic response of both species to defoliation differed in 2003 (significant, defoliation × species; Table 3) with defoliated larches showing a −18% reduction in photosynthesis, but pines showing no difference.

The potential for photosynthetic downregulation of all trees was measured in both years by manipulating the cuvette CO_2 concentration to the opposite treatment concentration that a particular tree was growing in. None of the pairwise contrasts showed any significant evidence of downregulation in both growing seasons for either species. However, undefoliated pines (but none of the larches) showed a tendency for downregulation (−14%) at elevated CO_2 concentrations. This pattern was stronger in 2003 and of marginal significance for contrasts at ambient (−21%; A_{max} [mass], $F_{1,8} = 3.40$, $P = 0.1$) and elevated CO_2 concentrations (−17%; A_{max} [mass], $F_{1,8} = 3.39$, $P = 0.1$).

Stomatal conductance differed significantly among species and was roughly twice as high in larch as compared to pine (Tables 2 and 3). Conductance did not differ among trees grown under elevated and ambient CO_2. However, defoliation in 2002 had a pronounced effect on stomatal conductance with higher values in remaining current-year needles of defoliated larches (+42%) and pines (+108%) compared to undefoliated trees (Table 3).

Needle and branch chemistry

With the exception of larches in 2003, both species increased nonstructural carbohydrate (NSC) concentration by increasing their starch fraction when grown under elevated CO_2 (Tables 4 and 5), which contributed to the reduction in specific leaf area as needles got heavier (Tables 2 and 3). Defoliation of larches resulted in a highly significant reduction in starch in the treatment year (−25%; Table 5). Defoliated larches also showed lower carbohydrate reserves in the following season (2003: −23% NSC; −27% sugar; −18% starch), but not in pines (2003: significant, defoliation × species for NSC and sugar; Table 5). NSC concentration of branch wood was significantly different for each species in the second and third seasons ($P < 0.001$), in the range of 10–15% dry matter (d.m.) in larch and 8–9% d.m. in pine, but showed no CO_2 or defoliation effect (*data not shown*). In both species, wood NSC responded differently to defoliation in 2002 (defoliation × species; starch, $F_{1,14} = 3.70$, $P < 0.1$) and in 2003 (defoliation × species; sugar, $F_{1,16} = 4.77$, $P < 0.05$). Wood of defoliated larch trees had higher starch (+16%; 2002) and sugar (+16%; 2003) concentrations, while wood of defoliated pine trees showed no difference in starch in 2002; but lower sugar (−8%; 2003) in contrast to undefoliated trees.

Nitrogen concentration of larch needles (2.1 ± 0.1% d.m.) showed no significant variation across treatments in 2002, but a significant −11% reduction occurred in response to defoliation in 2003 (Tables 4 and 5). Nitrogen concentration of larch wood (0.9 ± 0.1% d.m.) was similar across treatments and years (*data not shown*). Current-year pine needles grown under elevated CO_2 tended to have a lower N concentration (−9% d.m.) than those of ambient grown trees in the second and third year of the experiment, but the pattern was reversed when pines were defoliated (matching shoot growth response patterns, Fig. 2). Nitrogen concentration of current-year pine needles increased (+6%) in response to defoliation in 2002, but decreased (−17%) in 2003 (Table 4). Nitrogen concentration of pine wood (0.8–0.9% d.m.; *data not shown*) was similar across treatments in both years, but the relative species CO_2 response differed in 2003 (CO_2 × species; $F_{1,16} = 9.99$, $P < 0.001$): larch wood showed no difference, but wood of pines growing under elevated CO_2 had +17% greater N concentration than that of ambient grown trees.

Shoot growth

Annual shoot increment measured prior to the beginning of the experiment was a significant covariable in 2002 ($F_{1,7} = 6.32$, $P < 0.05$) and 2003 ($F_{1,7} = 7.66$,

TABLE 2. Summary of needle parameters pertaining to morphology and gas exchange of fully developed current-year needles of *Larix decidua* and *Pinus uncinata* under differing CO_2 concentrations and defoliation regimes monitored over two growing seasons.

	SLA (cm^2/g)		A_{max} (area) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		A_{max} (mass) ($mmol \cdot g^{-1} \cdot s^{-1}$)		g_s ($mmol \cdot m^{-2} \cdot s^{-1}$)		Needle length (mm)		Needle density (needles/fascicle)	
Treatment	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
Larix decidua												
Ambient CO_2	92.1	97.0	13.4	11.8	114	107	259	246	14.6	13.6	29	28
	(3.3)	(4.6)	(0.8)†	(0.5)	(8)†	(2)	(35)†	(38)	(0.8)	(0.8)	(2)	(3)
Elevated CO_2	85.0	84.5	17.4	18.2	170	145	195	200	16.9	15.9	31	32
	(4.0)	(3.2)	(1.0)	(1.1)	(6)	(10)	(28)	(26)	(0.6)	(1.2)	(1)	(1)
Ambient CO_2,	90.6	88.8	15.5	10.7	122	91	369	252	14.9	12.9	25	26
defoliated	(2.9)	(3.6)	(1.9)	(1.0)	(7)	(8)	(66)	(38)	(0.7)	(0.8)	(2)	(2)
Elevated CO_2,	93.1	88.3	21.6	17.2	184	143	285	241	15.8	13.0	23	26
defoliated	(5.5)	(2.8)	(3.0)†	(0.8)	(23)†	(10)	(57)†	(65)	(0.8)	(0.8)	(2)	(2)
Pinus uncinata												
Ambient CO_2	41.3	41.6	5.9	8.0	23	29	62	125	38.2	48.2	NA	NA
	(2.1)	(0.9)	(1.3)	(0.3)	(4)	(2)	(25)	(14)	(4.8)	(2.5)		
Elevated CO_2	38.7	40.7	9.4	10.7	32	36	79	98	36.4	44.3	NA	NA
	(1.9)	(1.9)	(1.5)	(0.5)	(5)	(1)	(21)	(9)	(0.8)	(1.1)		
Ambient CO_2,	40.4	40.3	10.1	7.9	35	28	129	159	34.6	43.0	NA	NA
defoliated	(1.8)	(1.8)	(0.8)†	(0.6)	(3)†	(2)	(18)†	(15)	(1.6)	(2.5)		
Elevated CO_2,	37.9	36.2	14.3	12.3	48	40	125	146	37.4	48.8	NA	NA
defoliated	(0.8)	(1.4)	(1.2)	(1.0)	(4)	(3)	(10)	(10)	(1.0)	(2.9)		

Notes: Gas-exchange-related measurements include: SLA, specific leaf area; A_{max} (area), light-saturated net photosynthesis expressed per unit needle area; A_{max} (mass), light-saturated net photosynthesis expressed per unit needle dry mass; and g_s, stomatal conductance. All values shown are means (±1 SE); $n = 5$ trees (except those indicated with a single dagger).

† $n = 4$.

$P < 0.05$). Across treatments, mean extension of lateral shoots was 56 ± 4 mm (2002) and 64 ± 4 mm (2003) in larch, and 45 ± 3 mm (2002) and 42 ± 3 mm (2003) in pine (Fig. 2). The response of shoot growth to elevated CO_2 was significant in 2002 only ($F_{1,7} = 6.17$, $P < 0.05$), driven by the +54% stimulation of larch trees. There was no significant overall defoliation response in either year. The response to carbon manipulation treatments was opposite in both species (2002; defoliation × species; $F_{1,15} = 7.32$, $P < 0.05$ and CO_2 × defoliation × species, $F_{1,15} = 4.31$, $P < 0.1$). Larches showed a +54% (2002) and +36% (2003) stimulation in response to elevated CO_2 alone (Fig. 2), however when defoliated and exposed to elevated CO_2 there was no stimulation. Conversely, pines showed no stimulation in response to elevated CO_2, but when defoliated and exposed to elevated CO_2, showed a +27% stimulation (in 2002 only; Fig. 2). Leading terminal shoots on the trees followed the same pattern of response, although variation was much stronger, given a typically higher incidence of winter damage. Leading shoot extension in larch was on average 158 ± 14 mm (2002) and 185 ± 15 mm (2003). In pine, mean leading shoot extension was 76 ± 11 mm (2002) and 67 ± 6 mm (2003).

DISCUSSION

This first-ever test of the influence of elevated CO_2 on trees at the alpine treeline revealed clear positive growth responses measured as annual shoot increment in both deciduous larch and evergreen pine in the first year (Hättenschwiler et al. 2002). Here we show that in larch the shoot increment response persisted into the third year of the experiment (2001–2003: +18, +54, +36%), but disappeared in pine (+13% in 2001, no effect thereafter). This striking species or life-strategy related response was modified significantly when part of the foliage was removed in the second year: defoliated larch no longer showed a positive shoot increment response to elevated CO_2, while in defoliated pine, the response resumed. These shoot growth responses were equally supported by detailed tree ring width data for both years (I. T. Handa, *unpublished data*). The shorter-term responses at the needle level for both seasons help to interpret these growth responses by accounting for the contrasting leaf and shoot life histories of both species.

Carbon source manipulation responses

In response to CO_2 enrichment or defoliation, one might predict that given the cheaper initial cost of production of deciduous foliage (Villar and Merino 2001), and higher rate of assimilation return per unit carbon investment, deciduous larch would show a more rapid and pronounced response to a carbon-source enhancement treatment than evergreen pine, and also suffer less of a loss than pine in response to carbon source removal. Furthermore, the indeterminate growth of larch, in contrast to the determinate growth of pine, might be expected to provide a greater flexibility in response to manipulations. Such a flexibility was observed in *Betula*, which, like larch, produces both long and short

TABLE 3. ANOVA results for a full factorial model testing for differences in the response of measured needle parameters to treatments of CO_2, defoliation, and species effects.

Needle parameter, by year	CO_2		Defoliation		Defoliation × CO_2		Species	
	F	df	*F*	df	*F*	df	*F*	df
SLA								
2002	NS	1, 8	NS	1, 8	NS	1, 8	529***	1, 16
2003	6.28*	1, 8	NS	1, 8	NS	1, 8	543***	1, 16
A_{max} (area)								
2002	7.64*	1, 5	12.2*	1, 5	NS	1, 5	57.5***	1, 13
2003	56.2***	1, 8	NS	1, 8	NS	1, 8	79.5***	1, 16
A_{max} (mass)								
2002	9.38*	1, 5	7.23*	1, 5	NS	1, 5	206***	1, 13
2003	45.3***	1, 8	NS	1, 8	NS	1, 8	451***	1, 16
g_s								
2002	NS	1, 5	10.1*	1, 5	NS	1, 5	21.9***	1, 13
2003	NS	1, 8	NS	1, 8	NS	1, 8	19.6***	1, 16
Needle length								
2002	5.33*	1, 7	NS	1, 7	NS	1, 7	517***	1, 15
2003	NS	1, 8	NS	1, 8	NS	1, 8	619***	1, 16
Needle density‡								
2002	NS	1, 8	31.8***	1, 8	NS	1, 8	NA	NA
2003	NS	1, 8	4.01†	1, 8	NS	1, 8	NA	NA

Notes: Gas-exchange-related measurements include: SLA, specific leaf area; A_{max} (area), light-saturated net photosynthesis expressed per unit needle area; A_{max} (mass), light-saturated net photosynthesis expressed per unit needle dry mass; and g_s, stomatal conductance. NA indicates not applicable.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS indicates not significant ($P > 0.1$).

† $0.1 < P < 0.05$.

‡ Number of needles per fascicle was tested in larch trees only (it is a fixed trait in pine trees); therefore no data are shown for the response of this parameter to effects including species.

shoots, and induced long shoot production from short shoot meristem primordia in response to warming and nitrogen fertilization treatments in Alaska (Bret-Harte et al. 2001). While both larch and pine showed enhanced rates of photosynthesis and an accumulation of storage nonstructural carbohydrate (NSC) reserves in needles in response to CO_2 enrichment, as has been observed in other conifers (Ellsworth et al. 1995, Wang et al. 1995, Griffin et al. 1996, Hättenschwiler and Körner 1996, Runion et al. 1999) and broadleaved species (Saxe et al. 1998, Medlyn et al. 1999, Norby et al. 1999), larch was arguably more responsive to carbon-source enhancement than pine in our experiment. A higher growth responsiveness for deciduous in contrast to evergreen woody species has been documented in other CO_2 enrichment studies (mostly with seedlings) under controlled environmental conditions (Poorter et al. 1996, Cornelissen et al. 1999).

TABLE 4. Summary of mean leaf quality measurements (±1 SE) of fully developed current-year needles of *Larix decidua* and *Pinus uncinata* under differing CO_2 concentrations and defoliation regimes.

Treatment	NSC		Sugar		Starch	
	2002	2003	2002	2003	2002	2003
Larix decidua						
Ambient CO_2	15.7 ± 0.7	21.6 ± 1.1	12.5 ± 0.4	11.6 ± 0.7	3.2 ± 0.4	9.9 ± 0.9
Elevated CO_2	17.9 ± 0.5	22.5 ± 1.4	12.9 ± 0.4	11.7 ± 0.3	5.0 ± 0.3	10.6 ± 1.2
Ambient CO_2, defoliated	14.7 ± 0.5	17.6 ± 0.6	12.3 ± 0.6	9.1 ± 0.1	2.4 ± 0.4	8.4 ± 0.7
Elevated CO_2, defoliated	15.5 ± 0.3	18.7 ± 1.1	12.5 ± 0.7	9.2 ± 0.5	3.0 ± 0.4	9.4 ± 1.3
Pinus uncinata						
Ambient CO_2	10.0 ± 0.9	12.0 ± 0.9	5.2 ± 0.3	4.7 ± 0.2	4.8 ± 1.0	7.1 ± 0.8
Elevated CO_2	12.5 ± 1.1	14.1 ± 1.4	5.4 ± 0.4	4.3 ± 0.3	7.1 ± 0.8	9.6 ± 1.4
Ambient CO_2, defoliated	8.9 ± 0.3	11.5 ± 1.3	5.1 ± 0.3	4.2 ± 0.4	3.8 ± 0.4	7.1 ± 1.0
Elevated CO_2, defoliated	10.8 ± 0.9	16.8 ± 0.8	4.9 ± 0.3	4.9 ± 0.4	5.8 ± 0.7	11.5 ± 0.9

Notes: All values are percentage of dry matter. Samples in 2002 were harvested on 18 September, with the exception of *Larix decidua* needles used for nonstructural carbohydrate (NSC) analyses that were harvested on 14 August. Samples in 2003 were harvested on 30 July. Samples comprised $n = 5$ trees except where indicated.

† For these entries, $n = 4$ trees.

Table 3. Extended.

CO$_2$ × species		Defoliation × species		CO$_2$ × defoliation × species	
F	df	*F*	df	*F*	df
NS	1, 16	NS	1, 16	NS	1, 16
NS	1, 16	NS	1, 16	3.42†	1, 16
NS	1, 13	NS	1, 13	NS	1, 13
NS	1, 16	7.39*	1, 16	NS	1, 16
3.65†	1, 13	NS	1, 13	NS	1, 13
NS	1, 16	18.0***	1, 16	NS	1, 16
NS	1, 13	NS	1, 13	NS	1, 13
NS	1, 16	NS	1, 16	NS	1, 16
NS	1, 15	NS	1, 15	NS	1, 15
NS	1, 16	NS	1, 16	7.45*	1, 16
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA

The greater responsiveness to CO$_2$ enrichment of larch as compared to pine was evident through several observations. Larch showed an improved assimilation potential in response to CO$_2$ enrichment through a slightly longer growing season and increased needle length, in contrast to pine which showed no phenological or needle-length response. Over the three years, larch showed a reduction in needle NSC reserves (+17%, +14%, no significant accumulation in 2003) and no significant changes in branch wood NSC concentrations produced under elevated CO$_2$, suggesting that extra assimilated carbon was used for growth rather than stored. Pine also showed a diminished increase in needle NSC reserves under elevated CO$_2$ concentrations over the three years (+38, +25, +17%), but needle NSC concentrations were still significantly higher under elevated CO$_2$ at the end of the third growing season suggesting a consistent surplus carbon gain over carbon demand for growth. The strong accumulation of NSC in pine needles co-explains the lower N concentrations per unit dry mass (through a dilution effect) that were observed in needles grown under elevated CO$_2$ and the tendency toward photosynthetic downregulation in pine in 2003, possibly through negative feedback on Rubisco activity and photochemical processes (Drake et al. 1997).

Table 4. Extended.

Nitrogen		C:N	
2002	2003	2002	2003
1.91 ± 0.14	2.00 ± 0.04	25 ± 2	25 ± 1
2.06 ± 0.11	2.13 ± 0.09	23 ± 1	24 ± 1
2.10 ± 0.12	1.80 ± 0.09	22 ± 2	28 ± 1
2.13 ± 0.18†	1.64 ± 0.06	23 ± 1†	31 ± 1
1.18 ± 0.06	1.29 ± 0.03†	42 ± 2	38 ± 1†
1.09 ± 0.02	1.18 ± 0.02	46 ± 1	41 ± 1
1.25 ± 0.02	1.10 ± 0.02	40 ± 1	45 ± 1
1.32 ± 0.06	1.25 ± 0.07	38 ± 2	40 ± 2

Contrary to our expectations, deciduous larches were more sensitive to defoliation than evergreen pines. In the year of defoliation, larch was only able to compensate foliage loss by a small +7% photosynthetic and +15% shoot growth stimulation, showed earlier needle senescence in the year of defoliation, fewer needles per fascicle in both 2002 and 2003, and shorter needle length in 2003. In contrast, pine showed a stronger compensatory response in the year of the defoliation treatment: photosynthesis was stimulated +52% in the remaining current-year needles, and there was a tendency toward stimulated shoot growth (+25%) in defoliated trees. Storage NSC reserves in remaining current-year needles were depleted in larch, but no change was seen in pine needles. However, toward the end of the season, NSC reserves were surprisingly high in the wood of new grown larch shoots, but depleted in pine shoots. Collectively, our results show that defoliated larches could not compensate for the foliage loss in the year of defoliation, even when grown under elevated CO$_2$, but were able to achieve photosynthesis, growth, and storage comparable to that of control trees in the following year. In contrast, defoliated pines showed rapid compensation in the year of defoliation, which was further enhanced in trees that were grown under elevated CO$_2$. Other defoliation experiments with larch and other pine species revealed the opposite. Ten-year-old *Pinus resinosa* showed more reduced growth than *Larix decidua* in a lowland experimental plantation (Krause and Raffa 1996), while no differential growth response was observed in *P. resinosa* and *Larix leptolepis* seedlings in an arboretum experiment (Vanderklein and Reich 1999). These inconsistencies may relate in part to the high sensitivity of growth response to defoliation pattern (Honkanen et al. 1994, 1999), intensity (Krause and Raffa 1996), timing (Millard et al. 2001) and environmental conditions under which defoliation occurs (none of these tests were performed at the treeline).

Carbon limitation at treeline

Source limitation of growth due to a shortage of photoassimilates is still a controversial hypothesis in the treeline debate. While there is agreement that rates of photosynthesis in general are not very sensitive to temperature and not less than those at lower elevations

TABLE 5. ANOVA results for a full factorial model testing for response difference in measured needle-quality parameters of larch (L) and pine (P) to treatments of CO_2, defoliation, and species effects.

Needle parameter, by year	CO_2		Defoliation		Defoliation × CO_2		Species	
	F	df	*F*	df	*F*	df	*F*	df
NSC								
2002 L	9.08*	1, 6	7.40*	1, 6	NS	1, 6	NA	NA
2002 P	9.28*	1, 8	NS	1, 8	NS	1, 8	NA	NA
2003	5.06*	1, 8	NS	1, 8	NS	1, 8	79.6***	1, 16
Sugar								
2002 L	NS	1, 6	7.40*	1, 6	NS	1, 6	NA	NA
2002 P	NS	1, 8	NS	1, 8	NS	1, 8	NA	NA
2003	NS	1, 8	NS	1, 8	NS	1, 8	712***	1, 16
Starch								
2002 L	10.5*	1, 6	14.5*	1, 6	4.76†	1, 6	NA	NA
2002 P	9.51*	1, 8	NS	1, 8	NS	1, 8	NA	NA
2003	4.42†	1, 8	NS	1, 8	NS	1, 8	NS	
N								
2002 L	NS	1, 7	NS	1, 7	NS	1, 7	NA	NA
2002 P	NS	1, 8	16.2**	1, 8	4.71†	1, 8	NA	NA
2003	203***	1, 7	335***	1, 7	NS	1, 7	105***	1, 15
C:N								
2002 L	NS	1, 7	NS	1, 7	NS	1, 7	NA	NA
2002 P	NS	1, 8	16.1**	1, 8	4.15†	1, 8	NA	NA
2003	NS	1, 7	18.7**	1, 7	NS	1, 7	402***	1, 15

Notes: Since needles were harvested on different dates for larch (L) and pine (P) trees in 2002, tests were conducted separately for each species. NA indicates not applicable.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS indicates not significant ($P > 0.1$).

† $0.1 < P < 0.05$.

(Häsler 1982, Goldstein et al. 1994, Grace et al. 2002), source limitation is still discussed as a possible explanation for treeline formation with arguments mostly focusing on seedlings, extreme events, or particular test systems. In the Rocky Mountains (USA), for instance, increased sky exposure induced low-temperature photoinhibition of photosynthesis and a high mortality of conifer treeline seedlings (Germino and Smith 2002), similar to what has been shown for herbaceous alpine plants (see Smith et al. 2003). Whether there is a carbon-balance problem for taller trees is unclear, but photoinhibition has been observed in older trees as well (Grace et al. 2002, Öquist and Huner 2003), though effects on actual growth remain unresolved. Significantly greater tissue damage (needle loss due to frost and wind exposure) has been reported for some exposed treeline sites (van Gardingen et al. 1991, Pereg and Payette 1998, Sveinbjörnsson 2000). Such mechanical damages can reduce the potential for assimilation, but are not ubiquitously occuring at all treelines, including the Alps (Turner 1968).

As was argued by Körner (1998) regarding the growth limitation hypothesis, treeline trees are less likely to be limited by the supply of photosynthetic assimilates, but rather by the rate at which the products can be utilized. In a comparison of different treeline *Pinus* species from three latitudes (Mexico, Swiss Alps, and Sweden), carbon charging (NSC and lipids) was not lower, but rather higher at the tree limit, compared to tall montane forests, supporting sink rather than source limitation (Hoch and Körner 2003). In line with these findings, our pines did not show evidence of carbon limitation at treeline. The growth stimulation seen initially under elevated CO_2 disappeared after the first season. However, under conditions of severe tissue loss due to defoliation, an improved carbon availability in a CO_2-enriched atmosphere stimulated recovery in pine and alleviated the negative effects of needle loss. This indicates that although pine in our study system is generally not carbon limited, carbon limitation may occur occasionally and might be important for long-term growth and survival depending on the frequency of such severe disturbances. In contrast to pine, source limitation persisted over the three-year experimental period in larch, as evident by the consistent photosynthetic and growth stimulation in response to elevated CO_2. Thus, our direct test of the carbon limitation hypothesis through in situ CO_2 enrichment at the treeline offers an ambiguous answer to the source–sink debate of treeline trees given the species-specific or tree-functional-type-specific response.

Treeline trees in a CO_2-enriched world

To date, the only alpine CO_2-enrichment study has been on grassland 300 m above treeline, where it has been shown that elevated CO_2 has prolonged positive effects on photosynthesis (Diemer 1994), as we report for our trees as well, but no effects on aboveground

TABLE 5. Extended.

CO$_2$ × species		Defoliation × species		CO$_2$ × defoliation × species	
F	df	*F*	df	*F*	df
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NS	1, 16	7.36*	1, 16	NS	1, 16
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NS	1, 16	32.7***	1, 16	NS	1, 16
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NS	1, 16	NS	1, 16	NS	1, 16
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NS	1, 15	3.73‡	1, 15	NS	1, 15
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NS	1, 15	6.20*	1, 15	15.3**	1, 15

plant biomass after four years of treatment and irrespective of nutrient status (Schäppi and Körner 1996). This response matches that observed in pine here and the growth response found in montane spruce (Hättenschwiler and Körner 1996). If the growth enhancement in larch persists under rising atmospheric CO$_2$ concentrations, increased abundance of larch, typically considered a pioneer species, could affect early plant establishment after disturbance and recruitment patterns of later successional species. Although this might point toward a direct CO$_2$ effect (in addition to the influence of climate warming) on the upward migration of the treeline, a shift in species dominance is likely to have more important consequences. Species-specific responses to elevated CO$_2$ are a common phenomenon in many ecosystems, and altered competitive balance among species and community composition are of major concern for biodiversity and ecosystem functioning (Körner 2003*b*). In the understory at our site, four species of dwarf shrubs also exhibited species-specific growth responses, with *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, and *Empetrum hermaphroditum* gaining in shoot length under elevated CO$_2$, but no effect was seen in *Vaccinium uliginosum* (T. Zumbrunn and S. Hättenschwiler, *unpublished data*). As well, the

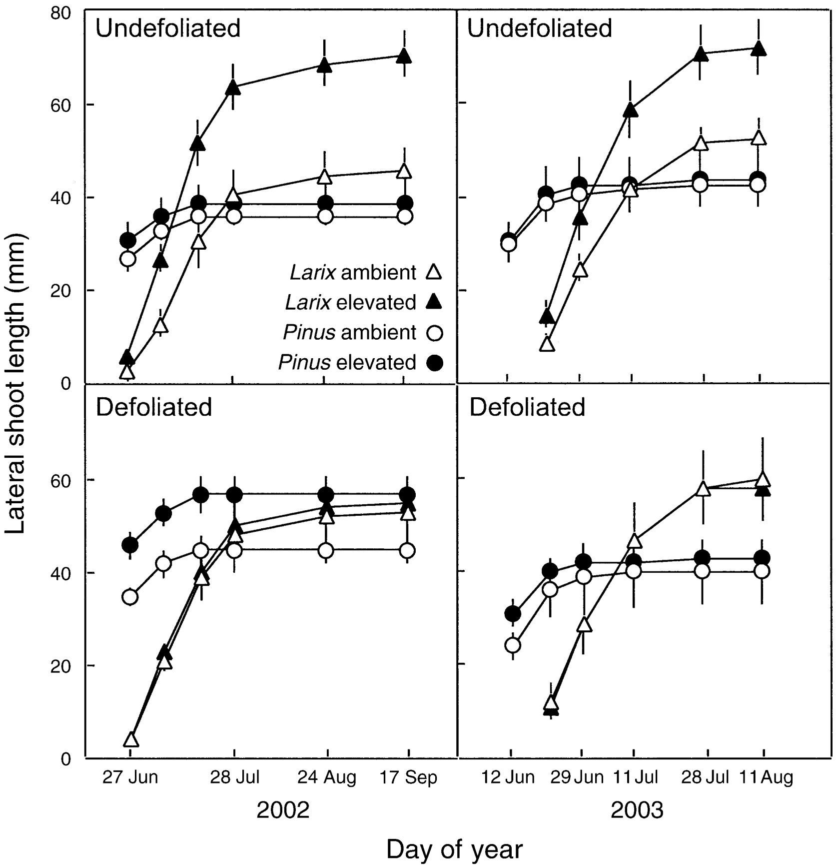

FIG. 2. Length increment of lateral shoots (n = 5) in the years 2002 and 2003 in the undefoliated and defoliated trees of *Larix decidua* and *Pinus uncinata* grown at ambient or elevated concentrations of atmospheric CO$_2$. Data points (mean ± 1 SE) are based on measurements taken from five mid-canopy branches per tree.

abundance and seed production of *Melampyrum pratense*, a hemiparasite annual, increased under elevated CO_2 in our plots (S. Hättenschwiler, *unpublished data*). All together, these observations point to biodiversity effects irrespective of plant functional type.

It is difficult to assess whether the higher CO_2 responsiveness that we observed of larch in contrast to pine is evident in the long-term record at treeline in parallel to the rising atmospheric CO_2 concentrations over the last 150 years. Although larch currently outnumbers pine at our site due to severe fungal attacks on pines since planting (Senn and Schönenberger 2001), the size and mass increment of both species after 23 years was not different (Bernoulli and Körner 1999). Rolland et al. (1998) documented the long-term dendrochronological record for treeline populations of our larch and pine species in the French Alps and found that pine showed brief periods of increasing growth separated by long periods of constant growth, while larch showed a more continuous long-term increasing trend, which was, however, ambiguous to interpret given the frequent (roughly decadal) larch-bud-moth outbreak years. The larch bud moth, which can defoliate entire stands (Baltensweiler et al. 1977, Weber and Schweingruber 1995), highlights the importance of plant–animal interaction factors which may modify growth responses, as demonstrated by the species-specific interactions between defoliation and elevated CO_2 treatments that we report. Furthermore, three years of CO_2 enrichment in a short-season environment may be insufficient to draw firm conclusions on long-term responses of such long-lived trees. In the short term, young trees often grow faster under elevated CO_2 through nutrient dilution or greater nutrient acquisition (Saxe et al. 1998, Norby et al. 1999). In the long term, it has been argued that tree and ecosystem responses will be determined by nutrient availability (Ceulemans et al. 1999, Körner 2003*b*). Several studies have shown that CO_2 effects typically decline over time, in part due to age-related dynamics of the stand leading to increased competition for above- and belowground resources (Hättenschwiler et al. 1997, Idso 1999, Oren et al. 2001, Spinnler et al. 2002). However, even if the CO_2 response in larch is restricted to the juvenile phase, a community composition in favor of larch could nonetheless occur. Collectively, these results suggest that rising atmospheric CO_2 concentrations can influence tree growth at the upper altitudinal limit, with potentially significant consequences for the structure and function of the treeline ecotone.

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3 Conifer stem growth at the altitudinal treeline in response to four years of CO_2 enrichment

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Conifer stem growth at the altitudinal treeline in response to four years of CO_2 enrichment

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Abstract

Northern latitude and upper altitude climatic treelines have received increasing attention given their potential sensitivity to atmospheric and climate change. While greater radial stem growth at treeline sites in recent decades has been attributed largely to increasing temperature, rising atmospheric CO_2 concentration may also be contributing to this growth stimulation. Tree ring increments of mature *Larix decidua* and *Pinus uncinata* were measured over 4 years in a free air CO_2 enrichment experiment at treeline in the Swiss Central Alps (2180 m a.s.l.). In addition, a one-time defoliation treatment in the second year (2002) of the experiment was used to simulate one of the common natural insect outbreak events. In response to elevated atmospheric CO_2, *Larix* showed a cumulative 4-year growth response of +41%, with particularly strong responses in the third and fourth year. This increase in radial stem wood growth was the result of more latewood production, in particular, the formation of larger tracheids, rather than a greater number of cells. In contrast, *Pinus* showed no change in ring width to elevated [CO_2], neither in each of the treatment years, nor in the cumulative response over 4 years, although an increase in tracheid size was observed in the third year. Defoliation led to a pronounced decrease in annual ring width of both species, marked in particular by less latewood production, in the treatment, as well as subsequent years. There was no significant interaction between defoliation and CO_2 enrichment. Although *Pinus* showed no growth response to CO_2, the positive growth response observed in *Larix* after 4 years of CO_2 enrichment implies that the sensitivity of treeline trees to global change may not be purely temperature driven. We conclude that the open sparse canopy in the treeline ecotone favours the indeterminate growth strategy of the early successional *Larix* when neither weather nor carbon are limiting, whereas the later successional *Pinus* does not show any indication of more vigorous growth under future higher atmospheric CO_2 concentrations.

Keywords: dendrochronology, earlywood, elevated CO_2, FACE, *Larix decidua*, latewood, *Pinus uncinata*, ring width, tracheid

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Introduction

Treeline conifers, growing at the edge of the boreal forest or at their climatic limit in mountain systems, have received particular attention in light of potential impacts of climate change, as tree growth at the natural boundary appears to be largely limited by the seasonal mean growing temperature (Körner, 1998; Körner & Paulsen, 2004). Treeline growth records are of exceptional value as annual ring width is highly sensitive to temperature, correlating well with the instrumental climate records (Gindl *et al*., 2000; Paulsen *et al*., 2000; Frank & Esper, 2005) and, thus, useful for example, in the reconstruction of past climate patterns (Schweingruber, 1996; Briffa *et al*., 2002). In the last 150 years, tree ring widths of upper montane (La Marche *et al*., 1984; Graybill & Idso, 1993; Nicolussi *et al*., 1995; Rolland *et al*., 1998; Paulsen *et al*., 2000; Motta & Nola, 2001; Büntgen *et al*., 2005) and arctic treeline conifers (MacDonald *et al*., 1998; Esper & Schweingruber, 2004) have shown an increasing trend. While it has been

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suggested that this increase reflects warming, it has also been argued that rising atmospheric CO_2 concentrations might be contributing to this growth stimulation (Wang *et al*., 2006), especially at high altitude mountain sites because of the lower partial pressure of CO_2 (La Marche *et al*., 1984). However, in addition to increasing temperature and atmospheric CO_2 concentration, other abiotic (e.g. radiation, nitrogen deposition, precipitation) and biotic factors (e.g. forest management, insect outbreaks) are also changing, creating multiple possibilities for confounding interactions (Graumlich, 1991; Grace *et al*., 2002; Trotter *et al*., 2002). Recently, an overall reduced sensitivity of North American and Eurasian treeline dendrochronological records to temperature was reported (Briffa *et al*., 1998), and there has been increasing caution towards assuming uniformitarianism as growth might be mainly limited by one climatic factor at one time and another factor in a subsequent period (Carrer & Urbinati, 2006; Vaganov *et al*., 2006). One case that has received particular attention is the Alaskan and north-west Canadian treeline, where drought seems to have reduced growth in warmer years (Barber *et al*., 2000; D'Arrigo *et al*., 2004; Wilmking *et al*., 2004). The experimental manipulation of such confounding factors is, thus, of key interest in understanding growth responses of treeline conifers to atmospheric and climate change, especially in their younger growth phase (Wang *et al*., 2006).

Owing to fossil fuel burning and land use change, the current atmospheric CO_2 concentration is higher than it has ever been in the last 25 million years and has exceeded more than double that of the glacial minima concentrations of the last 650 000 years (Pearson & Palmer, 2000; Siegenthaler *et al*., 2005). The potential effect of this continuing rise in atmospheric CO_2 concentration on trees, which store close to 85% of all carbon fixed in plant biomass (Saugier *et al*., 2001), has been studied earnestly by the scientific community in the last few decades in experimental growth chambers, open top chambers (OTC; see reviews by Curtis & Wang, 1998; Saxe *et al*., 1998; Ceulemans *et al*., 1999; Norby *et al*., 1999) or more recently, using field-based free air CO_2 enrichment (FACE) technology (Nowak *et al*., 2004; Ainsworth & Long, 2005). As discussed in detail in these reviews, multiple studies have reported biomass stimulation in tree saplings or fast growing plantations in response to CO_2 enrichment, although studies of trees grown under more natural conditions have challenged such projections (Hättenschwiler *et al*., 1997; Körner *et al*., 2005). However, the focus of these studies has been on lowland altitude forest sites and not on the potential response of trees growing at their existential limit such as in the treeline ecotone.

Beyond changes in growth, an elevated atmospheric CO_2 concentration might alter the structural and chemical constituency of conifer tissues with possible implications for herbivores (Zvereva & Kozlov, 2006), the timber and paper industries (Atwell *et al*., 2003; Kostiainen *et al*., 2004; Yazaki *et al*., 2004), the protective function of forests on steep slopes due to altered mechanical stability (Beismann *et al*., 2002), and ecosystem processes such as litter decomposition (Cotrufo & Ineson, 2000). Structural changes in wood can result from changes in meristematic activity and differentiation processes that can change density or water conduits (Schweingruber, 1996). In response to elevated CO_2 concentrations, some conifers have shown increased wood density (Hättenschwiler *et al*., 1996; Atwell *et al*., 2003), although this response is not consistent (Telewski *et al*., 1999; Ceulemans *et al*., 2002; Kilpeläinen *et al*., 2005). Studies of wood anatomical properties under elevated CO_2 concentration have shown that tracheid formation can increase (Yazaki *et al*., 2001, 2004; Ceulemans *et al*., 2002), decrease (Kostiainen *et al*., 2004) or show no change (Telewski *et al*., 1999). Similarly, cells might produce thicker walls (Yazaki *et al*., 2001) or show no difference in cell wall thickness under elevated CO_2 concentration (Kostiainen *et al*., 2004; Yazaki *et al*., 2004).

The establishment of a FACE experiment at the treeline in the Swiss Central Alps has made it possible to study the effect of elevated CO_2 concentration on tree growth of two ca. 30-year-old conifer species, *Larix decidua* and *Pinus uncinata* under natural conditions in an undisturbed treeline ecotone (Hättenschwiler *et al*., 2002). In an earlier contribution, we reported shoot increment growth data and tree physiological responses to 3 years of CO_2 enrichment in combination with a one-time defoliation event (Handa *et al*., 2005). In this study, we focus on tree ring responses and tracheid characteristics after 4 years of CO_2 enrichment and a one-time defoliation event (simulating an extreme natural disturbance) to understand (1) if growth of these trees is changed in a future CO_2 enriched atmosphere, (2) how tree growth might be modified by defoliation events and (3) if tracheid characteristics are influenced by elevated CO_2 according to, or independently of, any changes in stem diameter increment.

Materials and methods

Study site and treatment descriptions

The treeline FACE site was established in 2001 at Stillberg, Davos (Central Alps, Switzerland) on a NE-exposed 25–30° slope and has been described in detail by Hättenschwiler *et al*. (2002) and Handa *et al*. (2005). In the upper end (2180 m a.s.l.), corresponding to or slightly above the natural climatic treeline) of the long-term afforestation research area planted in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research, trees are not taller than ca. 1.5 m and form a sparse open canopy with a dense understory vegetation composed dominantly of dwarf shrubs such as *Vaccinium myrtillus*, *V. uliginosum*, *Empetrum hermaphroditum*, and common herbaceous species such as *Gentiana punctata*, *Homogyne alpina* and *Melampyrum pratense*.

In early June 2001, 20 trees each of *L. decidua* L. (27-year-old), the European larch, and *P. uncinata* Ramond (29-year-old), the mountain pine, were selected based on the following criteria for the experiment: one dominant stem, no serious signs of disease or herbivory, not more than one close (<80 cm) neighbouring tree, a distance of at least 2 m between each FACE tree, and a total tree height of 0.8–1.5 m. Using a split-plot approach, these 40 trees were assigned to 10 groups of four neighbouring trees (two each of *Larix* and *Pinus* trees per group) in order to facilitate the logistics of CO_2 distribution and regulation. Half of these groups were randomly assigned to an elevated CO_2 treatment (target concentration of $550\,\mu mol\,mol^{-1}$), while the remaining groups served as controls at ambient CO_2 concentration (ca. $370\,\mu mol\,mol^{-1}$). The CO_2 technology in use at our FACE site released pure CO_2 during daytime hours through laser-punched drip irrigation tubes hung vertically around the hexagonal ring surrounding each tree and lifted accordingly throughout the experiment to compensate for growth when required. Temporal and spatial variability of concentrations was measured and regulated at the group level (for more details on the system and its performance, see Hättenschwiler *et al.*, 2002). Here, we report the effects of CO_2 enrichment treatment for the first four growing seasons (ca. 15 June–15 September 2001–2004) during the daylight hours. Under poor weather conditions (e.g. photon flux density $<100\,\mu mol\,m^{-2}\,s^{-1}$, wind speeds $>75\,km\,h^{-1}$, temperatures $<5\,°C$, snow or sleet), we interrupted CO_2 release because of the enormous cost of CO_2 delivery. Technical failure (<5%) or such weather conditions meant that trees received CO_2 enrichment for 75%, 81%, 73% and 72% of the 2001–2004 respective seasonal treatment periods. Seasonal mean CO_2 concentrations in CO_2 enriched plots (2001–2004; ±SD; $n = 20$ measurement channels) were 566 ± 42, 582 ± 35, 579 ± 52 and $600 \pm 80\,\mu mol\,mol^{-1}$ for the CO_2 dispensing period only. Ambient CO_2 concentrations during this period ranged from 370 to $377\,\mu mol\,mol^{-1}$.

In late June of the second year of treatment (2002), following bud break but before full needle extension, half of the trees in each group of both species and CO_2 treatments were assigned to an 80% defoliation treatment ($n = 5$ per level of species, CO_2 and defoliation). *Larix* was defoliated by systematically cutting four out of every five short shoots above the meristem throughout the entire tree, while all new long shoots were left intact. *Pinus* was defoliated by cutting 80% of the proximal needles away within each of four age classes from 1999–2002 on the entire tree, while the distal 20% of the needles within each age class were left untouched (Handa *et al.*, 2005).

Tree coring and measurements

All 40 trees were cored with a 2 mm diameter increment puncher (Hättenschwiler *et al.*, 1996; Forster *et al.*, 2000) in late October 2003 (two cores per tree) and 2004 (one core per tree). Trees were always cored on the uphill side of the tree to avoid compression wood and the core was taken ca. 50 cm above tree base. Cores were mounted on wood, gently cut (moist) with a microtome and stained with 1% safranin to enhance the contrast. All measurements were made using an Olympus BX51 microscope (Olympus Schwelz AG, Volketswil, Switzerland) with UMPlanFL-BD universal objectives connected to an Olympus Camedia C3040-ADU zoom digital camera (Olympus Optical GmbH Europe, Hamburg, Germany) and personal computer where images (1600 × 1200 × 24 pixels) were processed with the software analySIS 3.0 (Soft Imaging System GmbH, Münster, Germany). Tree ring width measurements were done at a total magnification of ×50 or ×100, whichever allowed for a clear differentiation between earlywood and latewood segments, the latter defined as two times double wall thickness equal or greater than the width of the lumen (Denne, 1988). All ring widths from 1997 to 2003 are based on an average width of three cores per tree.

Tracheid characteristics were measured at a total magnification of ×200 within earlywood and latewood segments separately for the 20 undefoliated trees only. In order to count cell number, a 200 × 200 pixel grid was randomly fitted three times into the earlywood or latewood segment and the total number of cells falling within the grid were averaged. Five tracheids each for earlywood and latewood were randomly sampled and the contour cross-sectional area including the cell wall was drawn freehand using the computer software in order to estimate cell size.

Annual shoot length increment was measured at the end of each growing season (to the nearest millimetre) of five mid-canopy lateral branches per tree. On each branch in 2001, the length of the shoot increment in the year before CO_2 enrichment (2000) was measured as a covariable for pretreatment tree vigour.

Weather data were collected at a long-term weather station on Stillberg of the Swiss Federal Institute for

Forest, Snow and Landscape Research, slightly below our FACE site (2080 m a.s.l.).

Statistical analysis

Repeated measures analyses of covariance using a mixed effect linear model for the split plot design were used to test for overall growth responses to CO_2 enrichment and defoliation on log transformed tree ring width and shoot increment data after testing for normality and equal variance. Covariables that accounted for pretreatment differences in tree vigour consisted of mean ring width from 1997 to 2000 and shoot increment length in 2000. As tree species and year emerged as strong effects in the full model, tests were also carried out by species and year separately. Pearson's product moment correlation was used to test for the relation between weather variables and ring width every year. All analyses were performed with R (version 1.9.1, R Development Core Team, 2004). In the tree ring record, no outliers were observed in *Larix* ($n = 5$ for each treatment combination), but one defoliated *Pinus* growing at elevated CO_2 was removed for the entire chronology as we probably cored a stem segment with reaction wood only (ring widths were more than double all other trees) and a second undefoliated *Pinus* growing at ambient CO_2 showed signs of sickness and was removed in 2004 only. In the cumulative shoot record, some trees were identified as outliers at a certain time interval during the 4-year experimental period due to bud injury events that can produce inflated estimates of shoot length in *Larix*. Sample sizes were reduced from $n = 5$ to 3 (undefoliated) and $n = 4$ (defoliated) for *Larix* growing at ambient CO_2 concentrations in such cases. In *Pinus*, there was stunted shoot growth in one case due to herbivory damage from the black grouse, *Tetrao tetrix*, and in another case, due to a fungal attack. Sample sizes were thus reduced to $n = 4$ for both undefoliated *Pinus* at elevated CO_2 concentration and defoliated *Pinus* at ambient CO_2 concentration.

Results

Ring width response

The punched cores allowed us to measure ring widths from 1997 to 2004, that is 4 years before the initiation of CO_2 enrichment and 4 years of treatment response. There was a trend of increasing radial growth in both species irrespective of treatment in the last 4 years (Fig. 1). *Larix* showed double and *Pinus* one quarter more growth in ring width when compared with the previous 4 years. This increase in ring width did not show any significant correlation with the measured climate variables during a specific summer, although the summer 2003 was notably much warmer than any other (Fig. 1).

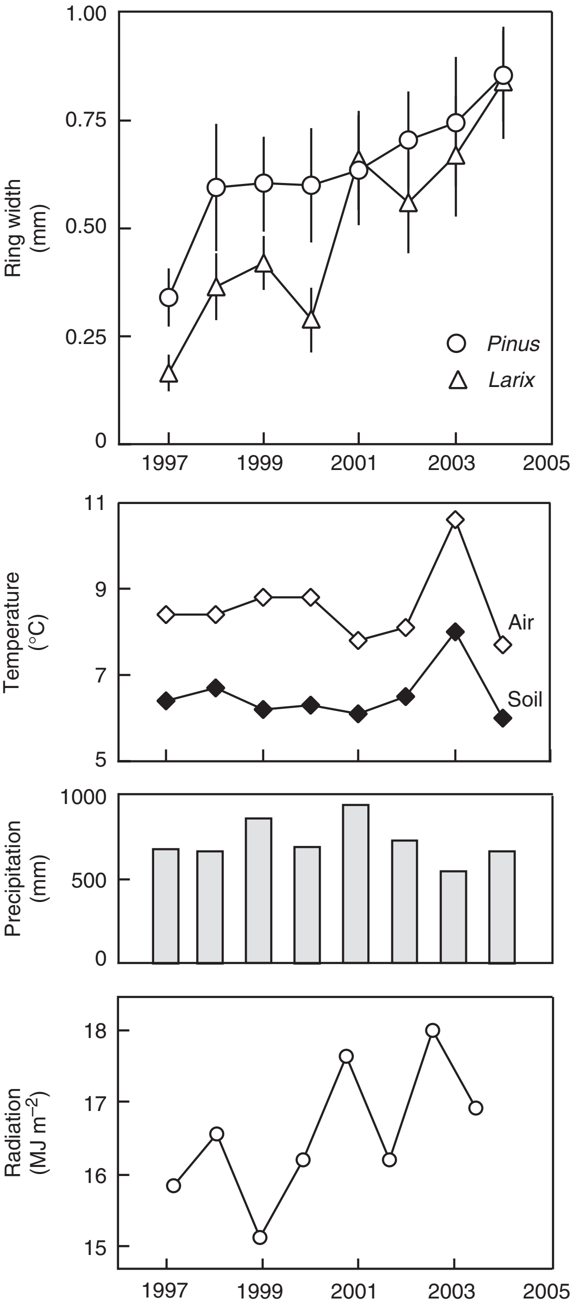

Fig. 1 Mean annual ring width (±SE, $n = 5$) of *Larix decidua* and *Pinus uncinata* grown at 2180 m a.s.l. under ambient CO_2. Temperatures (air at 2 m and soil at −10 cm), radiation (daily 24 h sums) and precipitation were recorded at a climate station at 2080 m a.s.l. (data courtesy of A. Streule). Seasonal means and cumulative precipitation are reported (1 May–30 September).

Ring width was significantly affected by all factors in the experiment (Tables 1 and 2), namely CO_2, defoliation, tree species and year. The CO_2 response interacted

Table 1 Summary of repeated measures mixed effects linear model testing for differences across *Larix decidua* and *Pinus uncinata* in the growth parameters annual lateral shoot length increment and total ring width following 4 years of CO_2 enrichment (2001–2004) and a one-time defoliation treatment (after snowmelt in 2002)

	Shoot length increment			Ring width	
	df	*F*	*P*	*F*	*P*
Covariable	1, 15	53.58	< 0.001***	15.57	0.001***
CO_2	1, 8	3.60	0.094$^{(*)}$	6.71	0.032*
Defoliation	1, 8	1.09	0.327	16.91	0.003**
Defoliation × CO_2	1, 8	1.77	0.220	0.27	0.621
Species (Sp)	1, 15	40.81	< 0.001***	10.95	0.005**
CO_2 × Sp	1, 15	0.25	0.627	0.06	0.806
Defoliation × Sp	1, 15	2.21	0.158	0.02	0.889
CO_2 × Defoliation × Sp	1, 15	2.23	0.156	1.47	0.243
Year	3, 91	16.34	< 0.001***	6.97	< 0.001***
CO_2 × Year	3, 91	0.91	0.439	0.74	0.529
Defoliation × Year	3, 91	3.07	0.032*	13.05	< 0.001***
Species × Year	3, 91	4.22	0.008**	7.18	< 0.001***
CO_2 × Defoliation × Year	3, 91	0.51	0.673	0.28	0.837
CO_2 × Species × Year	3, 91	0.40	0.752	2.28	0.085$^{(*)}$
Defoliation × Species × Year	3, 91	2.25	0.088$^{(*)}$	0.98	0.404
CO_2 × Defoliation × Species × Year	3, 91	1.80	0.153	0.63	0.597

Pretreatment tree growth is used as a covariable in the analyses.
$^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$, $^{(*)}P<0.1$.

marginally with species and year and was driven by the undefoliated *Larix* trees which showed a clear and increasing relative ring width response to elevated atmospheric CO_2 both when depicted annually (Fig. 2, Table 2a) or cumulatively as an integrated measure over the four experimental years (+41%; Fig. 3). *Pinus* trees did not grow wider rings in response to elevated CO_2 in any year or cumulatively (Figs 2 and 3, Table 2b).

Defoliation had a pronounced effect on relative ring width of both species (Table 1, Fig. 2) showing a reduction in the year of defoliation (2002) and an even more pronounced growth reduction in the subsequent year. The cumulative reduction in relative ring width in response to defoliation was −20% in *Larix* and −27% in *Pinus* (Fig. 3).

Ring width response to the treatments was reflected in the annual shoot increment (Table 1, Fig. 3). Although CO_2 and defoliation effects were less pronounced for annual shoot length increment than for ring width, there were equally strong species and year effects and interactions between defoliation and year that were observed (Table 1). The only significant effect of CO_2 on shoot increment, as in the ring width response, was found in undefoliated *Larix* trees (Table 1, + 15% expressed as cumulative relative growth over 4 years, Fig. 3).

Tracheid characteristics

The ring width response to both CO_2 and defoliation treatments was driven largely by differences in latewood formation (Table 2). In undefoliated *Larix* trees, elevated atmospheric CO_2 resulted in ca. 40–60% wider latewood in 2003 and 2004 (Table 2a). These wider latewood rings are likely the result of larger cells rather than more cells being produced; in 2003, fewer tracheids per unit area were measured and there was a trend towards greater lumen area in tracheids of the latewood produced under elevated CO_2 (Table 3). In 2003, earlywood width, in addition to latewood width, was affected by elevated CO_2 showing ca. 30% wider rings in undefoliated *Larix* (Table 2a).

Although ring width of *Pinus* did not increase under elevated CO_2, there was a marginally significant effect of elevated CO_2 on tracheid number in 2001 where 20% less cells per unit area of earlywood were counted (Table 3). In 2002, a significant effect on the earlywood to latewood ratio was observed (Table 2b), likely driven by a greater earlywood fraction in defoliated *Pinus* grown under elevated atmospheric CO_2, compared with all other treatment combinations. Similarly, in that same year, a marginally significant CO_2 × defoliation interaction on total ring width of *Pinus* was also observed (Table 2b) where trees growing at an elevated CO_2 concentration did not show a growth reduction to defoliation like observed in ambient grown trees. In the latewood of 2003, tracheids of *Pinus* growing under elevated atmospheric CO_2 had 26% greater cell area than those in the latewood of ambient grown trees (Table 3). Defoliation also significantly reduced

Table 2 Average tree ring width characteristics (±SE) and analysis of covariance for (a) *Larix decidua* and (b) *Pinus uncinata* trees exposed to four years of elevated CO_2 concentrations and a one-time defoliation treatment early in the second season)

Wood characteristic	Year	Ambient CO_2	Elevated CO_2	Ambient CO_2, defoliated	Elevated CO_2, defoliated	Pretreatment growth (1997–2000) df = 1, 7		CO_2 df = 1, 8		Defoliation df = 1, 7		CO_2 × Defoliation df = 1, 7	
						F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
(a) Larix deciduas													
Total ring (μm)	mean 1997–2000	311 ± 64	332 ± 57	364 ± 138	372 ± 66								
	2001	660 ± 114	797 ± 147	745 ± 144	792 ± 218	5.64	<0.05*	0.05	0.837	0.05	0.835	0.28	0.613
	2002	563 ± 115	647 ± 92	493 ± 91	572 ± 208	5.42	0.05*	0.04	0.849	1.68	0.236	0.27	0.622
	2003	671 ± 137	1034 ± 139	270 ± 47	393 ± 50	0.11	0.755	4.16	0.076	149	<0.001***	0.2	0.587
	2004	840 ± 128	1325 ± 147	619 ± 151	861 ± 63	0.86	0.385	4.05	0.079	13.2	<0.01**	0.00	0.974
Earlywood (μm)	mean 1997–2000	184 ± 40	209 ± 39	231 ± 100	245 ± 53								
	2001	363 ± 73	486 ± 91	523 ± 126	530 ± 195	5.99	<0.05*	0.00	0.998	0.05	0.832	0.94	0.364
	2002	333 ± 92	374 ± 50	369 ± 82	442 ± 188	4.49	0.072	0.01	0.929	0.02	0.891	0.27	0.621
	2003	367 ± 65	551 ± 72	143 ± 21	207 ± 36	2.06	0.194	4.23	0.074	292	<0.001***	0.62	0.458
	2004	491 ± 101	850 ± 144	384 ± 97	492 ± 62	1.03	0.343	3.03	0.120	9.53	<0.05*	0.43	0.533
Latewood (μm)	mean 1997–2000	127 ± 29	123 ± 21	133 ± 42	127 ± 17								
	2001	297 ± 62	311 ± 63	222 ± 32	262 ± 29	1.93	0.207	0.48	0.510	1.59	0.249	0.18	0.687
	2002	230 ± 35	273 ± 44	124 ± 17	130 ± 22	7.82	<0.05*	0.51	0.496	27.1	0.001**	0.35	0.572
	2003	304 ± 73	483 ± 71	126 ± 26	186 ± 24	2.05	0.196	4.07	0.078	48.4	<0.001***	0.31	0.648
	2004	349 ± 70	475 ± 46	234 ± 72	370 ± 25	25.7	0.001**	9.58	0.01**	13.9	<0.01**	1.31	0.289
Earlywood : latewood ratio	mean 1997–2000	1.40 ± 0.48	1.72 ± 0.42	1.79 ± 0.74	1.95 ± 0.59								
	2001	1.22 ± 0.35	1.56 ± 0.43	2.36 ± 0.66	2.03 ± 0.78	3.05	0.124	0.59	0.465	1.53	0.256	2.21	0.181
	2002	1.44 ± 0.46	1.37 ± 0.29	2.98 ± 0.78	3.39 ± 1.55	1.13	0.323	0.10	0.757	14.7	<0.01**	0.04	0.854
	2003	1.21 ± 0.36	1.14 ± 0.22	1.13 ± 0.28	1.12 ± 0.24	2.48	0.160	2.29	0.168	1.15	0.319	0.00	0.958
	2004	1.41 ± 0.41	1.79 ± 0.35	1.64 ± 0.65	1.33 ± 0.19	2.69	0.145	0.13	0.731	0.07	0.797	1.57	0.251

Wood characteristic	Year	Ambient CO_2	Elevated CO_2	Ambient CO_2, defoliated	[†]Elevated CO_2, defoliated	Pretreatment growth (1997–2000) df = 1, 6		CO_2 df = 1, 8		Defoliation df = 1, 6		CO_2 × Defoliation df = 1, 6	
						F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
(*b*) *Pinus uncinata*													
Total ring width (µm)	1997–2000	537 ± 115	644 ± 113	437 ± 92	663 ± 146								
	2001	638 ± 124	679 ± 101	413 ± 75	992 ± 176	28.3	<0.01**	0.50	0.499	0.21	0.660	3.73	0.102
	2002	707 ± 108	787 ± 99	411 ± 78	979 ± 108	40.0	<0.001***	1.74	0.224	2.12	0.195	4.32	0.083
	2003	749 ± 146	830 ± 71	302 ± 57	776 ± 340	10.3	<0.05*	0.02	0.905	16.9	<0.01**	0.02	0.887
	2004	703 ± 171	923 ± 178	360 ± 104	707 ± 187	8.5	<0.05*	0.08	0.785	2.22	0.187	0.10	0.765
Earlywood width (µm)	1997–2000	396 ± 102	480 ± 85	336 ± 87	518 ± 139								
	2001	487 ± 119	522 ± 78	324 ± 74	867 ± 185	21.8	<0.01**	0.67	0.438	0.06	0.817	2.78	0.147
	2002	527 ± 101	629 ± 80	326 ± 77	829 ± 86	40.5	<0.001***	2.40	0.160	1.61	0.251	3.67	0.104
	2003	515 ± 109	605 ± 58	220 ± 47	637 ± 324	9.51	<0.05*	0.14	0.723	16.0	<0.01**	0.10	0.767
	2004	519 ± 134	691 ± 138	279 ± 90	582 ± 171	10.7	<0.05*	0.07	0.792	1.99	0.208	0.11	0.753
Latewood width (µm)	1997–2000	141 ± 16	163 ± 34	101 ± 12	150 ± 15								
	2001	151 ± 12	157 ± 29	89 ± 7	125 ± 14	41.0	<0.001***	0.14	0.716	5.08	0.065	3.86	0.097
	2002	179 ± 26	158 ± 21	85 ± 8	150 ± 23	16.1	<0.01**	0.46	0.517	6.27	0.046	2.98	0.135
	2003	234 ± 47	226 ± 21	83 ± 12	138 ± 30	18.7	<0.01**	0.68	0.434	11.2	<0.05*	0.06	0.810
	2004	184 ± 39	232 ± 40	81 ± 32	125 ± 21	7.00	<0.05*	0.10	0.758	4.82	0.070	0.05	0.831
Earlywood : latewood ratio	1997–2000	2.77 ± 0.79	2.92 ± 0.79	3.27 ± 0.94	3.37 ± 0.91								
	2001	3.23 ± 0.83	3.33 ± 0.79	3.64 ± 0.88	6.96 ± 1.66	11.3	<0.05*	0.78	0.402	0.60	0.466	0.47	0.519
	2002	2.94 ± 0.71	4.00 ± 0.75	3.84 ± 0.98	5.53 ± 1.02	43.6	<0.001***	5.75	<0.05*	0.52	0.499	0.20	0.669
	2003	2.21 ± 0.64	2.68 ± 0.35	2.66 ± 0.69	4.60 ± 2.55	8.98	<0.05*	0.09	0.773	0.15	0.708	2.09	0.198
	2004	2.82 ± 0.95	2.98 ± 0.78	3.45 ± 1.78	4.66 ± 1.57	11.3	<0.05*	0.15	0.706	0.19	0.677	0.24	0.641

The mean of the pretreatment growth from 1997–2000 is used as a covariable in the analysis. Sample size was $n = 5$, [†]exception $n = 4$.
***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, $^{(*)}P < 0.1$.

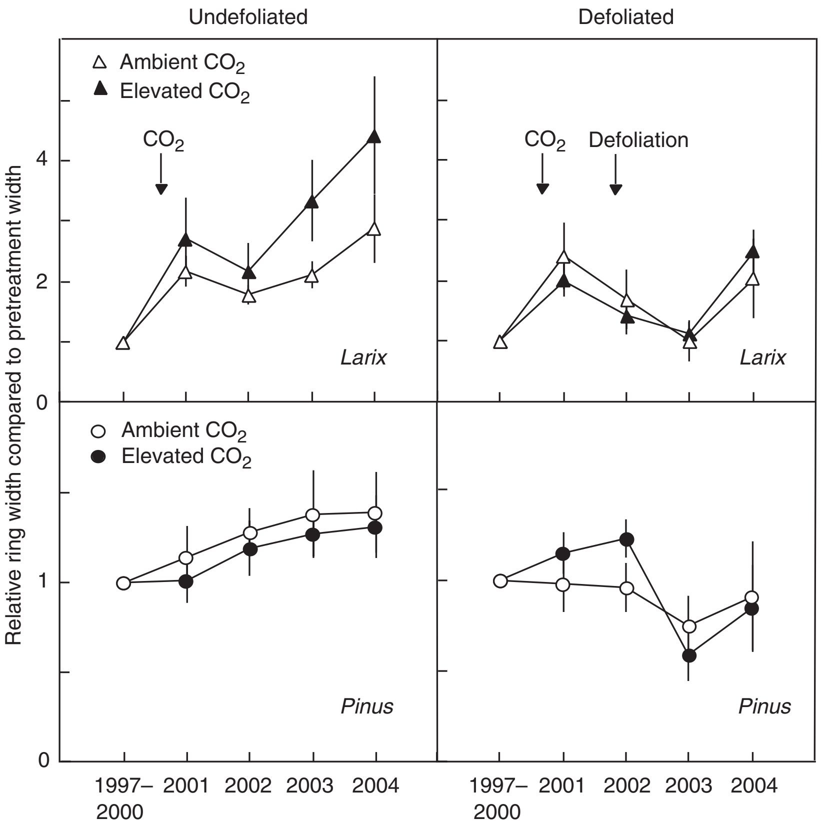

Fig. 2 Relative tree ring width of *Larix decidua* (top) and *Pinus uncinata* (bottom) expressed as a proportion of the mean ring width of 1997–2000 of each tree (± SE, $n = 5$ except defoliated + elevated *Pinus* where $n = 4$). Trees were grown at alpine treeline (2180 m a.s.l.) under ambient (open symbols) or elevated (closed symbols) CO_2 conditions (treatment began after snowmelt in 2001 as indicated by the arrows). Defoliated trees (right) were subject to a one-time 80% foliage removal in June 2002.

earlywood production by 57% in *Pinus* trees in 2003 (Table 2b).

The defoliation treatment reduced latewood width in both species in the year of the treatment (2002) and both subsequent years (Tables 2a and b). In *Larix*, defoliation significantly reduced latewood width by ca. 40–60% (Table 2a), in *Pinus*, latewood width was reduced by ca. 50–65% (Table 2b). However, we urge caution in the interpretation of this latter result as the random group of *Pinus* trees that received the defoliation treatment already showed, by coincidence, less latewood production (marginally significant) in 2001, the year before the defoliation treatment.

Discussion

Species-specific CO_2 response

While trees have been argued to be the most responsive functional plant group to elevated CO_2 (Ainsworth & Long, 2005), it has equally been emphasized that species identity, tree age and resource availability are important factors determining whether a CO_2 response is observed at all (Nowak *et al*., 2004; Körner *et al*., 2005). Following 4 years of FACE, we found that *L. decidua* shows a positive ring width stimulation to elevated CO_2 concentration while *P. uncinata* does not, reflecting the same species specific response that has been previously observed in the shoot increment record of the first three experimental years at our site (Handa *et al*., 2005). When contrasted to other studies on CO_2 responses in stem wood increment or biomass production of conifers, responses reported across young and old individuals are varied. There are also differences depending on nutrient regimes, as well as CO_2 experimental treatment type (i.e. phytotron, OTC or FACE studies), making it challenging to formulate broad patterns. As equally noted in a recent meta-analysis on all plant functional types to CO_2 enrichment by Poorter & Navas (2003), no clear response pattern appears to exist across deciduous or evergreen species, maybe because responses have not been ranked by the nutritional status of the test plants (Körner, 2003a).

Studies in which conifers showed a clear ring width or biomass response to elevated CO_2 treatment, like we observe after four years in *L. decidua*, include a 4-year

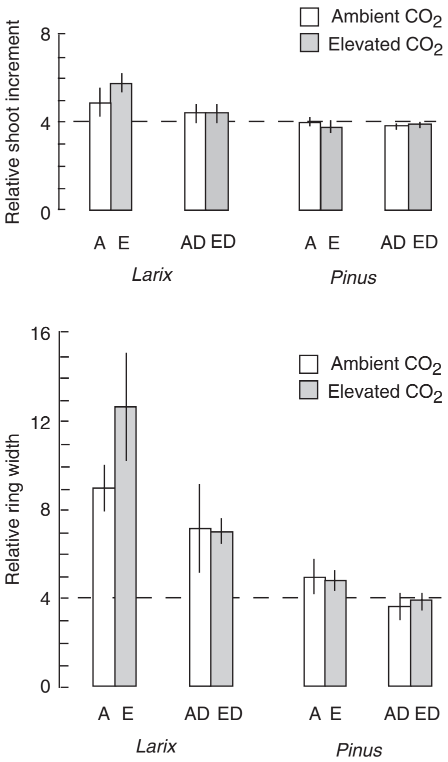

Fig. 3 Relative cumulative growth (2001–2004) for trees grown under ambient (A) or elevated (E) CO_2 conditions or with a one-time defoliation treatment (D) in 2002 (± SE, $n = 3$–5 depending on the treatment combination). Shoot increment is expressed relative to shoot increment in 2000, while ring width is expressed relative to a mean of 1997–2000 ring widths. The dashed line indicates what the theoretical value would be if there was equal growth for 4 years relative to the measured pretreatment year.

OTC experiment of *P. taeda* seedlings growing under nonlimiting nutrient conditions (Telewski *et al.*, 1999), as well as a 4-year study starting with 13-year-old *P. taeda* growing on old fields (Hamilton *et al.*, 2002), a 3-year OTC study starting with 3-year-old *P. sylvestris* (Ceulemans *et al.*, 2002) and a 4-year phytotron study starting with 1-year-old *P. sylvestris* (Ziche & Overdieck, 2004), both growing in unamended but fertile soils, and a 4-year model ecosystem study starting with 2- to 3-year-old *Picea abies* grown in natural forest soils of low and high fertility (Spinnler *et al.*, 2002). In contrast, conifer studies showing no significant response to CO_2 enrichment, like we observe in *P. uncinata*, include a 4-year OTC study starting with 2-year-old *Pseudotsuga menziesii* under low nutrients (Olszyk *et al.*, 2005), a 3-year model ecosystem study of 4-year-old *P. abies* under low and high nutrients (Hättenschwiler *et al.*, 1996), a 4-year OTC study of varying provenances of *P. sylvestris* and *P. abies* seedlings under nonlimiting nutrient conditions (Vanhatalo *et al.*, 2003) and two phytotron studies on *Larix* species, *L. sibirica* and *L. kampferi* seedlings, under low and high fertility (Yazaki *et al.*, 2001, 2004). Four further studies where no overall significant growth response to elevated CO_2 is reported (although singular year effects or marginal effects were observed) include a 3-year OTC study of tissue cultured *P. radiata* seedlings under low and high nutrients where a significant effect was observed under high nutrients in the first year only (Atwell *et al.*, 2003), a 6-year OTC study of *P. ponderosa* seedlings under varying N levels where an increase in biomass was also observed in the first year only (Johnson *et al.*, 2006), a 3-year OTC study of 15-year-old *P. sylvestris* under low nutrients where although no significant effect was reported due to large variance, a doubling of radial growth was observed (Kilpeläinen *et al.*, 2003), and a 3-year whole tree chamber study of 41-year-old *P. abies* where significantly wider rings were only observed in the second year of a 3-year experiment under low nutrient treatment (Kostiainen *et al.*, 2004).

Why results of CO_2 enrichment studies are inconsistent across species remains one of the key questions in our understanding of this aspect of global atmospheric change. Loehle (1995) suggested that these anomalies in CO_2 responses might be best understood by considering results within a life history framework of the species in question, in particular, whether or not natural selection might have favoured a risk-averse strategy with consequent unresponsiveness to nutrient enrichment (in harsh environments) or rather, might have favoured maximal growth responsiveness (in naturally enriched environments). The treeline ecotone is considered in many respects a harsh environment (Körner, 1998), such that the lack of responsiveness to CO_2 by *Pinus*, the more classic of the two treeline genera in our study and which is arguably not carbon limited according to a global comparison of treeline *Pinus* (Hoch & Körner, 2003), would fit this interpretation. Vanhatalo *et al.* (2003) also found less responsiveness in the northern (more harsh environment) of two provenances of 10-year-old *P. sylvestris* in their 4-year OTC study in Finland. In the only other long term *in situ* test of elevated CO_2 concentrations in harsh high mountain conditions (a *Carex* sward at 2500 m a.s.l. in the Alps), no positive growth effect or interaction with nutrient or temperature was observed over 4 years (Körner *et al.*, 1997), also supporting such an interpretation. *Larix*, on the other hand, although it occurs in the alpine and Siberian treeline under extreme conditions, is a typical pioneer, fast-growing, conifer that colonizes raw substrate on fresh glacier moraines or after disturbances such as avalanches, landslides or fire; a life strategy perhaps favouring a rapid responsiveness to CO_2 enrichment. Another possibility to explain the positive CO_2 growth response that we report in *Larix*, might be that as trees

Table 3 Tracheid characteristics for *Larix decidua* and *Pinus uncinata* trees (± SE, $n = 5$) exposed to 4 years of elevated CO_2 concentrations

Tracheid characteristic	Year	Ambient CO_2	Elevated CO_2
L. deciduas			
Cell number per fixed area in earlywood	2001	15 ± 2	13 ± 2
	2002	14 ± 2	14 ± 2
	2003	13 ± 2	13 ± 2
Cell number per fixed area in latewood	2001	25 ± 2	24 ± 2
	2002	24 ± 2	22 ± 2
	2003	25 ± 2	21 ± $2^{(*)}$
Cell area ($pixel^2$) in earlywood	2001	2100 ± 105	2279 ± 435
	2002	2357 ± 137	2100 ± 258
	2003	2624 ± 340	2351 ± 209
Cell area ($pixel^2$) in latewood	2001	811 ± 16	932 ± 76
	2002	955 ± 59	943 ± 87
	2003	904 ± 77	1035 ± 96
P. uncinata			
Cell number per fixed area in earlywood	2001	20 ± 2	16 ± $2^{(*)}$
	2002	20 ± 2	18 ± 2
	2003	19 ± 2	17 ± 2
Cell number per fixed area in latewood	2001	30 ± 2	25 ± 2
	2002	26 ± 2	26 ± 2
	2003	27 ± 2	27 ± 2
Cell area ($pixel^2$) in earlywood	2001	1551 ± 75	1687 ± 85
	2002	1687 ± 194	1967 ± 172
	2003	1595 ± 210	1937 ± 108
Cell area ($pixel^2$) in latewood	2001	1016 ± 84	965 ± 46
	2002	1016 ± 80	1060 ± 75
	2003	847 ± 74	1068 ± 14*

One-way analysis of variance results are shown ($F_{1,8}$).
$^*P<0.05$, $^{(*)}P \leq 0.1$.

are growing spatially isolated from one another, there is no steady state leaf area index of the tree canopy and, hence, differences due largely to accelerated above-ground growth in the early stages of the experiment (Loehle, 1995) can result in compound interest effects (Norby *et al.*, 1999; Körner, 2003b), also noted explicitly by Spinnler *et al.* (2002) and Atwell *et al.* (2003) in their above-mentioned experiments. These studies, however, differ from ours in so much that they started with planting young plants in their model systems and showed strong significant CO_2 growth responses in the early phases of CO_2 enrichment. Our trees were planted ca. 30 years before the beginning of experimental CO_2 enrichment and the stem increment data for *Larix* that we report here do not show a significant CO_2 response in the first 2 years of the experiment.

Defoliation

Our 80% defoliation treatment in 2002, which created an extreme disturbance similar to an insect outbreak event, resulted in a cumulative reduction in relative ring width of −20% in *Larix* and −27% in *Pinus* trees evident in the treatment and subsequent years (Figs 2 and 3). The decrease in ring width we observed was more substantial than what was observed in a 66% defoliation study with 10-year-old saplings of *L. decidua* and *P. resinosa*: stem radial growth was not at all reduced in *L. decidua*, but reduced by −12% after 2 months in *P. resinosa* (Krause & Raffa, 1996). Defoliation outbreak events are known to occur cyclically in many conifer species and show clear signals in the dendrochronological record; i.e. the larch bud moth, *Zeiraphera diniana*, and larch sawfly, *Prostiphora erichsonii*, on *Larix* species (Weber, 1997; Rolland *et al.*, 2001; Girardin *et al.*, 2005; Nola *et al.*, 2006), the spruce bud worm, *Choristoneura* sp., and spruce bark beetle, *Dendroctonus micans* on *Picea* species (Weber & Schweingruber, 1995; Jardon *et al.*, 2003; Rolland & Lemperiere, 2004) or the pandora moth, *Coloradia pandora* and pine processionary moth, *Thaumetopoea pityocampa*, on *Pinus* species (Laurent-Hervoüet, 1986; Speer *et al.*, 2001). Although the latter moth examples do not occur on treeline conifers, recent studies have suggested that under warming scenarios, the range of *Thaumetopea*, for example, could expand (Battisti *et al.*, 2005). Our factorial experimental design allowed us to test the combined effects of defoliation and CO_2 enrichment on ring width of both species. Although the shoot increment record previously suggested an interaction of elevated CO_2, defoliation and species (Handa *et al.*, 2005), no such interaction is apparent in the more robust and longer term stem growth record that we present in the full model or in any of the individual 4 years for *Larix* or *Pinus* (Tables 1 and 2). Effects of CO_2 were more pronounced in defoliated treatments in 2002 for *Pinus*, where CO_2-enriched defoliated trees produced wider rings than CO_2-enriched undefoliated trees. However, this tree ring response must be interpreted with caution as a marginally significant interaction is observed in the year before any defoliation treatment (Table 2b), thus, suggesting that the stem growth response of trees to elevated CO_2 is not altered by an extreme defoliation event in contrast to what we previously reported for shoot length increment (Handa *et al.*, 2005).

Tracheid characteristics

As a result of changes in radial growth, elevated atmospheric CO_2 concentration could change the structure and chemical constituency of conifer wood through altered cell differentiation processes with effects on cell lumen area, cell wall thickness and lignification of the cell wall, all of which are known to be subject to the influence of climatic variability (Schweingruber, 1996; Gindl *et al.*, 2000). We found that responses to both treatments were driven primarily by changes in latewood properties (Table 2). Wider latewood rings were measured in both 2003 and 2004 in *Larix* in response to CO_2 enrichment, while in response to defoliation, both species produced only half as wide latewood rings as in undefoliated trees. In the wider latewood rings in CO_2 enriched *Larix* trees, fewer tracheids per unit cross-sectional area were measured in 2003 implying larger tracheid diameter (Table 3). Although no changes in ring width were observed in CO_2 enriched *Pinus*, tracheids in the latewood in 2003 had one third greater cell area than ambient grown trees (Table 3). Studies of wood anatomical properties under elevated CO_2 concentration have shown the formation of larger tracheids in *P. sylvestris* (Ceulemans *et al*., 2002), as well as in *L. kampferi* and *L. sibirica*, which in the latter case was accompanied by a decrease in cell wall thickness (Yazaki *et al*., 2001, 2004). In *P. abies*, however, tracheid size tended to decrease in response to elevated CO_2 concentration with no effect on cell wall thickness (Kostiainen *et al*., 2004). Such changes can impact wood density, in turn with possible mechanical implications given the snow pressure these treeline trees face (Körner, 1998). We did not directly measure wood density in our study, but in other CO_2 enrichment conifer studies, there have been increases in wood density (Hättenschwiler *et al*., 1996; Atwell *et al*., 2003) and wood toughness (Beismann *et al*., 2002), although this response is not consistent in conifers (Telewski *et al*., 1999; Ceulemans *et al*., 2002; Ziche & Overdieck, 2004) or in hardwoods (Beismann *et al*., 2002; Luo *et al*., 2005). At the tree level, differences in tracheid diameter resulting from elevated CO_2 might affect water conductivity in conifers (Roderick & Berry, 2001), particularly in cold climates where narrow diameters protect against freezing-induced embolism (Mayr *et al*., 2003; Pittermann & Sperry, 2003). At the ecosystem level, changes in wood quality could impact ecosystem processes like decomposition (Hättenschwiler *et al*., 1996; Cotrufo & Ineson, 2000).

Global climate change and the treeline ecotone. Independent of CO_2 enrichment in our experiment, *Larix* showed double and *Pinus* one quarter more growth in ring width from 2001–2004 when compared with the previous 4 years (Fig. 1). This rapid increase in vigour during the experimental years may reflect a shift in developmental stage as is often seen in conifers after years of slow initial growth. However, the summer 2003 was notably the hottest and driest summer that Europe experienced in >500 years and the years 2000, 2002 and 2003 were among the four warmest years since 1500 (Casty *et al*., 2005). Büntgen *et al*. (2005) also report from their tree-ring proxy dendroclimatological reconstruction of the central Alps that summer temperatures in the last decade are unprecedented over the past millennium. Although growth of forests at low elevations was significantly reduced in 2003 because of the associated drought, subalpine forests, not suffering from large reductions in precipitation, showed more radial growth than average in 2003 (Jolly *et al*., 2005). Much of the discussion regarding global climate change and the treeline ecotone has focused on potential warming which is speculated to result in forest expansion in high latitude and mountain regions through treeline advance (Gamache & Payette, 2005) with further possible feedbacks on global climate (Bonan *et al*., 1992; Foley *et al*., 2000). The growth record of *L. decidua*, in particular, produces the highest correlations with the instrumental climate record when compared with other treeline species in the European Alps (Frank & Esper, 2005), indicating a high sensitivity to temperature change, particularly evident in older tree individuals (Carrer & Urbinati, 2004). Although no CO_2 and temperature interactions have been observed in the growth response of other evergreen conifer experiments (Kilpeläinen *et al*., 2003; Olszyk *et al*., 2005) and elevated temperatures might actually mitigate changes in plant chemistry resulting from elevated CO_2 in gymnosperms (Zvereva & Kozlov, 2006), the high sensitivity of *L. decidua* to both elevated CO_2 concentration observed in our study and to temperature lends to the hypothesis that such a positive $CO_2 \times$ temperature interaction could exist in this fast-growing conifer species, opening the question for an *in situ* combined CO_2 and temperature experimental manipulation, and suggesting potential significant changes in plant community structure for this ecotone.

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4 No stimulation in root production in response to four years of *in situ* CO_2 enrichment at the Swiss treeline

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No stimulation in root production in response to 4 years of *in situ* CO_2 enrichment at the Swiss treeline

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Summary

1. Plants are frequently observed to increase carbon allocation to below-ground sinks and particularly, to accelerate fine root turnover in response to rising atmospheric CO_2 concentration. While these strong below-ground responses have predominantly been observed in rapidly expanding systems, late successional plant communities have rarely been studied.

2. In an ongoing free air CO_2 enrichment (FACE) experiment, we assessed below-ground responses to elevated CO_2 after 4 years, in a treeline ecosystem in the Swiss Central Alps (2180 m a.s.l.) dominated by a late successional ericaceous dwarf shrub community (*Vaccinium myrtillus*, *V. uliginosum*, *Empetrum hermaphroditum*), and a sparse overstorey of 30-year-old *Larix decidua* and *Pinus uncinata* trees. Measurements included quantification of fine root growth using ingrowth root cores and parallel standing crop harvests and decomposition of roots using litter bags.

3. Elevated CO_2 did not stimulate root growth of the treated vegetation (although some significant above-ground growth responses were observed), nor did altered root decomposition occur. Root quality measurements indicated that elevated CO_2 resulted in significantly higher starch concentrations, but no change in N concentration, or root dehydrogenase activity.

4. The use of the stable isotope $\delta^{13}C$ permitted us to trace the new carbon entering the system through our CO_2 enrichment treatment. We observed that only *c.* 30% of new root biomass (< 2 mm) was formed by new carbon indicating a rather slow root turnover in this system.

5. Our data show that fine root growth may be much less stimulated by elevated CO_2 in systems with late successional elements than has been reported in ecosystems with a rapidly expanding plant community biomass.

Key-words: *Larix decidua*, *Pinus uncinata*, ericaceous dwarf shrubs, fine roots, turnover, stable carbon isotope, elevated CO_2, FACE

Introduction

The current atmospheric CO_2 concentration is higher than it has ever been in the last 25 million years and has exceeded more than double that of the glacial minima concentrations of the last 650 000 years due to fossil fuel burning and land use change (Siegenthaler *et al*. 2005). The potential effect of this continuing rise in atmospheric CO_2 concentration on trees, which store 85% of all carbon fixed in terrestrial plant biomass (Saugier, Roy & Mooney 2001), has been studied earnestly by the scientific community in the last two decades in experimental growth chambers, open top chambers (see reviews by Curtis & Wang 1998; Saxe, Ellsworth & Heath 1998; Ceulemans, Janssens & Jach 1999; Norby *et al*. 1999) and more recently, using field-based free air CO_2 enrichment (FACE) technology in forests (Schäfer *et al*. 2003; Nowak, Ellsworth & Smith 2004; Körner *et al*. 2005). To date, a more widely documented effort has gone towards characterizing above-ground plant responses, in part due to the methodological challenges of observing *in situ* root dynamics (Tingey, Phillips & Johnson 2000; Pregitzer 2002). Nonetheless, it is estimated that as much as one-third of global annual net primary production is allocated to fine roots, making their role key in the terrestrial carbon cycle (Jackson, Mooney & Schulze 1997).

During the last decade, an increasing number of studies have begun to address root production and mortality (Norby & Jackson 2000; Matamala *et al*. 2003; Pendall *et al*. 2004). Central questions include understanding whether atmospheric change, itself or in combination with nutrient and/or climatic

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*Correspondence author. E-mail: tanya.handa@cefe.cnrs.fr change, might alter root turnover, and whether such changes might have consequences for plant physiological processes, soil microbial communities and the fate of carbon in soils (Norby & Jackson 2000). At the plant level, root turnover is critical to internal plant C allocation, as greater root longevity implies a trade-off: allocating less C to the production of new roots is at the cost of a higher energy expenditure (i.e. root respiration) associated with maintaining roots that are potentially less efficient at nutrient uptake (Eissenstat *et al.* 2000). At the ecosystem level, root mortality is a major input pathway of organic matter into the soil, and roots mediate other processes that influence the soil C cycle such as exudation, microbial activity (Hu, Firestone & Chapin 1999; Pendall *et al.* 2004), and mycorrhizal associations which have been typically shown to increase under elevated CO_2 (Tingey *et al.* 2000; Treseder 2004). Furthermore, it has been argued that gaps in our understanding of fine root biology, in particular potential species specific responses, limit our ability to predict the response of soil microorganisms to rising CO_2, which could trigger divergent responses of soil C and N cycling within ecosystems (Zak *et al.* 2000).

The majority of existing studies on root dynamics to elevated CO_2 have observed stimulation in fine root production, although there is a paucity of long-term studies focusing particularly on natural ecosystems with mature trees (Norby & Jackson 2000; Tingey *et al.* 2000; Olsrud *et al.* 2004). In their review on the response of woody plants to elevated CO_2, Curtis & Wang (1998) reported roughly +40% stimulation in root biomass, relative to an overall +30% stimulation of total plant biomass. Similarly, based on six studies under field conditions with deciduous tree species, Norby *et al.* (1999) summarized an absolute +60% to +140% stimulation in fine root density, and in every case, an increase that was superior to any change in leaf area, implying that CO_2 enrichment led to a higher allocation below-ground than above-ground. In contrast, in their review on coniferous trees, Tingey *et al.* (2000) reported that although there might be brief perturbations of the root:needle ratio in early stages of CO_2 enrichment, there does not appear to be any fundamental change in patterns of C allocation. Their review showed that the absolute response to elevated CO_2 on coniferous root biomass ranged from –27% to +225% with a median response of +52%. However, these results are based on studies (> 75%) that lasted < 1 year, were conducted in growth chambers or glass houses, and where almost all plants were in a seedling or juvenile stage.

At the Swiss treeline FACE site (Hättenschwiler *et al.* 2002), we have an ongoing experiment close to the natural treeline boundary in which ecosystem responses have been studied *in situ* since 2001. For two *c.* 30-year-old conifer tree species, we have documented a clear species specific above-ground growth response to elevated CO_2. After 4 years of CO_2 enrichment, *Larix decidua* L., an early successional species within the treeline ecotone, has shown a sustained positive growth response as evident in the shoot increment and tree ring records, while *Pinus uncinata* Ramond, a later successional species, has shown no such response (Handa, Körner & Hättenschwiler 2005, 2006). In this study, we sought to understand not only the root dynamics of this ecosystem, which include tree roots, but also those of the mature ericaceous dwarf shrub understorey and other herbaceous, graminoid plants, as well as moss rhizoids that occur at a lower frequency in our plots. Specifically, we asked: (i) is there an increase in below-ground C allocation as evident in new root ingrowth and standing crop root biomass in response to elevated CO_2, (ii) how much and how rapidly is new C being incorporated into the root system as evident through the $\delta^{13}C$ stable isotope tracer added by CO_2 enrichment, (iii) how does the isotope based root turnover estimate compare to turnover estimates based on measurements of new root production, standing crop and root decomposition, (iv) can the $\delta^{15}N$ stable isotope be used to differentiate trees from ericoid dwarf shrubs that have different means of acquiring nutrients (e.g. Körner 2003; Olsrud *et al.* 2004), and (v) is there an effect of elevated CO_2 on root quality as measured through starch and N concentration with potential implications for root activity?

Materials and methods

STUDY SITE AND TREATMENT DESCRIPTIONS

The treeline FACE site was established in 2001 at Stillberg, Davos (Central Alps, Switzerland) on a 25°–30° slope with a northeast aspect at 2180 m a.s.l., corresponding to, or slightly above, the natural climatic treeline (described in detail by Hättenschwiler *et al.* 2002; Handa *et al.* 2005). The site is part of a long-term afforestation research area planted in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research. Trees are 1–2 m tall, have stems with diameters < 10 cm and form a sparse open canopy with dense understorey vegetation composed predominantly of dwarf shrubs such as *Vaccinium myrtillus*, *V. uliginosum*, *Empetrum hermaphroditum*, and common herbaceous species such as *Gentiana punctata*, *Homogyne alpina* and *Melampyrum pratense*. Dwarf shrubs represent a mature community with a canopy height of 30–40 cm and stem diameters < 1 cm. Soils are Rankers (US: Lithic Haplumbrept), derived from silicaceous Paragneiss parent material, and dominated by an organic Humimor layer of 5–15 cm with a pH ($CaCl_2$) of 3·5 and a C : N ratio of 27 (detailed soil description in Hagedorn *et al.* 2008).

In early June 2001, 20 trees each of *L. decidua* (27-year-old), the European larch and *P. uncinata* (29-year-old), the mountain pine, were selected based on the following criteria for the experiment: one dominant stem, no serious signs of disease or herbivory, not more than one close (< 80 cm) neighbouring tree, a distance of at least 2 m between each FACE tree and a total tree height of 0·8–1·5 m. Using a split-plot approach, these 40 trees were assigned to 10 plots of four neighbouring trees (two each of *Larix* and *Pinus* trees per plot) in order to facilitate the logistics of CO_2 distribution and regulation. Within each plot, four 1·1 m^2 subplots, each separated by a distance of 1–5 m, were defined by a hexagonal ring suspended from three wooden posts surrounding each tree and extending to the soil surface. The size of these subplots was considered appropriate for the smaller spatial scales existing in alpine ecosystems. Half of the plots were randomly assigned to an elevated CO_2 treatment (target concentration of 550 µmol mol^{-1}), while the remaining plots served as controls at ambient CO_2 concentration (*c.* 370 µmol mol^{-1}). The CO_2 technology in use at our FACE site released pure CO_2 during day-time hours through laser-punched drip irrigation tubes hung vertically around the hexagonal ring such that the tree canopy and understorey plants received treatment. Subplot height and tubing was lifted accordingly throughout the experiment to compensate for growth when required. Temporal and spatial variability of concentrations was measured and regulated at the plot level (for more details on the system and its performance, see Hättenschwiler *et al.* 2002). Here, we report the effects of CO_2 enrichment treatment for the first four growing seasons (*c.* 15 June to 15 September, 2001–2004) during the daylight hours. Under poor weather conditions, for example, photon flux density $< 100\ \mu mol\ m^{-2}\ s^{-1}$, wind speeds $> 75\ km\ h^{-1}$, temperatures < 5 °C, snow or sleet, we interrupted CO_2 release because of the enormous cost of CO_2 delivery. Technical failure (< 5%) or weather conditions meant that trees received CO_2 enrichment for 75%, 81%, 73% and 78% of the 2001–2004 respective seasonal treatment periods. Seasonal mean CO_2 concentrations (2001–2004; ±1 SD) were 566 ± 42, 582 ± 35, 579 ± 52 and 578 ± 41 $\mu mol\ mol^{-1}$.

As part of the general experiment at the site, an 80% defoliation treatment was applied to one tree of each species within each CO_2 treatment plot in order to simulate an extreme disturbance event as occurs during insect outbreaks through a one time cutting of needles in late June 2002. *Larix* was defoliated by systematically cutting four out of every five short shoots above the meristem throughout the entire tree, while all new long shoots were left intact. *Pinus* was defoliated by cutting 80% of the proximal needles away within each of four age classes from 1999 to 2002 on the entire tree, while the distal 20% of the needles within each age class were left untouched (Handa *et al.* 2005). Although we did not expect to see strong effects in the ecosystem root biomass (as part of the root biomass that we studied was from the dwarf shrubs and not the trees), some effects of defoliation were recognized in various measured soil parameters indicating the tight coupling of above- to below-ground processes at our selected spatial scale (F. Hagedorn, unpublished data). For this reason, we included defoliation as a parameter in our full statistical model.

ROOT SAMPLING AND PROCESSING

Ten ingrowth root cores were established in each of the 40 subplots in June 2001 by extracting 10 soil cores (diameter of 2 cm installed to a depth of 10 cm) and filling the holes with sieved soil that was dug up adjacent to the study site. Each core was marked with an aluminium cylinder inserted 1 cm into the top layer of the soil. These 10 original soil cores were pooled for each subplot and washed to estimate the standing crop of roots at the beginning of the experiment. On every sequential spring (*c.* June 20) and autumn (*c.* Sept 30) harvest date in 2002 and 2003, two ingrowth cores per subplot were extracted and pooled to estimate new root production for all 40 subplots. Parallel to all ingrowth samplings (exception of 20 June 2002) and in autumn 2004, two supplemental cores were extracted and pooled per subplot to estimate standing crop for all 40 subplots. In autumn 2005, two cores per subplot were taken for stable isotope analysis only. In order to quantify the imprecision associated with recoring the marked ingrowth cores, four control cores were installed in June 2002 and resampled after 3 weeks to estimate the margin of error of our field technique (precision of recoring the same hole). This was determined to be < 10% of the mean standing crop biomass that had been harvested in June 2001. No cores during any sampling event were taken within 20 cm from the subplot edge to minimize possible edge effects.

Soil cores were processed by gently washing away organic soil on a 400 × 65 mm test sieve of 500 µm mesh size (Retsch DIN ISO 3310–1, Schieritz & Hauenstein AG, Arlesheim, Switzerland). Samples were then floated in a basin and decanted to facilitate separation of rock sediment or floating leaf litter and subsequently transferred back to the sieve where living root material that was structurally identifiable and not in an apparent advanced stage of decomposition was kept (no attempt was made to quantify dead roots within the sample). Roots were separated into three size classes of fine roots, corresponding roughly to functional groups: (i) < 100 µm 'non-woody' roots were white and fleshy, typical of graminoids and moss rhizoids; (ii) < 100 µm 'woody' roots were darker, slightly woody and typical of tree or understorey shrubs; (iii) 0·1–2·0 mm roots were predominantly larger 'woody' roots and large tuberous roots like that of *G. punctata*. Samples were stored at 5 °C and processed within 4–6 weeks of soil coring. The only exception was the initial 400 samples from June 2001 that were taken for the establishment of the ingrowth cores. These were washed over a time span of 5 months to estimate standing crop and for subsequent use in the root litter decomposition experiment. A statistical test showed that date of processing did not affect standing crop estimates so all data were included in the final analyses. All root samples were air-dried prior to weighing. A subsample for each root size class was subsequently oven-dried at 80 °C and all biomass values were corrected accordingly.

Samples for chemical analyses ($n = 3$ for each species and CO_2 treatment combination from 2001 to 2004, $n = 6$ in 2005) were ground to a homogenous powder and 8 mg were analysed for N, ^{13}C and ^{15}N by combustion in an elemental analyzer (EA-1110, Carlo Erba Thermoquest, Italy) transferred via an open split interface (Conflo II, Finnigan MAT, Germany) to a mass spectrometer (Delta S, Thermo Finnigan MAT, Germany). The depleted $\delta^{13}C$ (–30‰) in the CO_2 used at our FACE site decreased the $\delta^{13}C$ of CO_2 within the plots from –7·9‰ under ambient conditions to –15·7‰ under elevated CO_2. This difference in ^{13}C acted like a tracer within the ecosystem, and enabled us to see how rapidly recently assimilated CO_2 (new C) entered the root system. The fraction of new C in roots was estimated by relating the difference in root $\delta^{13}C$ to that in annual cress plants (6·6 ± 0·6‰) that germinated and grew their entire life cycle in ambient or elevated CO_2 concentration in our plots (von Felten *et al.* 2007). In order to roughly estimate the relative contribution of tree and dwarf shrub roots in the < 100 µm 'woody' roots and the 0·1–2·0 mm size class, we assumed: (i) that the strong majority of roots in these size classes were from trees or dwarf shrubs that comprise > 90% of the plant cover, and (ii) that none of the other vegetation had as negative isotope signatures as the dwarf shrubs (as a consequence, we possibly overestimate the relative abundance of tree roots in the samples). Sample averages were calculated for each size class for years 2003–2005 of ^{13}C and ^{15}N relative to the average of measured tree and dwarf shrub 'standards' that we excavated in the field adjacent to the site for this purpose.

Roots for the measurement of starch concentration and root dehydrogenase activity ('root vitality') were sampled in an independent event in September 2003. These were separated from bulk soil, freeze-dried with liquid N and stored at –80 °C, after which they were cleaned with distilled water subsequent to thawing and analysed. Starch concentration was determined by digesting starch enzymatically, adding a tri-ethanol-amin-ATP-NADP-Mg buffer and reading the absorption at 340 nm (Boehringer Mannheim 1989). For measuring root dehydrogenase activity, roots were cut into 1–2 mm fragments, put into 6 mL TTC-solution (0·6% triphenyltetrazoliumchloride in 0·06 M Na_2HPO_4–KH_2PO_4), degassed for 15 min under vacuum, and incubated 20 h at 30 °C. Then, dehydrogenases were extracted for 15 min in 95% ethanol at 80 °C and absorption was measured at 520 nm (Clemensson-Lindell 1994).

For the root litter decomposition experiment, root material from the June 2001 harvest (*c.* 20–30 mg of the < 100 µm 'non-woody' roots and 50–60 mg of the < 100 µm 'woody' roots) were sewn into 3 × 3 cm litter bags made of 500 µm Sefar Propyltex 05 280/41 (Sefar Holding, Rüschlikon, Switzerland). The initial root material was harvested from the subplots prior to the beginning of CO_2 enrichment, such that any potential differences in decomposition in response to CO_2 would not have been a direct result of any potential change in root quality, but rather would have represented indirect changes of the soil environment, such as moisture or microbial activity. Five bags for each size class under each of *L. decidua* and *P. uncinata* for both CO_2 treatment levels were buried in the top 5 cm of soil in 20 subplots on 22–23 June 2002. One bag of each size class was then randomly sampled from each subplot on 28 August 2002, 17 October 2002, 8 June 2003, 30 July 2003 and 9 September 2004. Bags were air-dried, soil was removed by gentle shaking and scratching of the mesh. Bags were then opened and remaining samples and bags were weighed separately to the nearest milligram.

STATISTICAL ANALYSIS

Analysis of variance, based on the split plot experimental design, was used to test for differences in CO_2, defoliation and tree species on measured root biomass of ingrowth, standing crop and decomposition measurements on every date. Defoliation was not a significant factor in the latter two series of tests, such that data were pooled across defoliation treatments and a reduced model testing only for CO_2 and tree species differences was used. This same reduced model (CO_2 and tree species) was used to test for differences in all chemical root parameters ($\delta^{13}C$, $\delta^{15}N$, N concentration, starch, dehydrogenase activity). All analyses were performed with R (Version 1·6·1, R Development Core Team 2004).

Results

STANDING CROP ROOT BIOMASS

Total root standing crop remained fairly constant over the 4 years, ranging normally from *c.* 500 to 1000 g m^{-2} with the exception of certain autumn measurements in 2002 and 2003 that were up to 1500 g m^{-2} (Fig. 1). Dwarf shrub roots had more negative $\delta^{13}C$ and $\delta^{15}N$ values than tree roots (Table 1), which allowed us to estimate the relative contributions of the functional groups to the sorted size classes: *c.* 60% tree and 40% dwarf shrub roots in the '< 100 µm woody' size class, and *c.* 40% tree roots, 55% dwarf shrub roots and 5% tuberous roots of perennial plants, for example, *G. punctata* in the 0·1–2·0 mm size class. The '< 100 µm non-woody' size class was considered to be composed of only grasses and herbaceous plants.

There were no treatment effects of elevated CO_2 concentration or defoliation on total root standing crop or in any of the size classes, although there was a trend towards higher biomass in the largest size class on the fall sampling dates in response to elevated CO_2 (Fig. 1). There was a significant tree species effect in June 2003 for < 100 µm 'woody' roots ($F_{1,22} = 4{\cdot}36$, $P < 0{\cdot}05$) where there was *c.* +25% higher biomass under *Larix* than under *Pinus* trees. This trend was reversed in the larger root class (0·1–2·0 mm) in September 2003 ($F_{1,21} = 8{\cdot}11$, $P < 0{\cdot}01$) and September 2004 ($F_{1,8} = 3{\cdot}66$, $P = 0{\cdot}09$) where there was a +40% to +70% higher root biomass under *Pinus* rather than *Larix* trees in this root class only. This particularly strong difference in the largest root class in September 2003 influenced total root biomass where a significant species effect was also observed ($F_{1,21} = 10{\cdot}3$, $P < 0{\cdot}01$).

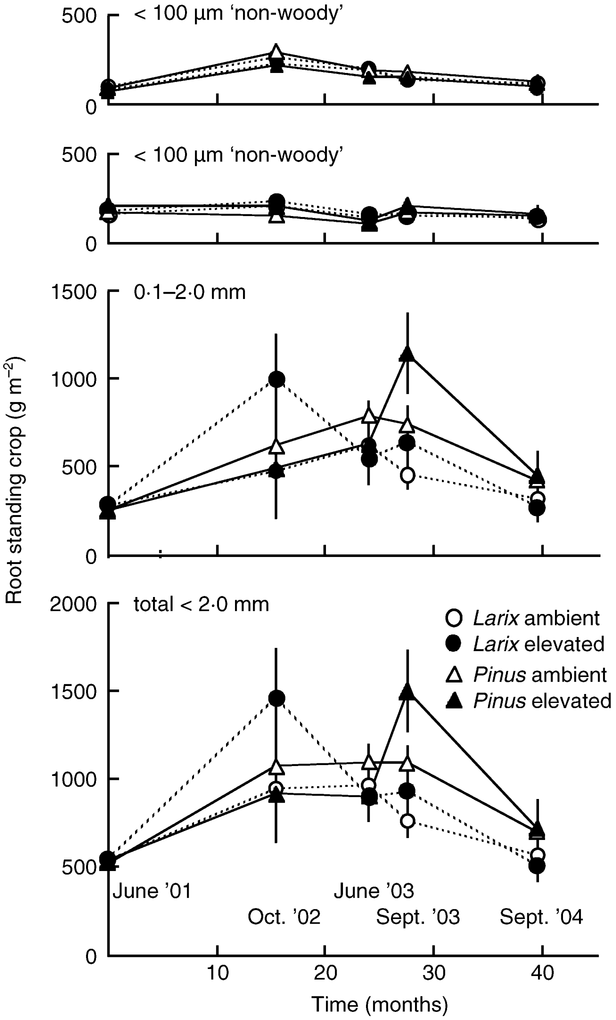

Fig. 1. Total root standing crop under ambient (open symbols) and elevated (close symbols) CO_2 based on sequential harvests in June 2001 ($n = 10$), October 2002 ($n = 6$), June 2003 ($n = 8$), September 2003 ($n = 7$–9) and September 2004 ($n = 5$) for three size classes and total biomass over all size classes (± SE). Please note that error bars are smaller than symbols in the top two panels.

INGROWTH CORE NEW ROOT PRODUCTION

Root ingrowth core production was *c.* 150 g m^{-2} during the growing seasons (from spring to fall) in 2002 and 2003 with 70 g m^{-2} root mortality over the dormant season from October 2002 to June 2003 (Table 2). There was no significant effect of elevated CO_2 concentration on new root growth. However, there was an impact of the defoliation treatment and its interactions with other factors. In June 2003, 1 year after the

Table 1. Stable isotope signatures (‰, ± SE) of fine root biomass (< 2 mm) of different woody species in the tree line ecotone (n = 3 to 5 individuals for tree species) and pooled material from multiple individuals for dwarf shrubs (n = 2)

Species	$\delta^{13}C$	$\delta^{15}N$ (‰)
Larix decidua	–27·03 ± 0·30	–2·15 ± 1·25
Pinus uncinata	–26·34 ± 0·41	–3·90 ± 0·58
Pinus cembra†	–25·57 ± 0·33	–4·35 ± 0·95
Vaccinium myrtillus	–28·80 ± 0·36	–5·99 ± 0·16
Vaccinium uliginosum	–28·16 ± 0·09	–7·22 ± 0·35
Empetrum hermaphroditum	–25·50 ± 0·20	–7·26 ± 0·44

†*Pinus cembra* exists in low densities at this site, although it is not included within the experimental plots.

defoliation treatment, there was a marginally significant CO_2 × defoliation interaction on < 100 µm 'non-woody' roots ($F_{1,5} = 5\cdot06$, $P = 0\cdot07$) where there was 35% less biomass in the undefoliated elevated CO_2 plots but equal or slightly higher (+20% to +35%) biomass in the defoliated, elevated CO_2 plots compared to respective control plots. In September 2003, the year after defoliation, however, a significant defoliation and interaction effect with tree species was observed among the < 100 µm 'woody' roots (Defol: $F_{1,7} = 4\cdot34$, $P = 0\cdot08$; Defol × Sp: $F_{1,15} = 5\cdot65$, $P < 0\cdot05$) and 0·1–2·0 mm roots (Defol: $F_{1,7} = 4\cdot17$, $P = 0\cdot08$; Defol × Sp: $F_{1,15} = 5\cdot47$, $P < 0\cdot05$) thus driving the differences in total root biomass (Defol: $F_{1,7} = 9\cdot00$, $P < 0\cdot05$; Defol × Sp: $F_{1,15} = 4\cdot39$, $P < 0\cdot05$). Under *Larix*, defoliation decreased new root growth by 40%–60% at ambient, but not when exposed to elevated CO_2 concentration at the same time. Under *Pinus*, defoliation tripled the amount of new root growth at ambient CO_2 in < 100 µm and 0·1–2·0 mm 'woody' roots. Under elevated CO_2 concentration, this increase was supplemented further by a +20% to +105% stimulation (Table 2). Species effects, independent of defoliation in 0·1–2·0 mm roots were evident in June 2002 ($F_{1,15} = 4\cdot15$, $P = 0\cdot06$), thus also driving differences in total root ingrowth ($F_{1,16} = 4\cdot62$, $P < 0\cdot05$). Under *Larix* trees, total fine root ingrowth biomass was 40% higher than under *Pinus* trees (Table 2).

ROOT QUALITY

Roots growing in elevated CO_2 plots had +32% higher starch concentration than those in ambient grown plots (Table 3; $F_{1,35} = 36\cdot2$, $P < 0\cdot05$). Neither nitrogen concentration in any of the years from 2002 to 2005, nor root dehydrogenase activity was significantly influenced by elevated CO_2. There was however a significant tree species effect on root dehydrogenase activity ($F_{1,34} = 240\cdot5$, $P < 0\cdot01$) where roots growing under *Pinus* trees showed + 55% more dehydrogenase activity than those growing under *Larix* (Table 3).

ROOT DECOMPOSITION

There was no difference between ambient and elevated CO_2 treatments in the decomposition of < 100 µm 'non-woody'

Table 2. Ingrowth fine root biomass (g m^{-2}, ± SE) for sequential harvests of soil cores installed in June 2001 at the beginning of the experiment and sorted by root diameter class (n = 5 in June 2002, n = 3 in October 2002, n = 3 to 5 in June 2003 and n = 5 in September 2003) growing in ambient (A) or elevated (E) CO_2 concentration

	Larix decidua				*Pinus uncinata*			
	Undefoliated		Defoliated		Undefoliated		Defoliated	
	A	E	A	E	A	E	A	E
< 100 µm 'non-woody'								
June 2002	30 ± 7	27 ± 5	35 ± 4	25 ± 2	27 ± 4	25 ± 4	24 ± 2	32 ± 5
October 2002	59 ± 22	77 ± 19	61 ± 16	62 ± 11	85 ± 11	92 ± 8	42 ± 2	105 ± 31
June 2003	63 ± 4	41 ± 7	38 ± 2	45 ± 6	50 ± 12	31 ± 7	40 ± 7	54 ± 21
September 2003	45 ± 7	43 ± 10	74 ± 25	61 ± 24	72 ± 13	75 ± 10	60 ± 17	95 ± 26
< 100 µm 'woody'								
June 2002	18 ± 6	66 ± 34	21 ± 3	22 ± 1	14 ± 6	21 ± 8	18 ± 3	16 ± 5
October 2002	53 ± 15	48 ± 10	59 ± 19	35 ± 18	53 ± 24	56 ± 13	22 ± 7	44 ± 12
June 2003	19 ± 1	35 ± 13	19 ± 7	28 ± 7	21 ± 6	13 ± 4	23 ± 10	31 ± 15
September 2003	60 ± 23	54 ± 11	36 ± 9	52 ± 11	28 ± 4	60 ± 28	76 ± 10	103 ± 23
0·1–2·0 mm								
June 2002	88 ± 39	47 ± 42	69 ± 25	53 ± 36	40 ± 20	22 ± 17	19 ± 15	27 ± 16
October 2002	40 ± 31	53 ± 41	185 ± 110	212 ± 202	18 ± 13	255 ± 206	83 ± 32	143 ± 59
June 2003	228 ± 138	105 ± 41	52 ± 19	126 ± 42	91 ± 36	76 ± 22	111 ± 39	51 ± 19
September 2003	195 ± 86	125 ± 31	71 ± 31	108 ± 57	70 ± 22	151 ± 59	216 ± 52	443 ± 171
Total < 2 mm								
June 2002	136 ± 48	139 ± 71	124 ± 24	100 ± 36	81 ± 25	67 ± 17	61 ± 16	76 ± 24
October 2002	152 ± 66	177 ± 60	305 ± 144	309 ± 230	155 ± 16	403 ± 212	147 ± 26	292 ± 94
June 2003	310 ± 140	181 ± 55	109 ± 26	199 ± 47	162 ± 53	120 ± 31	174 ± 49	136 ± 47
September 2003	300 ± 111	223 ± 34	181 ± 65	220 ± 62	171 ± 28	285 ± 82	352 ± 56	641 ± 203

Table 3. Root quality parameters under *Larix decidua* or *Pinus uncinata* at ambient (A) or elevated (E) CO_2 concentration (± SE). These include starch concentration and dehydrogenase activity measured once in September 2003. Nitrogen concentration was measured at the end of all growing seasons from 2002 to 2005

	Larix decidua		*Pinus uncinata*	
	A	E	A	E
Starch (% dry matter)	5·55 ± 0·64	7·87 ± 1·21	6·46 ± 0·51	7·95 ± 0·72
Dehydrogenase activity†	34·0 ± 4·0	34·8 ± 3·9	49·4 ± 6·7	57·5 ± 8·1
N (% dry matter)				
2002	0·82 ± 0·08	0·69 ± 0·06	0·73 ± 0·08	0·71 ± 0·08
2003	0·88 ± 0·08	0·91 ± 0·09	0·83 ± 0·08	0·88 ± 0·07
2004	0·84 ± 0·11	0·79 ± 0·08	0·65 ± 0·07	0·80 ± 0·10
2005	0·88 ± 0·05	0·81 ± 0·04	0·68 ± 0·05	0·73 ± 0·04

†Absorbance of Formazan at 520 nm g d m^{-1}.

and ‘woody’ root fractions. On 28 August 2002 (after one 3-month growing season), *c.* 90% of the roots were remaining in both treatments and size classes. This 90% remained unchanged as observed through samplings on 17 October 2002 and 30 July 2003. On 9 September 2004, after 26 months in the soil, there were 66 ± 5% and 68 ± 3% of < 100 µm ‘non-woody’ roots remaining and 67 ± 3 and 67 ± 3 of < 100 µm ‘woody’ roots in ambient and elevated CO_2 plots respectively.

STABLE ISOTOPES

The standing crop samples showed progressive incorporation of new C from 2002 to 2005 within the roots starting from the finest size class and eventually detectable in the largest size class. There was a significant CO_2 effect on $\delta^{13}C$ of total root biomass from 2003 onwards ($P < 0·05$). In the < 100 µm ‘non-woody’ roots, the CO_2 effect on $\delta^{13}C$ was significant as of 2002 ($P < 0·05$) and became stronger in subsequent years ($P < 0·01$–$P < 0·001$). In the < 100 µm ‘woody’ roots, the CO_2 effect was marginally significant as of 2003 ($P = 0·06$) and significant as of 2004 ($P < 0·01$). In the 0·1–2 mm roots, the CO_2 effect was only significant after 3 years of CO_2 enrichment in 2004 and 2005 ($P < 0·05$). After 5 years of CO_2 enrichment, the fraction of new C in roots (pooled across species) was 25 ± 4% in the < 100 µm ‘non-woody’ roots, 25 ± 7% in the < 100 µm ‘woody’ roots and 15 ± 12% in the 0·1–2·0 mm roots. Tree species had a significant influence on $\delta^{13}C$ in total root biomass in 2004 only ($F_{1,122} = 5·74$, $P < 0·05$), where $\delta^{13}C$ values were more negative under *Larix* than under *Pinus* trees independant of CO_2, and in 2005 there was a significant CO_2 × species interaction ($F_{1,60} = 7·69$, $P < 0·01$), where the difference in $\delta^{13}C$ between roots grown under ambient or elevated CO_2 was less under *Larix* trees than under *Pinus* trees (Fig. 2). In the ingrowth cores, the new C was already evident in 2002, even in the larger root size classes, as evident in the significant CO_2 effect on $\delta^{13}C$ (Table 4).

In the standing crop root samples, no significant effects were observed in 2002 or 2003 on $\delta^{15}N$ (Fig. 2). In 2004, however, a marginally significant CO_2 × species interaction was observed ($F_{1,23} = 7·33$, $P = 0·07$) whereby under *Larix*, $\delta^{15}N$ was more negative than under *Pinus* trees (Fig. 2). In 2005, when a significant tree species effect ($F_{1,60} = 5·68$, $P < 0·05$) was observed, the $\delta^{15}N$ was more negative under *Pinus* than *Larix* trees (Fig. 2), as was the case for the needles as well (unpublished data). In ingrowth cores, in 2003 only, there was a significant tree species effect on $\delta^{15}N$ ($F_{1,26} = 8·29$, $P < 0·01$), where $\delta^{15}N$ was more negative under *Pinus* than under *Larix* trees (Table 4).

Discussion

ROOT BIOMASS RESPONSES

Measurements of above-ground growth in our study system have indicated that while some tree (*P. uncinata*) or understorey dwarf shrub species (*E. hermaphroditum*, *V. uliginosum*) showed no stimulation in their shoot length growth when exposed to elevated CO_2, other tree species (*L. decidua*) and understorey shrubs (*V. myrtillus*, *V. vitis-idaea*) exhibited more vigorous growth during the first 5 experimental years (Handa *et al.* 2005, 2006, T. Zumbrunn, unpublished data). This rapid above-ground growth response by some species may seem counterintuitive in the harsh environment of the treeline ecotone, where plants are often believed to exhibit conservative growth strategies. However, despite these differences observed above-ground, our data set demonstrates that below-ground, there was no overall difference in total root standing crop after 4 years (Fig. 1), in new root production measured over 3 years (Table 2), in root decomposition measured over 26 months, and thus in root turnover (when calculated as new production for one growing season/maximum standing crop for the same growing season; 0·07 ± 0·04 in 2002 and 0·08 ± 0·05 in 2003). These data also do not offer support for any increase in plant allocation to roots in response to elevated CO_2.

At first glance, this lack of stimulation in root biomass may appear to contrast with the majority of studies that have reported positive root growth responses for trees to elevated CO_2 concentrations (Curtis & Wang 1998; Norby *et al.* 1999; Tingey *et al.* 2000), although as emphasized in their reviews, plant growth stage, experimental duration and the soil environment may have strongly biased the reported

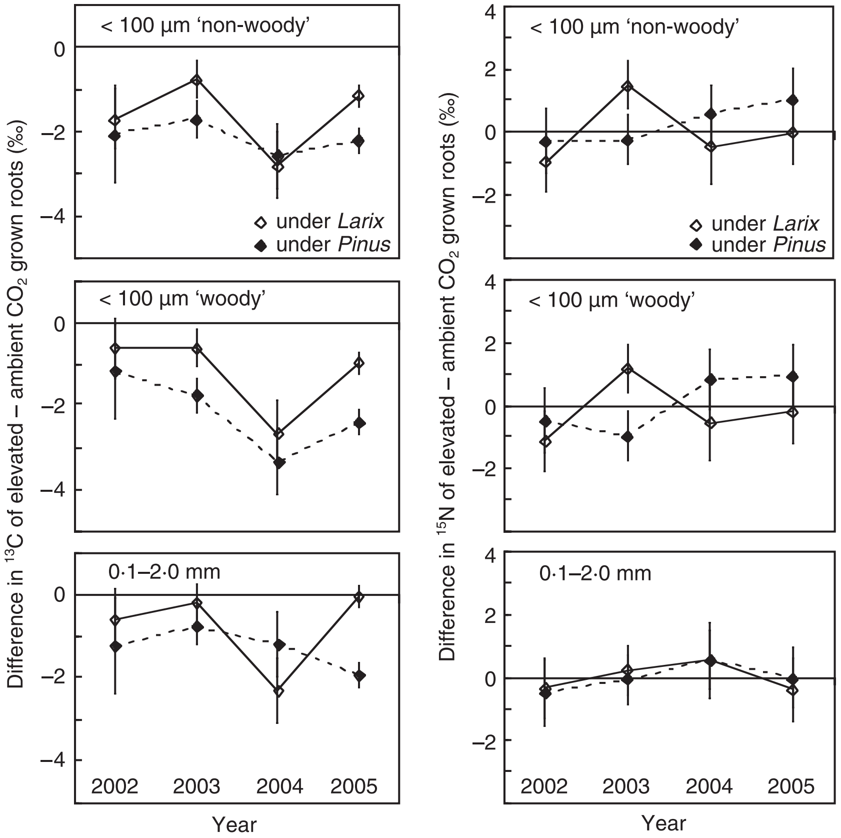

Fig. 2. Difference (elevated CO_2 grown–ambient CO_2 grown) in the stable isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ root communities (± SE) under *Larix* (white diamonds) and *Pinus* (black diamonds) trees ($n = 3$ for 2001 to 2004, $n = 6$ for 2005). The solid horizontal line represents no difference in between the CO_2 treatments.

Table 4. Stable isotope signatures of ingrowth fine root biomass (‰) for sequential harvests of soil cores installed in June 2001 at the beginning of the experiment and sorted by root diameter class ($n = 3$ in October 2002, $n = 6$ in September 2003) in ambient (A) and elevated (E) CO_2 concentrations

				‡*Larix decidua*		*Pinus uncinata*	
	$\delta^{13}C$ A	$\delta^{13}C$ E	CO_2 *F*-test	$\delta^{15}N$ A	$\delta^{15}N$ E	$\delta^{15}N$ A	$\delta^{15}N$ E
< 100 µm 'non-woody'							
Pre-treatment	–27·82 ± 0·11†	–	–	–2·93 ± 0·15†	–	–	–
October 2002	–27·79 ± 0·17	–30·70 ± 0·79	*P* = 0·040*	–3·10 ± 0·60	–2·08 ± 0·97	–3·41 ± 0·59	–3·59 ± 0·65
September 2003	–27·74 ± 0·23	–30·42 ± 0·90	*P* = 0·041*	–2·10 ± 0·30	–2·43 ± 0·25	–3·06 ± 0·22	–3·23 ± 0·15
< 100 µm 'woody'							
Pre-treatment	–28·17 ± 0·37†	–	–	–5·02 ± 0·19†	–	–	–
October 2002	–27·69 ± 0·36	–28·74 ± 0·53	*P* = 0·258	–3·83 ± 0·79	–4·39 ± 0·60	–2·97 ± 2·11	–4·82 ± 1·00
September 2003	–27·63 ± 0·23	–29·07 ± 0·36	*P* = 0·022*	–1·13 ± 1·47	–4·33 ± 0·41	–4·23 ± 0·15	–4·40 ± 0·40
0·1–2·0 mm							
Pre-treatment	–28·04 ± 0·12†	–	–	–5·02 ± 0·85†	–	–	–
October 2002	–28·22 ± 0·16	–30·51 ± 0·85	*P* = 0·019*	–3·97 ± 1·26	–5·79	–5·29 ± 1·28	–5·22 ± 0·66
September 2003	–28·21 ± 0·31	–29·69 ± 0·87	*P* = 0·169	–3·87 ± 0·75	–4·71 ± 0·48	–5·60 ± 0·28	–4·41 ± 0·76
Total < 2·0 mm							
Pre-treatment	–28·01 ± 0·13†	–	–	–4·32 ± 0·43†	–	–	–
October 2002	–27·89 ± 0·15	–28·16 ± 0·49	*P* = 0·045*	–3·60 ± 0·46	–3·60 ± 0·71	–3·89 ± 0·81	–4·54 ± 0·46
September 2003	–27·86 ± 0·15	–29·69 ± 0·41	*P* = 0·040*	–2·36 ± 0·63	–3·92 ± 0·43	–4·30 ± 0·38	–4·05 ± 0·31

†Pre-treatment material was harvested in spring 2001 prior to CO_2 enrichment and pooled across treatment combinations. We thus report only one baseline value.

‡Data for ^{15}N are separated by species because a significant species effect ($P < 0.01$) was found in 2003.

responses. In his recent review, Körner (2006) proposed to interpret system responses to CO_2 enrichment by groups depending on soil conditions as belonging to either 'decoupled' systems (where plants have access to a high abundance of major resources other than carbon), 'expanding' systems (where there is free space available above- and/or below-ground that has not yet been fully explored by plants due to available space/time and/or plant development stage) and 'coupled' systems (where plants live in a steady-state system with constant leaf area index and root turnover). Most tree root responses to elevated CO_2 exposure reported to date (citations refer to a minimum 2-year study duration) fall in the 'decoupled' or 'expanding' systems categories. For these two categories, absolute increases in root biomass were generally observed, reflecting increased total biomass responses and no change in the root:shoot allocation (Crookshanks, Taylor & Broadmeadow 1998; Calfapietra *et al*. 2003; Shinano *et al*. 2007 but see Jach, Laureysens & Ceulemans 2000, where 8% shift in root allocation was observed). Such examples of absolute root production in response to CO_2 enrichment include conifers such as *P. sylvestris* (Crookshanks *et al*. 1998; Janssens *et al*. 1998; Jach *et al*. 2000), *P. ponderosa* (Phillips *et al*. 2006), *L. kaempferi* (Shinano *et al*. 2007), broad-leaved species such as *Fraxinus exelsior*, *Quercus petraea* (Crookshanks *et al*. 1998), and various *Populus* species (Pregitzer *et al*. 2000; Calfapietra *et al*. 2003; King *et al*. 2005), although for *Populus tremuloides,* this enhancement of root production was observed at high resource availability only (Pregitzer *et al*. 2000; King *et al*. 2005).

Examples of 'expanding' systems where no increase in root production was observed in response to elevated CO_2 also exist. In a study system with similar properties to ours ('expanding' trees in a late successional 'steady state' dwarf shrub community), 14–24 year old naturally-established *P. sylvestris* trees grew in a *Vaccinium* dominated heath with low plant available nitrogen in soils. After 3 years of CO_2 enrichment, no change in coarse or fine root production of tree or dwarf shrubs was reported (Kasurinen, Helmisaari & Holopainen 1999). No increase in root production was observed either in a Swiss montane forest model system of *Picea abies* with soils similar to ours (Hättenschwiler & Körner 1998) or in a nutrient-poor post-burn scrub oak system consisting of various *Quercus* species after 6 years (Brown *et al*. 2007). In addition, soil pH can have a decisive role on root dynamics. Spinnler, Egli & Körner (2002) found that after 4 years of CO_2 enrichment in *Picea abies-Fagus sylvatica* model ecosystems, root production was enhanced only in calcareous, but not in acidic soils.

In the 'coupled' steady-state systems, the data are still few. At the *P. taeda* Duke FACE site after 8 years, a 13%–27% stimulation in annual basal area of the trees is reported above-ground (Moore *et al*. 2006) and a +25% increase in root standing crop below-ground based on mini-rhizotron data (S. Pritchard, personal communication). This long-term data set confirms the previously reported stimulation to root production after 2 years using destructive methods (Allen *et al*. 2000; Matamala & Schlesinger 2000). At the Swiss deciduous web-FACE forest, measurements of fine root production show no response to CO_2 enrichment after 7 years (M. Bader & Ch. Körner, personal communication). The best documented experiment is the *Liquidambar styraciflua* Oak Ridge FACE site (Norby *et al*. 2004), where the situation is notably different from the great majority of cases outlined above. While the strong initial above-ground growth rapidly disappeared after 1 year, fine root production remained stimulated after 6 years leading to a clear shift in plant allocation (Norby *et al*. 2004). This increased production of roots has been interpreted as a mechanism to increase N uptake or increase resistance to drought. The deeper rooting in the soil profile that Norby *et al*. (2004) observed in response to elevated CO_2 has been seen in other studies (Johnson *et al*. 2006), although upward shifts in the soil profile have also been observed (Arnone *et al*. 2000). Our results indicating no response after 4 years of CO_2 enrichment belong in part in this 'coupled' system category given that the dwarf shrub matrix, where our trees grow, represents a 'steady-state' state.

While elevated CO_2 did not influence root production in our study, species identity of the tree in the plot did (Fig. 1, Table 2), although according to our stable isotope estimates, tree roots comprised only about half of the total root biomass. In the spring of 2002, there was more root production (+40%) in ingrowth cores in *Larix* than *Pinus* plots, as was the case with the < 100 µm 'woody' standing crop root measurements (+25%) in June 2003. In autumn 2003 and 2004, the pattern was the inverse, higher standing crop was observed under *Pinus* and not under *Larix* trees and dehydrogenase activity of the live root tissue was +55% higher under *Pinus* trees. In autumn 2003, there was also a significant defoliation by species interaction evident in the ingrowth core data set, where under defoliated *Larix*, there was a –40% to 60% reduction in new roots (Table 2) matched by much higher NO_3^- concentrations in those plots (F. Hagedorn, unpublished data). In contrast, under defoliated *Pinus* trees, root production tripled and was even further enhanced by elevated CO_2.

Conifers growing under harsh conditions tend to invest their annual carbon gains largely in needle and root production at the expense of above-ground woody parts (e.g. *L. gmelinii* in nutrient-poor permafrost soil; Kajimoto *et al*. 1999), perhaps particularly, in the case of *Larix*, in the spring when high rates of photosynthesis of the young N-rich needle tissue allow for it. These responses that we attribute to the trees in the subplots are possibly not only due to the tree root system, but are a combined consequence of differential growth of dwarf shrub roots under different host trees, as observed in the greater shoot increments of dwarf shrubs under *Larix* trees (T. Zumbrunn, unpublished data). Any shifts in dominance of root biomass of the different species means that although our standing crop remains unchanged by elevated CO_2, we cannot preclude the possibility of shifts in dominance of root production within a community of one plant at the cost of another. In a 1·5 year model community study with *P. palustris* seedlings and its naturally occuring understorey plants, Pritchard *et al*. (2001) found +62% higher root biomass of *Pinus* at the expense of the root biomass of the understorey plants. We have made use of the $\delta^{15}N$ values to estimate if elevated CO_2 induced a change in the contribution of different species to the overall root biomass, as trees had substantially higher $\delta^{15}N$ values than dwarf shrubs (*c.* 5‰ in the case of *Larix*; Table 1). However, neither the $\delta^{15}N$ in the new roots nor that of the standing crop biomass changed significantly (Table 4, Fig. 2), suggesting little change in species dominance or composition.

ROOT QUALITY RESPONSES

Root chemistry measurements in response to elevated CO_2 showed a significant increase in starch concentration in the roots (Table 3), but no change in N concentration or in root dehydrogenase activity. Higher starch concentration is a common response of plants growing under elevated CO_2 (Norby *et al.* 1999) and was also observed in the needles of our trees (Handa *et al.* 2005). This increase of stored carbon below-ground may well have consequences for mycorrhizae, or hemi-parasitic plants that can tap on the carbon resources of their hosts (Press 1989; Tennakoon & Pate 1996). The abundance of the hemiparasites, *M. pratense* and *M. sylvaticum* that co-occur at our site, effectively doubled under elevated CO_2 and the number of seeds and average seed mass increased, suggesting that these plants were able to profit from the supplement in stored carbon (Hättenschwiler & Zumbrunn 2006). Mycorrhization has not been studied in detail on the adult trees of our site, however an increase in mycorrhizal colonization is a common response to CO_2 enrichment (Olsrud *et al.* 2004; Treseder 2004). It has also been argued that the increased root total non-structural carbohydrates under elevated CO_2 favours NO_3^- uptake, which is more costly than NH_4^+ uptake (BassiriRad *et al.* 1996). In our soils (in 2003 only), CO_2 enrichment decreased the concentration of NO_3^- and increased NH_4^+ concentration (*c.* +60%), which could indeed reflect a shift in plant N uptake (F. Hagedorn, unpublished data). The change in soil N form, could however also be caused by a change in N transformation due to plant-microbial feedbacks such as suppressed nitrification (Luo *et al.* 2004; Hagedorn *et al.* 2005). Other studies have also shown higher starch (Janssens *et al.* 1998) and lower N concentrations in roots under elevated CO_2 (Janssens *et al.* 1998; Pregitzer *et al.* 2000; Wan *et al.* 2004). However, this result is not ubiquitous. As in our study, where no change in N concentration was observed, Tingey *et al.* (2003) reported no change in principal carbon constituents or N allocation between needle, woody or root tissue after 4 years of CO_2 enrichment of *Pseudotsuga menziesii*. Similarly, in their 2·5 year study with *P. tremuloides* and *Acer saccharum*, King *et al.* (2005) found that absolute changes in root chemical composition in responses to increases in C and soil resource availability were small. They observed no effects on soil fungal biomass or specific rates of root decomposition, concluding that root contributions to soil C cycling will be more strongly influenced by fine root production and turnover rather than any changes in substrate chemistry. Soil nitrogen availability can play a decisive role on fine root N concentration under elevated CO_2. Hagedorn *et al.* (2002) found that after 4 years of CO_2 enrichment in spruce-beech model ecosystems, concentrations of root N decreased in N-rich soils, but remained unchanged in N-poor soils as the ones at our site.

ROOT TURNOVER

Our stable isotope tracer signal showed that newly fixed carbon was already evident in the ingrowth cores in 2002 in all root size classes (Table 4), and was progressively detectable in the general standing crop with significant differences in $\delta^{13}C$ evident in the smallest size class (< 100 µm 'non-woody') in 2002 and in the large size class (0·1–2·0 mm) by 2004. However, only one-third of the carbon used for new root production in ingrowth cores carried the signature of the CO_2 treatment. Similarly, in the standing crop the maximum difference in $\delta^{13}C$ occured in 2004 and only represented *c.* 30% of the tracer fraction in our canopies' carbon supply in the roots. Contrary to our expectations, there was no further increase in new carbon in 2005. This overall delay in signal appearance likely reflects slow root turnover but also a mixing process of new C into an intermediate C pool that is only subsequently re-invested into tree biomass (Keel, Siegwolf & Körner 2006, Keel *et al.* 2007). Some recent estimates of mean residence time for conifers suggest that fine roots can live on the order of 4–6 years (Matamala *et al.* 2003; Johnsen, Maier & Kress 2005) or even up to 10 years (Steinmann *et al.* 2004; Joslin *et al.* 2006) in warm temperate low elevation forests, which may also be reflected by the results that we present with so little of the new carbon cycling through the roots.

The total new root production in the ingrowth cores was *c.* 150 g m^{-2} during each growing season although, we recognize that ingrowth cores might not provide an accurate estimate given the associated soil disturbance. Nonetheless, this estimate is only slightly more than the measured total needle litter fall of 130 g m^{-2} (T. Handa, unpublished data). These data support the assumption made by soil C models that litter inputs via roots equal those via above-ground litter (e.g. Michalzik *et al.* 2003). The apparent root turnover rate (new root production/standing crop biomass) approaches 0·1, suggesting that *c.* 10% of the standing root biomass are renewed every growing season. The decomposition experiment supports this estimate, with 30% mass loss during three seasons corresponding to a decomposition rate of 10% per year. Thus, when we assume the standing biomass being in steady state, it equals a 10% production of new roots. Turnover rates of roots are crucial for the input of carbon into soils, but they are in general poorly documented (Matamala *et al.* 2003). To date, it is controversial whether or not soils may sequester more C under elevated CO_2 (Hagedorn *et al.* 2003; Loya *et al.* 2003), particularly in light of the progressive nitrogen limitation debate (Luo *et al.* 2004; Gill *et al.* 2006). In our study, CO_2 enrichment did not alter the production of new roots, decomposition, and turnover rates of roots. Consequently, we conclude that elevated CO_2 did not affect the input of C into soils via altered root production and that the impact on C sequestration was small for this late successional tree line system.

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5 Atmospheric CO_2 enrichment of alpine treeline conifers

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Atmospheric CO_2 enrichment of alpine treeline conifers

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Summary

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- 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 non-structural 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.

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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

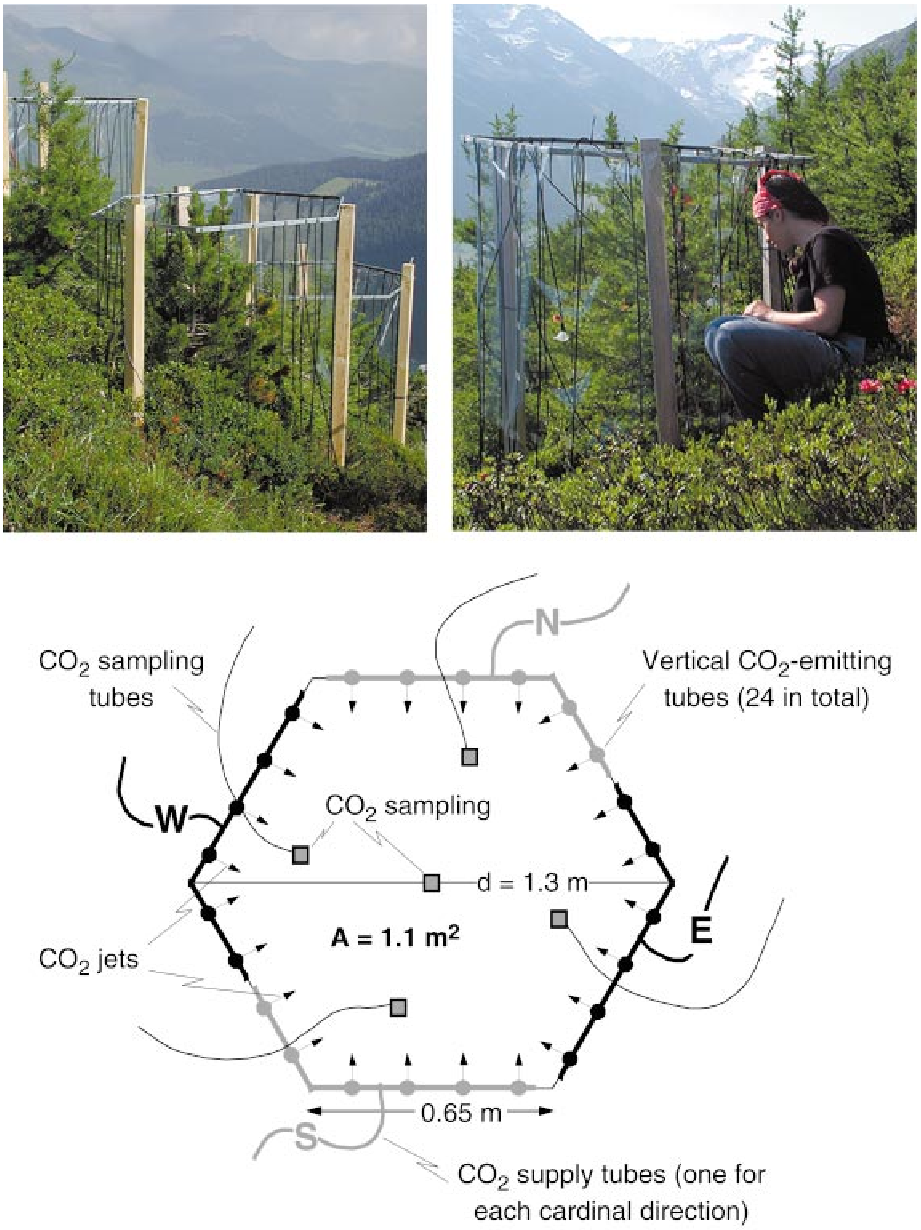

Fig. 1 Experimental plots at the research site Stillberg, Davos, Switzerland, 2180 m asl. The construction for support of CO_2 emitting tubes surrounding a tree in the center of each plot and its understory vegetation is shown in the two pictures on top. Tubes for CO_2 emission extend from the top (slightly 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

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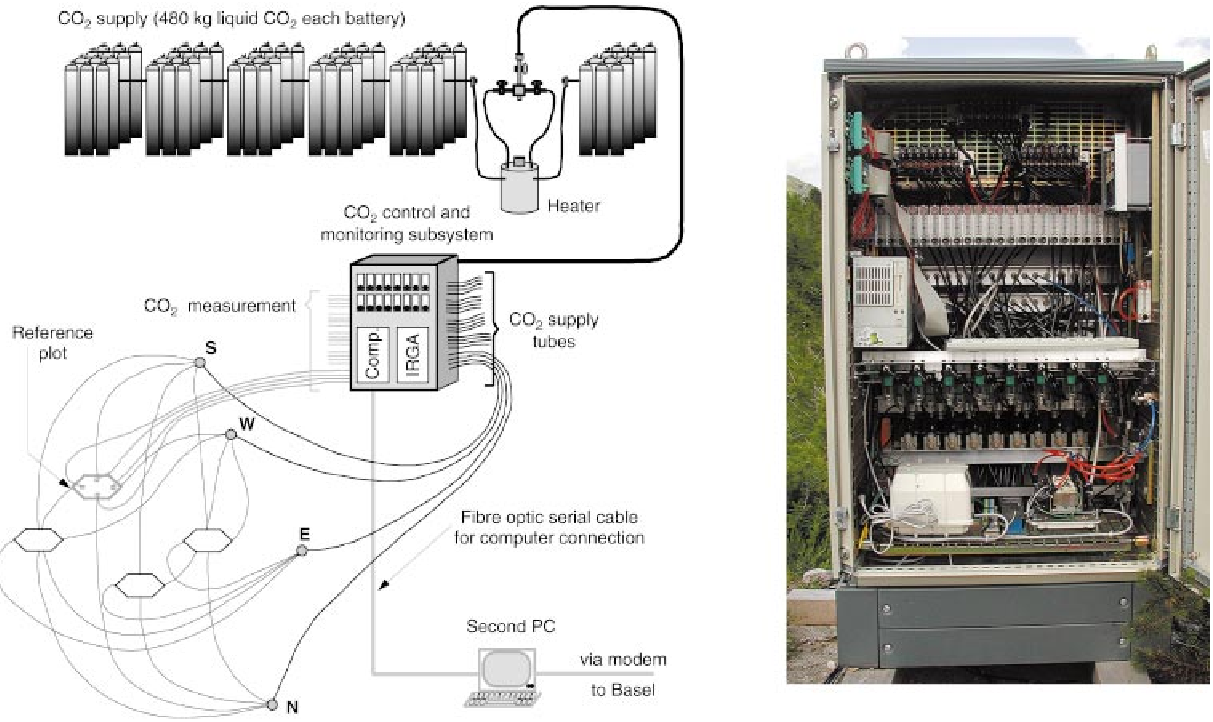

Fig. 2 Experimental setup of the supply, control, monitoring and field enrichment of CO_2. The control and monitoring system in the field contained the infrared gas analyzer (IRGA) for CO_2 measurement, the industrial control PC and the solenoid valves for CO_2 flow control (shown in detail on the right). 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

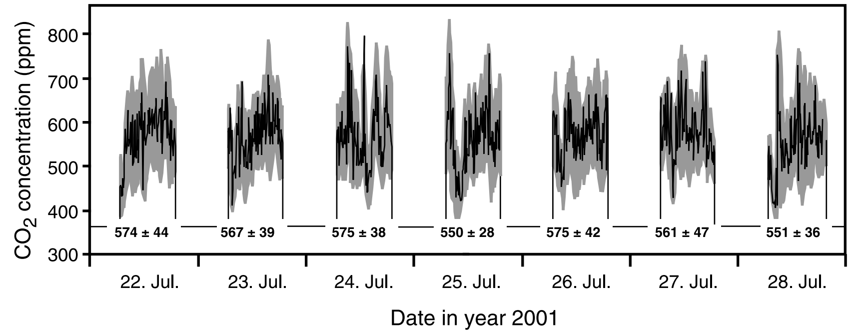

Fig. 3 Diurnal course of CO_2 concentrations (enriched for 12 h from 7 am to 7 pm) measured within five CO_2-enriched plots over a representative mid summer week in July 2001 (measurements are recorded every six minutes). The mean of five reference plots (black hairline) ± the standard deviation (grey area) are shown. Bold numbers at the bottom of the graph indicate mean daytime CO_2 concentrations (± SD).

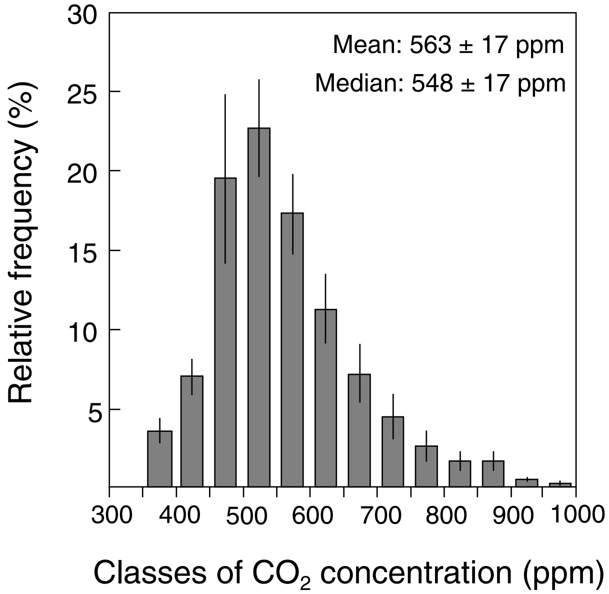

Fig. 4 Frequency distribution of daytime CO_2 concentrations (1 s readings) measured within the five reference plots during a representative week in July (22.–28.7. 2001). Bars represent mean values ± SE of 5 plots.

(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

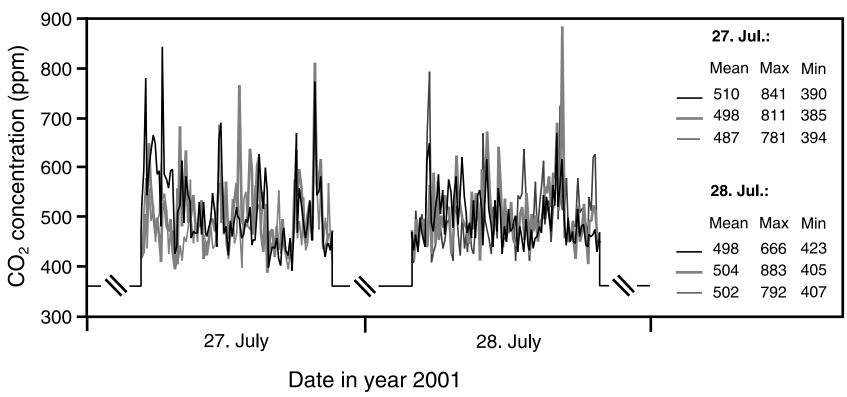

Fig. 5 Diurnal course of CO_2 concentrations measured in the center of three CO_2-enriched plots over two days in July 2001. The solid black line shows the CO_2 concentrations measured in the center of the reference plot used to control and regulate the CO_2 concentrations for the other plots. The grey 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%
$A_{max\ (area)}$ (μmol m^{-2} s^{-1})	10.1 ± 0.3	14.7 ± 0.6	+46%	7.1 ± 0.2	11.1 ± 0.4	+56%
$A_{max\ (mass)}$ (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 $A_{max\ (mass)}$. 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

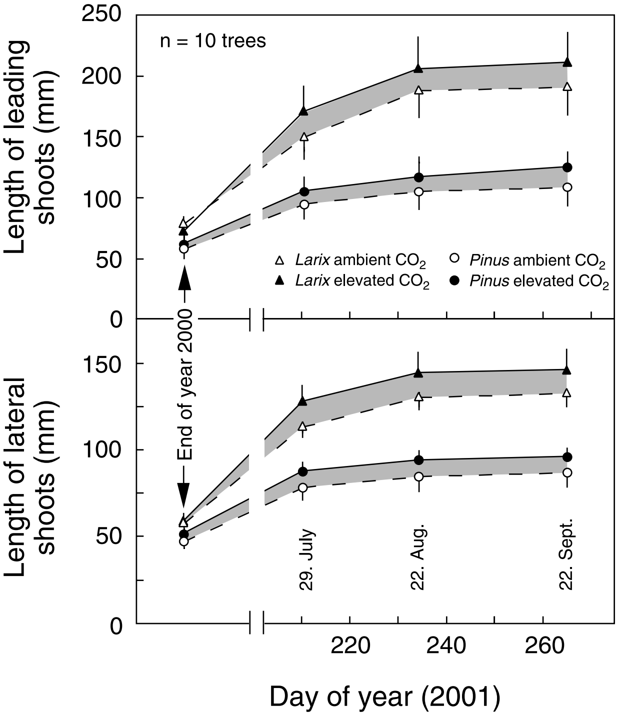

Fig. 6 Length increment of leading shoots (top) and lateral shoots (bottom) of *Larix decidua* (triangles) and *Pinus uncinata* (circles) grown at either current ambient CO_2 concentrations (open symbols) or at elevated CO_2 concentrations (closed symbols) during the first growing season of treatment 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.

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6 Elevated atmospheric CO_2 fuels leaching of old dissolved organic carbon at the alpine treeline

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Abstract

Dissolved organic matter (DOM), the mobile form of soil organic matter (SOM), plays an important role in soil C cycling and in nutrient transport. We investigated the effects of five years of CO_2 enrichment (370 vs. 570 µmol CO_2 mol^{-1}) on DOM dynamics at the alpine treeline, including the analysis of fast-cycling components such as low-molecular weight organic acids (LMWOAs), DOC biodegradability, and the decomposition of ^{14}C-labelled oxalate. Concentrations of DOC in canopy throughfall were 20% higher at elevated CO_2, probably driven by higher carbohydrate concentrations in leaves. In the organic soil layer, five years of CO_2 enrichment increased water-extractable organic C by 17% and soil solution DOC at 5 cm depth by 20%. The ^{13}C-tracing of recently assimilated CO_2 revealed that the input of recent plant-derived C (<15% of total DOC) was smaller than the CO_2-induced increase in DOC. This strongly suggests that CO_2 enrichment enhanced the mobilization of native DOC, which is supported by significant increases in DON. We mainly attribute these increases to a stimulated microbial activity as indicated by higher basal and soil respiration rates (+27%). ^{14}C-labelled oxalate was more rapidly mineralized from high CO_2-soils. The concentrations of LMWOAs, but also those of 'hydrophilic' DOC, and biodegradable DOC (6% of total DOC) were, however, not affected by elevated CO_2, suggesting that production and consumption of 'labile' DOC were in balance. In summary, our data suggest that five years of CO_2 enrichment speeded up the cycling of 'labile' DOM and SOM in a late-successional treeline ecosystem, and increased the mobilisation of older DOM through a stimulated microbial

activity. Such a ‘priming effect’ implies that elevated CO_2 can accelerate the turnover of native SOM, and thus, it may induce increasing losses of old C from thick organic layers.

1. Introduction

As the mobile fraction of soil organic matter (SOM), dissolved organic matter (DOM) represents a key vehicle for the transport of nutrients in ecosystems and determines the solubility and mobility of metals and organic compounds [e.g. *Kalbitz et al.*, 2000; *Hagedorn et al.*, 2000]. Fluxes of DOM through the soil are a small but potentially important pathway of C loss [*Neff and Asner*, 2001] and sorption of DOM in the mineral soil might contribute significantly to the accumulation and preservation of SOM [*Kaiser and Guggenberger*, 2000]. The current increase in atmospheric CO_2 by 1.5 ppm per year could potentially change DOM dynamics. Previous studies have demonstrated that experimental CO_2 enrichment alters the functioning of ecosystems and consequently DOM mobility in the following ways: (1) plant species specific changes in growth [*Saxe et al.*, 1998; *Körner*, 2000]; (2) a higher C allocation to below-ground sinks often followed by accelerated microbial activities [e.g. *Norby et al.*, 2004; *King et al.*, 2004]; (3) altered plant chemistry with decreasing N and increasing carbohydrate concentrations [*Körner*, 2000], and thus, changes in litter quality that could affect decomposition processes [e.g. *Hättenschwiler and Bretscher*, 2001].

Although the effects of elevated CO_2 on plants and ecosystems have been intensively studied, little attention has been paid to the response in DOM. A better understanding of DOM dynamics under elevated CO_2 could help to elucidate the role of soils as C sinks/sources with ongoing atmospheric change. To date it is controversial whether or not soils may sequester more C under elevated CO_2 [*Schlesinger and Lichter*, 2001; *Hagedorn et al.*, 2003; *Loya et al.*, 2003; *Carney et al.*, 2003]. Changes in SOM are difficult to detect, because C stocks are large and spatially highly variable [*Hungate et al.*, 1996]. However, since DOM is the leaching product of SOM and its sources - litter and roots – [*Kalbitz et al.*, 2000; *Hagedorn et al.*, 2004], DOM in soil waters might actually represent a highly sensitive indicator for how elevated CO_2 might affect SOM.

In planted microcosms, CO_2 enrichment increased water- and salt-extractable organic C of soils [*Cheng and Johnson*, 1998; *van Ginkel and Gorisson*, 1998], suggesting higher soil C solubilization, which agrees with the hypothesis of stimulated below-ground activity under elevated CO_2 [*Zak et al.*, 1993; *Hungate et al.*, 1997]. Recently, *Freeman et al.* [2004] observed that 3 years of CO_2 enrichment increased DOC concentrations in peatland microcosm by as much as 60%. They hypothesized that rising CO_2 might even be responsible for the increase in DOC in surface waters of Northern Europe. Other studies, however, observed only negligible CO_2 effects on DOC concentrations in solutions of disturbed mineral soils in aggrading forests and forested mesocosms [*King et al.*, 2001; *Hagedorn et al.*, 2002]. So far, none of the CO_2-studies has looked at different fractions of DOM. For instance, rapidly cycling compounds such as low molecular weight organic acids (LMWOAs) and biodegradable DOM are closely linked to root turnover and litter decay [*Yano et al.*, 2000; *van Hees et al.*, 2005]. Consequently, these DOM fractions are likely responding more sensitively to elevated CO_2 than bulk DOM.

Essentially all published data on CO_2 effects on DOM and SOM are based on artificial ecosystems with highly disturbed soils or plantations of rapidly growing plant communities. It is highly uncertain how well-established plant communities and undisturbed soils will respond to a CO_2-enriched atmosphere. Here, we have studied the effects of five years of CO_2 enrichment on DOM dynamics in a late-successional treeline ecotone in the Swiss Alps. Ecosystems at the alpine treeline are characterized by a relatively low net primary productivity, little developed soils with typically a thick organic layer [*Bednorz et al.*, 2000]. Soil organic matter dynamics and DOM fluxes are generally very little understood in alpine soils. A recent study at the Norwegian treeline suggests that the relative importance of DOC leaching as compared to other C fluxes is greater at the treeline than at lower altitudes [*Clarke et al.*, 2005].

In our study we specifically aimed to determine (1) how elevated CO_2 affects concentrations and characteristics of DOM at the alpine treeline, (2) how sensitive rapidly cycling DOM components respond to high CO_2 concentration, (3) if CO_2 enrichment rather affects recent plant-derived DOM or older DOM leached from SOM, and (3) the consequences of altered DOM dynamics for the overall SOM cycling under elevated CO_2.

2. Study site

The study was carried out at 2180 m a.s.l. at Stillberg in the Central Alps near Davos, Switzerland, where a long-term research site was established in the late 50s to study climate-plant growth relationships [*Schönenberger and Frey*, 1988]. The terrain is rather steep with north-east exposed slopes of 25 to 30°. Long-term average annual precipitation is 1050 mm, mean maximum snow depth is 1.50 m, and average January and July temperatures are -5.8 ºC and 9.4 ºC, respectively. Parent rock material is Paragneiss. Soil types are Rankers and weakly developed Podzols [*Blaser*, 1980] with characteristic properties shown in Table 1. The organic layers are Humimors dominated by an Oa horizon [*Bednorz et al.*, 2000] and have thicknesses between 5 and 15 cm. The plant community is dominated by ericaceous dwarf shrubs such as *Vaccinium myrtillus*, *Vaccinium uliginosum*,and *Empetrum hermaphroditum*. Common herbaceous species are *Gentiana punctata*, *Homogyne alpina* ,*Melampyrum pratense, Avenella flexuosa,* and *Leontodon helveticus.* Individual trees not taller than 1.5 -2.5 m of the two species *Larix decidua* and *Pinus uncinata* form a sparse open canopy. The trees originate from a large-scale afforestation experiment established in 1975 [*Schönenberger and Frey*, 1998], when small seedlings (1- to 3-yrs old) were planted into the intact and undisturbed dwarf shrub community.

Table 1

3. Methods

3.1. CO_2 enrichment

Our free air CO_2 enrichment (FACE) study site is located at or slightly above the actual natural treeline within a relatively homogeneous 2500 m^2 area [*Hättenschwiler et al.*, 2002]. Forty plots were assigned to 10 groups of four neighbouring plots (two each with an individual *Larix* and *Pinus* tree in the plot centre per group) in order to facilitate logistics of CO_2 distribution and regulation [*Hättenschwiler et al.*, 2002]. Half of these groups were randomly assigned to an elevated CO_2 treatment, while remaining groups served as controls, resulting in a split-plot design. The CO_2 release system was installed by fixing a hexagonal stainless steel frame with an area of 1.1 m^2 on three wooden posts. From each frame, 24 laser-punched drip irrigation tubes were hung vertically around the ring (15 cm apart from each other), weighted with a 3 mm stainless steel rod (to maintain rigidity) and the bottom ends were buried 2-4 cm into the soil, which resulted in a CO_2 enrichment of the trees and all understory vegetation in the plot. CO_2 concentrations were measured and regulated at the group level (four trees) with a control system. Growing season average was 566±75 ppm_v CO_2 under elevated CO_2 and 370±3 ppm under ambient CO_2. More details about the experimental set-up and the performance of the CO_2 enrichment are given by *Hättenschwiler et al.* [2002].

3.2. Sampling

Soil solution was collected in all plots by installing two ceramic suction cups (SoilMoisture Equipment Corp., Santa Barbara, USA) diagonally (60° to surface) at 3 to 7 cm depth in each plot. All of the suction cups were located within the Oa horizon that dominated the organic layer. In addition, we installed 10 suction cups (5 per treatment) at depths of 15 cm (E horizon) and 30 cm depth (BC horizon). The suction cups were installed at fixed depths and not at defined horizons, because space within the 1.1 m^2 plots was too limited to open pits for identifying diagnostic horizons and inserting horizontal lysimeters. In order to minimize sorption of DOC to the ceramic cups, we only used 'aged' suction cups that had been used in other experiments before. Prior to their installation, they were flushed first with 1 M HCl, and then with distilled water. All lysimeters at a given depth were connected to one 1-l glass bottle per plot buried in the ground. Residence time of soil waters in the sampling devices was kept as short as possible. At each sampling, we collected soil waters by evacuating suction cups with a constant 400 hPa for about 16 hours (overnight). However, during the dry summer 2003, we had to apply the suction for a week to get sufficient amounts of water.

Throughfall was sampled with small polyethylene (PE)-funnels (Ø 10 cm) just above the soil surface connected to 250 ml aluminium-foliated PE-bottles. In order to capture the spatial variability, we changed the placement of the PE-funnels after each sampling (n=4 per season) within each plot. Samples from throughfall and soil waters were collected every month during the snow-free season. The site is not accessible in winter. All samples of soil and throughfall waters were transported in cooling boxes to the institute, filtered through 0.45-*μ*m cellulose-acetate filters (Schleicher & Schuell, ME25) within the next two days and then stored at 4°C until analysis.

Soils and roots were sampled with a corer (Ø 2 cm, *n*=6 per plot), then stored in a cooling box. Within 12 hours, roots were removed and soils were homogenised with a 4 mm sieve. For soil microbial biomass and extracts with water, soils were directly processed. Aliquots of soils were dried at 105°C to determine soil water contents. For soil C analysis, soils were dried at 40°C and sieved at 2 mm and all visible roots were removed from small subsamples using a binocular. Finally, the root-free soil samples were ground with a ball mill (Retsch MM 2000).

Soil respiration was measured in the field with permanently installed PVC-collars (10-cm ID and a height of 5 cm) and a LI-COR 6400-09 soil chamber connected to a LI-COR-820 portable system for data collection. One PVC collar per plot (total n=40) was pressed to a depth of 2 cm into the organic layer in between dwarf shrubs. *Basal respiration* was determined by measuring CO_2 evolution from 8 g of field fresh soils. Homogenised soil samples were placed in 100 ml tubes and after one day they were incubated in 250 ml air-tight glass vessels at 20°C for 24 hours. The CO_2 produced was absorbed in NaOH and determined titrimetrically.

3.3. Incubation experiments

To determine the effects of elevated CO_2 on DOC turnover, we have conducted two incubation experiments: one, measuring the biodegradability of DOC in soil solution, and the other one, quantifying the mineralization of ^{14}C-labelled oxalate in soils. *Biodegradable DOC* was quantified by incubating 'fresh' soil solution from the Oa-horizon (collected for 24 hours and filtered to 0.45 µm) in the laboratory for four weeks. Soil solution (230 ml) was filled into 300 ml incubation flasks. To avoid nutrient limitation, we added 4 ml of a standard solution (4 mM $CaCl_2$, 2 mM K_2HPO_4, 1 mM K_2SO_4, 1 mM $MgSO_4$, 25 µM H_3BO_3, 2 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$) and NH_4NO_3 yielding 15 mg N l^{-1} in the soil solution. Then all samples were inoculated with 5 ml extracts of fresh Oa-horizon material collected outside the experimental plots (extraction of soils with 5 mM $CaCl_2$ in a ratio 1:5 and filtration through a Schleicher&Schuell 790 1/2). To provide surfaces for microbial growth, we added 5 g of fibre-glass and one fibre-glass filter to each flask. The flasks were sealed, incubated in the dark at 20°C for four weeks and gently shaken by hand every day. In order to check the activity of microorganisms, we used a glucose solution of 30 mg C l^{-1} as a control. Another control with ultrapure water was used to quantify the CO_2 production from the added inoculum. Biodegraded

DOC was quantified by two methods: first, by determining the difference in DOC in 0.45 µm filtrates before and after incubation; and second by measuring the increase in CO_2 in the headspace of the flasks during two biweekly intervals. The samples and headspace were flushed with CO_2-free compressed air before the incubation. The CO_2 concentration in the gas phase was calculated using the general gas equation, from which we calculated the CO_2 in the liquid phase by using solubility constants and the measured pH.

Oxalate biodegradation kinetics in soil: In late August 2003, after three years of CO_2 enrichment, composite Oa horizon samples were taken from all plots and bulked for all blocks, yielding *n*=5 for both soils from ambient and elevated CO_2 plots, respectively. ^{14}C-oxalate (1,2-^{14}C; 185 MBq mmol^{-1}; ARC, Saint Lewis, MO, USA) was used in the assay. A ^{14}C-radiolabelled solution (100 *µ*l, specific activity 1.7 kBq ml^{-1}) with concentrations ranging from 10 to 1000 *µ*M (1–100 nmol; pH 4.5) was added to 1.00±0.02 g of moist soil contained in 50 ml polypropylene tubes. Following addition, the soil was gently shaken to ensure mixing and incubated at 4 °C in sealed tubes. The $^{14}CO_2$ produced by biodegradation of the substrate was collected by placing a plastic scintillation vial containing 1.0 ml of 1 M NaOH inside the tube, on top of the soil but separated from direct contact with the soil by a spacer. $^{14}CO_2$ trapped as $NaH^{14}CO_3$ in the NaOH was determined by liquid scintillation (Wallac 1414 scintillation counter, Wallac, Tampere, Finland) using alkali compatible scintillation fluid (Wallac Optiphase 3; Wallac, Tampere, Finland). ^{14}C-CO_2 production was measured during the linear initial phase of mineralization, which was assessed in a separate experiment (not shown). The sampling time for $^{14}CO_2$ trap removal was 1 h.

The concentration-dependent experimental data were fitted to a single Michaelis–Menten equation:

$V = (V_{max}C_s) / (C_s + K_M)$

where V is microbial substrate mineralization rate, V_{max} the maximum mineralization rate, C_s soil solution concentration and K_M is the concentration at which half maximal mineralization occurs. C_s was calculated assuming perfect mixing of the added oxalate with the solution present in the soil (estimated from the moisture content) and sorption onto the solid phase. Sorption characteristic (Langmuir) were taken from an Oe horizon of a South Swedish forest soil [*van Hees et al.*, 2003].

3.4. Chemical Analysis

[*Hydrophobic* and *hydrophilic DOM* was estimated by passing samples acidified to a pH of 2 through XAD-8 columns [*Aiken and Leenheer*, 1993]. In the effluent of the XAD-8 columns, representing the hydrophilic fraction, C concentration was measured. The hydrophobic fraction of DOC was calculated by difference. The *molar UV absorptivity* at 285 nm in DOC, a measure for aromaticity [*Chin et al.*, 1994] was determined with a Cary 50 UV-Spectrophotometer (Varian, Inc., Palo Alto, CA, USA). *Phenol* concentrations were measured with the Folin-Denis assay according to *Swaine and Hills* [1959]. *Low molecular weight organic acids* (LMWOAs) were determined in soil solutions sampled in mid September 2003 and early September 2004 by capillary electrophoresis using the method of *Dahlén et al.* [2000] employing electrokinetic injection. To determine oxalate and citrate, EDTA (final concentration 250 *µ*M at pH 9) was added in a separate run to eliminate interference from Al and Fe ions. Sampling time for the soil solution was kept as short as possible, three days in the relatively dry year 2003 and 12 hours in 2004. Unfiltered samples were immediately frozen after collection.

[*Water-extractable organic carbon (WEOC)* was measured by gently shaking 5 g of field fresh soils with 100 ml 0.05 mM NaCl using an overhead shaker within 12 h after sampling. Roots were removed and soils were homogenised prior to the extraction. We chose a 0.05 mM NaCl solution because it had approximately the same ionic strength as throughfall.

Concentrations of dissolved and water-extractable organic C and total dissolved N (TDN) was determined with a Shimadzu TOC/TN analyser (TOC-V, Shimadzu Corp. Tokyo, Japan). Dissolved organic N was estimated by subtracting concentrations of DIN from those of TDN. In all soil waters, the error of this indirect estimate was small because the fraction of DIN in TN was below 5% (Table 1). When inorganic N concentrations were below the detection limits (0.008 and 0.010 mg N l^{-1} for NO_3^- and NH_4^+, respectively), we subtracted half of these values from TDN. Nitrate concentrations were determined by ion chromatography (DX-120, Dionex, Sunnyvale, CA), those of NH_4^+ were measured colorimetrically by automated flow injection analysis (PE FIAS-300, PerkinElmer, Inc., Waltham, MA, USA). The *$\delta^{13}C$ values* of soil solution were analysed by freeze-drying aliquots in order to quantify the fraction of 'reecent plant-derived' C in DOM. Small amounts of K_2SO_4 were added prior to the freeze-drying to facilitate the recovery and weighing of samples. The $\delta^{13}C$ values of plant and freeze-dried samples were determined with an automated elemental analyser–continuous flow isotope ratio mass spectrometer (EA-1110, Carlo Erba, Milan, interfaced with a Delta-S Finnigan

MAT, Bremen). Results of the C isotope analysis are expressed in δ units (‰). The $\delta^{13}C$ values were referenced to the Pee Dee Belemnite (PDB) standard.

3.5. Calculations and Statistical Analysis

Fluxes of DOM from the organic layer were roughly estimated by multiplying modelled water fluxes with DOM concentrations. Water fluxes were modelled with DyDOC [*Michalzik et al.*, 2003], its hydrological subroutine is a simple 'bucket' model assuming that precipitation falls as snow at temperature below 0°C and snow melts above 5°C. Since we did not have DOM concentrations for the winter when the study site was not accessible, we roughly estimated them by assuming a Q_{10} of 2.

Effects of CO_2 enrichment on all measured parameters were tested by ANOVA using a full factorial split-plot model. Concentrations of DOC, DON, water-extractable OC, and *in situ* soil respiration were log transformed before the analysis. All statistical analyses were performed with R [version 1.9.1, R Development Core Team, 2004].

4. Results

4.1. DOM concentrations and fluxes

Concentrations of DOC and DON show a typical depth distribution with concentrations peaking in throughfall and the organic layer (Figure 1). In the sandy and stony mineral horizons (E and Bhs), concentrations of DOC decreased, but they still had values of approximately 10 mg C l^{-1} at 30 cm depth. Dissolved organic N was the dominant form of N in all soil waters at 5, 15, and 30 cm depth comprising more than 95% of total dissolved N. The DOC to DON ratios increased with passage through the soil from 30±5 in throughfall to 57±3 in the mineral soil at 30 cm depth. Within the Oa horizon, DOC/DON was almost twice as high as the C to N ratio of solid organic matter (Table 1 and 2).

Fluxes. Modelled drainage from the organic layer was 1200, 650, and 1000 mm y^1 in 2002, 2003, and 2004, respectively. Evapotranspiration was estimated to be between 250 to 300 mm y^{-1}, which closely matched evapotranpiration losses measured in lysimeter experiments with dwarf shrub communities at our study site ranging from 200 to 300 mm y^{-1} [*De Jong et al.*, 2002]. Under the assumption that DOC concentrations during winter months followed a Q_{10} of 2 [*Michalzik et al.*, 2003], we estimated that the DOC exports from the organic layer ranged between 20 and 35 g C m^{-2} y^{-1}. The DON export was about 6 g N m^{-2} y^{-1} making up more than 95% of the total N export (inorganic + organic N).

CO_2 enrichment significantly increased concentrations of DOC in throughfall (Figure 1; +20%; $P<0.05$). The CO_2 effect on throughfall DON was smaller (+12%) and not significant. The slightly different response of DOC and DON shows that the DOC to DON ratio in throughfall increased under elevated CO_2 (35±2 vs. 31±1) but this difference was only marginally significant ($P<0.06$).

[24] In the soil solution of the organic layer at 3 to 7 cm depth, CO_2 enrichment clearly increased DOC concentrations over time (Figure 2). While the DOC concentrations were approximately the same in 2002 in both CO_2 treatments (+3% at high CO_2), the CO_2 effect on DOC increased from year to year (2003: +10%, 2004: +13%; 2005: +23%). Due to the large spatial variability, the CO_2-induced increase in DOC was not significant,

not even in 2005 (main CO_2-effect: $P=0.12$). However, the increase in DOC concentrations of DOC between 2002 and 2005 (Figure 2) was significantly greater at elevated CO_2 (+36%) as compared to ambient CO_2 (+14%, $P<0.05$). The response in DON concentration was essentially the same as found for DOC. Again, the main CO_2 effect was not statistically significant in either year ($P=0.11$ in 2005), but the increase in DON concentrations between 2002 and 2005 was significantly greater under elevated (+27%) than under ambient CO_2 (+ 5%; $P<0.05$). The increase in DOC and DON concentrations under elevated CO_2 did not result from a concentration effect through decreasing amounts of water. On the contrary, measured water contents were slightly greater at elevated CO_2 (+9 and +7% in 2003 and 2004; n.s.). At the lower soil depths, 15 and 30 cm, CO_2 enrichment did not affect DOC and DON concentrations (Figure 1). The DOC/DON ratios were not affected by the CO_2 enrichment in none of the soil depths.

Water-extractable organic C from ‘root-free fresh’ organic layers comprised roughly 0.25% of total soil C. In line with *in situ* DOM, water extractable organic C in 2003 and 2004 was on average 17% higher under elevated CO_2 (Figure 3, $P<0.05$). Since concentrations of solid SOM (measured from the same samples as water-extractable organic C) were the same in both CO_2 treatments, the larger soluble C pool indicates that elevated CO_2 increased DOM production.

Soil respiration. CO_2 enrichment stimulated basal respiration from ‘root-free, fresh’ Oa horizons by 30% (Figure 4; $P<0.05$). Consistently, mean soil CO_2 efflux during the fifth season in 2005 was 27% greater under elevated CO_2, but this effect was only marginally significant ($P<0.07$).

4.2. DOM properties

Enrichment with CO_2 had no effects on the molar UV absorptivity, the concentrations of phenolics, and on the fractions of hydrophilic DOC in the organic layer (Table 2). A number of LMWOAs were identified in the soil solutions (Table 3). Average concentrations varied between <0.1-3 μM with the highest values seen for the monoprotic acids (1-3 C atoms per molecule). In addition to the LMWOAs presented, propionate was occasionally detected (<1.1 μM; not shown). The contribution of LMWOAs to total DOC was small (0.42% ± 0.04) and overall little affected by CO_2 enrichment (Table 3). The LMWOA composition is comparable to most studies involving forest soils, but the concentrations are at the lower end of the scale [*Strobel et al.*, 2001]. Concentrations of acetate were significantly lower at elevated CO_2, while those of lactate were significantly higher in 2003.

4.3. DOM turnover

Biodegradation. The four-week incubation of 'field-fresh' soil solution sampled during one day from the Oa horizon showed that only a relative small fraction - 6% of total DOC- was rapidly biodegradable (Table 2). The low mineralization rate cannot be attributed to an insufficient activity of microbes since 90% of the glucose added as a control was lost during the incubation (and the rest was very likely tied-up in microbial biomass; data for glucose not shown). Mineralization of DOC did not change in response to the previous four-year CO_2 exposure of soils.

Mineralization of ^{14}C-labelled oxalate was fast and conformed to a hyperbolic (Michaelis-Menten) type of kinetics over the concentration interval studied ($R^2 > 0.8$; Figure 5). Both maximum mineralization rates (V_{max}) and the concentration at which half maximal mineralization occurs (K_M) are within the range reported for forest soils (van Hees et al., 2005). On average, soils from elevated CO_2 plots showed marginally significant larger V_{max} values (+43%) and higher K_M values (+29%), indicating that high CO_2 stimulated mineralization of added ^{14}C-labelled oxalate in soils. In order to get an estimate of the CO_2-effects on the oxalate mineralization under field conditions, we used the measured mineralization kinetics to calculate the mineralization rates for *in situ* soil oxalate concentrations measured in 2003 (Table 3). Figure 5 (inset) shows that they were 40% higher at elevated CO_2 compared to ambient CO_2 ($P<0.05$).

Recent plant-derived DOC. Using ^{13}C depleted CO_2 (-30‰) for CO_2 enrichment, yields an arithmetically calculated decrease in ^{13}C by 7.2‰ in atmospheric CO_2 in elevated CO_2 plots. Accordingly, $\delta^{13}C$ values in pine and larch needles decreased substantially by 6.1‰. This decrease was also reflected in DOC: after the fifth year, the $\delta^{13}C$ values in DOC were 0.9‰ smaller under elevated than under ambient CO_2. Applying a simple mixing model with two end-members - the ‘recent’ plant-derived C and old SOM - yields a contribution of ‘recent’ C to DOC of 15±1% after five years of CO_2 enrichment.

5. Discussion

5.1. DOM fluxes in a CO_2 enriched treeline ecotone

Our first estimates of DOM fluxes at an alpine treeline indicate that DOM in throughfall and leachates from the organic layer contributes significantly to C and N fluxes in these ecosystems. DOC inputs via throughfall amounted to 5 g C m^{-2} during the 4-month growing season corresponding to 10% of the annual C input through leaf-litterfall [*T.*

Handa, unpublished data]. The DOC export of about 20 to 35 g C $m^{-2}y^{-1}$ from the organic layer found here is in the range of values reported for temperate forest ecosystems [*Michalzik et al.,* 2001]. However, in relative terms, DOC appears to be far more important for the overall C cycle in the treeline ecotone than in temperate forests which are typically characterized by other large C fluxes through litterfall and SOM mineralization. At our study site, *Reichstein et al.* [2000] estimated a mineralization rate of 70 to 120 g C $m^{-2}y^{-1}$ from the organic layer, which agrees well with our measured heterotrophic soil CO_2 effluxes (for the vegetation period see Figure 4). Thus, DOC export accounted for approximately 20 to 30% of the total annual C losses from the organic layer.

Fluxes of DOM appear to have increased in response to five years of CO_2 enrichment in this late-successional treeline ecotone. Throughfall DOC, water-extractable OC in the organic layer, and concentrations of '*in situ*' DOC and DON in soil solutions at 3 to 7 cm depth in the Oa horizon, all increased under elevated CO_2. The composition of '*in situ*' DOM did, however, not change. The 20%-increase in throughfall DOC in response to elevated CO_2 can probably be attributed to an increased availability of soluble C in and on leaves. Concentrations of non-structural carbohydrates, in particular starch, increased significantly in tree needles and dwarf shrub leaves under elevated CO_2 [*Handa et al.*, 2005; *Asshoff and Hättenschwiler*, 2005]. The wider DOC/DON ratios at elevated CO_2 supports the idea that an enhanced accumulation of 'labile' C compounds was mainly responsible for the increase in throughfall DOC. In addition, enhanced above-ground growth, and thus, increased canopy area might have contributed to the increase in throughfall DOC at the higher CO_2 level [Table 4; *Handa et al.*, 2006; *T. Zumbrunn*, unpublished data].

Our results are in line with the 50%-increase in throughfall DOC under elevated CO_2 reported from a loblolly pine plantation [*Lichter et al.*, 2000]. They suggested that although the absolute increase in throughfall DOC is small as compared to other C fluxes, it may fuel soil microbial activity because it is composed of mainly metabolically readily accessible forms of organic matter. We indeed found strong evidence for a stimulating CO_2 effect on belowground activity. Basal respiration increased by 30% (Figure 4), indicating that more substrate was available under higher CO_2 concentrations. Likewise, we measured a marginally significant 27% greater CO_2 efflux from soils of high CO_2 plots. Such a stimulation of soil microbial activity by elevated CO_2 can be explained by the greater direct input of labile throughfall DOC, but also by an increased C allocation to roots, which could lead to increased root exudation. Although total root biomass remained unchanged [*Handa et al.*, 2008], we found a 32% higher accumulation of starch in fine roots [Table 4].

5.2. Elevated CO_2 induced mobilisation of native DOM

Since we exposed late-successional plant communities on naturally developed thick organic soil layers to elevated CO_2 and given that bulk DOM is mainly made up of relatively ‘old’ components [*Fröberg et al.*, 2003; *Hagedorn et al.*, 2004], we did not expect a CO_2 effect on DOM after a relatively short experimental duration of five years. The increased DOM concentrations in response to CO_2 enrichment found here is in contrast to other experiments with forest ecosystems on mineral soils with juvenile and expanding tree communities. After two to four years of CO_2 treatment, no effects on DOC concentrations in topsoils have been found, despite considerable increases in fine-root biomass and greater soil respiration rates [*King et al.*, 2001; *Hagedorn et al.*, 2002]. However, our results agree well with a microcosm experiment in peatlands where DOC concentrations increased by 14 to 61% after three years of CO_2 enrichment [*Freeman et al.*, 2004]. Integrating across these few existing CO_2 studies suggests that the type of soil plays a decisive role on the CO_2 effects on DOM: in pure organic horizons - peatland soils in the *Freeman et al.* [2004] study and organic layers in our experiment – elevated CO_2 increased DOC leaching, while DOC concentrations in mineral soils did not respond. Apparently, mineral surfaces sorbing DOM particularly the lignin-derived DOM fraction [*Kaiser and Guggenberger*, 2000] are dampening the response of DOM to CO_2 enrichment.

A higher production of ‘recent’ DOM through increased plant growth, increased belowground C allocation, and higher DOM inputs via throughfall is the most evident mechanism for the increased DOC and DON concentrations under elevated CO_2. Interestingly, our data show that the fraction of ‘recent’ labile DOC is too small in bulk DOC to account for the observed increase. The ‘labile’ DOM fractions such as low-molecular weight organic acids and biodegradable DOC, which both presumably derive from recent C inputs from roots and decomposing litter, were below 10% of total DOM and did not respond to CO_2 enrichment. Moreover, tracing the ^{13}C signal added by the CO_2 treatment indicates that recent less than five-year old photosynthates contributed only to 15% to total DOM, which is actually smaller than the CO_2-induced increase in DOC (+23%; Figure 6). The water-extractable OC in 2003 after three years of CO_2

enrichment shows the same pattern with an increase of 17% under elevated CO_2, while the net input of recent C amounted only to 10% of total water-extractable OC (Figure 3). These small fractions of recent C and 'labile' C in DOM provide evidence for an accelerated mobilisation of 'old' DOM through a stimulated microbial breakdown, as an additional more indirect mechanism for increased DOM under elevated CO_2. Unfortunately, we cannot compare the fractions of plant-derived recent C in DOM at the two CO_2 concentrations, because the ^{13}C-label lacks at ambient CO_2. However, even under the extreme assumption that elevated CO_2 would increase the input of recent C as much as photosynthesis (+50%, *Handa et al.* [*2005*]), it would increase total DOC leaching by a mere 5%, which strongly suggests that the major fraction of the 23%-increase in DOC at elevated CO_2 was derived from an accelerated mobilisation of older native SOM. Our conclusion is supported by the similar increase in DON leaching by the CO_2 enrichment (Figure 2), which cannot be attributed to higher plant inputs since (i) N concentrations of dwarf shrubs and trees declined by 14% under elevated CO_2 [*Handa et al.*, 2005; *Asshoff and Hättenschwiler*, 2005], (ii) the DOC-to-DON ratio in throughfall was greater under CO_2-enriched plants and (iii) DOM leached from decomposing litter was significantly depleted in N as compared to C [*Hagedorn and Machwitz*, 2007].

An accelerated mobilisation of old DOC through a stimulated microbial activity - the so-called 'priming'-effect is discussed controversial [see reviews by *Kuzyakov*, 2000; *Fontaine*, 2003]. A common explanation for priming is that an increased availability of microbial substrate induces enzyme production and/or increases enzyme activity leading to a co-metabolic decomposition of soil organic matter. Although the production of DOM is assumed to be closely linked to enzymatic activity [*Pregitzer et al.*, 2004] and to be a by-product of lignin degradation [*Kalbitz et al.*, 2006], very little is known about priming effects on the release of DOM. *Park and Matzner* [2003] showed that experimental additions of glucose to forest floors stimulated microbial activity and doubled fluxes of DOC and DON far beyond an increase from the rapidly-decomposing glucose itself. Altered soil microbial communities at elevated CO_2 might have contributed to the greater mobilisation of older DOM. For instance, *Carney et al.* [2007] observed that CO_2 enrichment increased the abundance of fungi and accelerated losses of old SOM. Since fungi are the main lignin degrader in soils, it could be that a change in microbial communities under elevated CO_2 led to an increased production of lignin-derived DOM. Our ^{13}C-based study is the first to suggest that elevated CO_2 can induce 'priming' by mobilising old DOM in undisturbed organic layers. The composition of the DOM being typical for DOM leached from organic layers into mineral soils with the high-molecular, lignin-derived hydrophobic fraction dominating (Table 2) suggests that similar effects might occur in other forest soils. The CO_2-induced increase in DOC concentration found here, corresponds to an increase in DOC export from the organic layer by 4 to 6 g C m^{-2}, which is negligible in comparison to other C fluxes. With respect to N, however, the 25%-increase in DON leaching at elevated CO_2 is more relevant, because the treeline ecosystem is N-poor with undetectable inorganic N concentrations in soil solutions and the DON export from the organic layer is similar in magnitude as total atmospheric N deposition in alpine regions [*Schmitt et al.*, 2005].

5.3. Increased turnover of 'labile' DOC

Initially, we hypothesized that 'labile' DOM compounds would respond most sensitively to the CO_2-induced stimulation of belowground activity and to increased C inputs via throughfall. Our analysis of DOM composition, however, revealed that bulk DOC but none of the DOC fractions, not even the most labile ones were affected by elevated CO_2. Neither the 'hydrophilic' DOC, dominated by carbohydrates from decomposing plant residues and microbial metabolites [*Kaiser et al.*, 2001], nor the rapidly cycling DOC compounds such as LMWOAs and biodegradable DOC showed a response to increased atmospheric CO_2.

Measured concentrations of labile DOC, however, must not necessarily correspond with production rates of labile DOC, because elevated CO_2 might have simultaneously stimulated the consumption of 'labile' compounds. Indeed, our mineralization experiment with ^{14}C-labelled oxalate, a common root and fungal exudate, showed that the added oxalate decomposed more rapidly from soils that were previously exposed to higher atmospheric CO_2 concentration. This result provides evidence for a faster cycling of labile DOM with no net effect on labile DOM content in soil solutions. The stimulated oxalate mineralization supports our conclusion that the observed increase in DOM at elevated CO_2 was not primarily caused by an increased input of recent labile organic matter into DOM, because an accelerated microbial activity under CO_2 enrichment led to a more rapid decomposition of labile DOM but apparently induced an increased DOM mobilization (Figure 7).

Although concentrations of LMWOAs are small, they may contribute significantly to the CO_2 effluxes from soils due to their rapid mineralization, usually within hours. For instance, van Hees et al. (2005) estimated that LMWOA mineralization in a boreal forest accounts for approximately 45 g C $m^{-2}y^{-1}$. Therefore, our result showing that CO_2 enrichment stimulated oxalate mineralization by 40% suggests that an increased turnover of ‘labile’ DOM could substantially contribute to the CO_2-induced increase in soils C availability and respiration rates (+60 g CO_2-C m^{-2} during the growing season 2005). In contrast to soil respiration, however, labile rapidly cycling DOM plays a less important direct role for DOM leaching from the 5 to 15 cm thick organic layer at this alpine treeline, because Oa horizon DOM is dominated by older non-readily biodegradable organic matter (Table 2; Figure 6).

5.4. Implications for soil carbon cycling

In contrast to most previous studies on the response of SOM to elevated CO_2 in aggrading ecosystems on disturbed soils [e.g. *Schlesinger & Lichter*, 2001; *Hagedorn et al.*, 2003; *Loya et al.*, 2003], we have run our CO_2 enrichment experiment in an unproductive treeline ecotone with naturally developed alpine soils. Since spatial heterogeneity is high and C stocks of the 5 to 15 cm thick organic layers are big, the direct detection of any CO_2 effect on SOM is impossible after some years. In our study, however, we may make use of our measured DOC dynamics to infer indirectly on SOM cycling because DOC comprises a small fraction of SOM, which is in a microbially-driven dynamic equilibrium with the much greater solid SOM fraction [*Kalbitz et al.*, 2000]. Our results showed that elevated CO_2 increased DOC concentrations by 20% and that this increase was mainly related to a stimulated microbial activity and not to a greater net input of recent C into DOC. It implies that CO_2 enrichment accelerated the mobilization of native SOM and thus, the turnover of SOM. This so-called ‘priming’-effect counteracts an increased storage of C in soils. If we can translate the increased leaching of DOM to SOM cycling, it would indicate that elevated CO_2 induces losses of older C from soils. Several studies have indicated that living roots and greater inputs of labile C stimulate native SOM decomposition [*Cheng and Coleman*, 1990; *Kuzyakov*, 2000]. Microcosm studies and plantations on agricultural C4-soils indicate that CO_2 enrichment can induce priming through an increased input of labile C [*Cheng and Johnson*, 1998; *Hoosbeek et al.*, 2004]. Recently, *Carney et al.* [2007] found in young scrub-oak ecosystems that elevated CO_2 reduced soil organic matter storage by altering soil microbial communities with higher abundances of fungi and higher activities of soil carbon-degrading enzymes. There is, however, no experimental evidence that elevated CO_2 leads to priming in natural ‘old’ ecosystems, because the SOM pool is too large and/or the fractions of recent C are not known under ambient CO_2. Therefore, our result suggesting that CO_2 enrichment induces an increased mobilisation of older DOM in thick organic layers is a first indication for priming in undisturbed nutrient-poor acidic soils. As the DOM in the Oa horizon was very typical for DOM exported from organic layers with the lignin-derived hydrophobic fraction dominating, CO_2-induced priming might occur in many forest soils. How quantitatively relevant it is for total soil C storage and thus for C sequestration remains, however, uncertain.

6. Conclusions

Atmospheric CO_2 enrichment in a late-successional treeline ecotone with thick organic layers on acidic soils influenced a number of key soil processes. Higher basal and *in situ* soil respiration rates indicate a sustained increased microbial activity after five years of CO_2 enrichment. This stimulation was also reflected in a higher turnover of the labile DOC fraction: ^{14}C-oxalate was more rapidly mineralized in high CO_2-soils, but as the concentrations of labile DOC components (including oxalate) remained unchanged, elevated CO_2 increased both the microbial consumption and the production of 'labile' DOC. In the Oa horizon, CO_2 enrichment significantly increased concentrations of total DOC which had a typical composition with an 80%-contribution of the high-molecular, lignin-derived hydrophobic fraction. A dominance of older C in DOM and an accompanying increase in DON suggests that this increase was mainly caused by an accelerated mobilisation of native SOM through a stimulated microbial activity under elevated CO_2. This so-called 'priming'-effect would counteract an increased storage of C in soils, but its quantitative importance for different ecosystems with other soil types needs to be explored.

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Table 1: Properties of organic layer material (mainly Oa-horizon) collected from 0-5 cm depth.

	Soil organic C %	C/N	pH ($CaCl_2$)	CEC eff $mmol_C$/kg	BS %
Ambient CO_2	40.8 ± 2.5	27.2 ± 0.7	4.2 ± 0.1	169 ± 8	83 ± 3
Elevated CO_2	40.3 ± 2.4	27.2 ± 0.5	4.2 ± 0.1	167 ± 13	80 ± 3

Table 2: Effects of 4 years of CO_2 enrichment on DOM properties of soil solutions of the Oa horizons (3 to 7 cm from the surface). Means and standard errors of n=20.

	DOC/DON	Molar UV absorptivity $l\ cm^{-1}mol^{-1}$	Hydrophilic DOC (% of DOC)	Phenolics (% of DOC)	∑LMWOAs (% of DOC)	Biodegradable DOC (% of DOC)
Ambient CO_2	47.0 ± 2.4	402 ± 20	19.4 ± 1.0	12.6 ± 1.7	0.46 ± 0.05	6.0 ± 0.7
Elevated CO_2	45.4 ± 1.8	411 ± 8	19.5 ± 1.4	12.1 ± 0.7	0.39 ± 0.06	5.9 ± 0.7

Table 3: Effects of three and four year of CO_2 enrichment on low-molecular weight organic acids in soil solutions of Oa horizons (3 to 7 cm from the surface). Means and standard errors of n=20.

	Low molecular weight organic acids						
	Acetic	Butyric	Citric	Formic	Malonic	Lactic	Oxalic
	-- μM ---						
2003							
Ambient CO_2	2.2±0.7^a	0.6±0.7^a	<0.1^a	1.9±0.5^a	0.1±0.0^a	1.0±0.2^a	1.0±0.2^a
Elevated CO_2	0.8±0.2^a	1.3±0.4^a	0.2±0.1^a	1.1±0.3^a	0.1±0.0^a	1.0±0.3^a	1.3±0.4^a
2004							
Ambient CO_2	1.6±0.4^a	1.1±0.1^a	0.1±0.1^a	1.1±0.2^a	0.1±0.0^a	1.8±0.6^a	0.9±0.1^a
Elevated CO_2	0.8±0.2^a	1.3±0.4^a	0.2±0.1^a	1.1±0.3^a	0.1±0.0^a	1.0±0.3^a	0.9±0.1^a
P_{CO2}	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 4: Effects of elevated CO_2 on cumulative shoot growth (2001-2004) and starch contents in needles and fine roots.

		Shoot [1] growth mm	Starch [1] Needles %	Starch Fine Roots %	Fine roots-protein %
Ambient CO_2	*Larix*	274 ± 8	9.9 ± 0.9	5.6 ± 0.5	1.2 ± 0.1
Elevated CO_2	*Larix*	319 ± 10	10.6 ± 1.2	7.9 ± 1.0	1.2 ± 0.1
Ambient CO_2	*Pinus*	157 ± 7	7.1 ± 0.8	6.5 ± 0.4	1.0 ± 0.0
Elevated CO_2	*Pinus*	183 ± 21	9.6 ± 1.4	8.0 ± 0.6	0.7 ± 0.0
PCO_2		<0.05	<0.05	<0.05	<0.05

[1] from Handa et al. (2005)

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Figure 6: Effects of elevated CO_2 on organic layer DOC and the ‘recent’ DOC originating from the recently assimilated CO_2. Means and standard errors of 18 plots.

Figure 7: Conceptual model of CO_2-effects on DOM dynamics. The CO_2-induced increase in belowground C allocation accelerates the production of labile DOM fraction. Microbial activity is also stimulated by elevated CO_2, which has two effects on the dynamics of DOM: firstly, it increases the decomposition of labile DOM which balances

out the greater production of labile compounds. Secondly, an enhanced enzymatic activity induces ‘priming’ - a greater mobilisation of DOM being dominated by ‘older’ components during the co-metabolic decomposition of SOM.

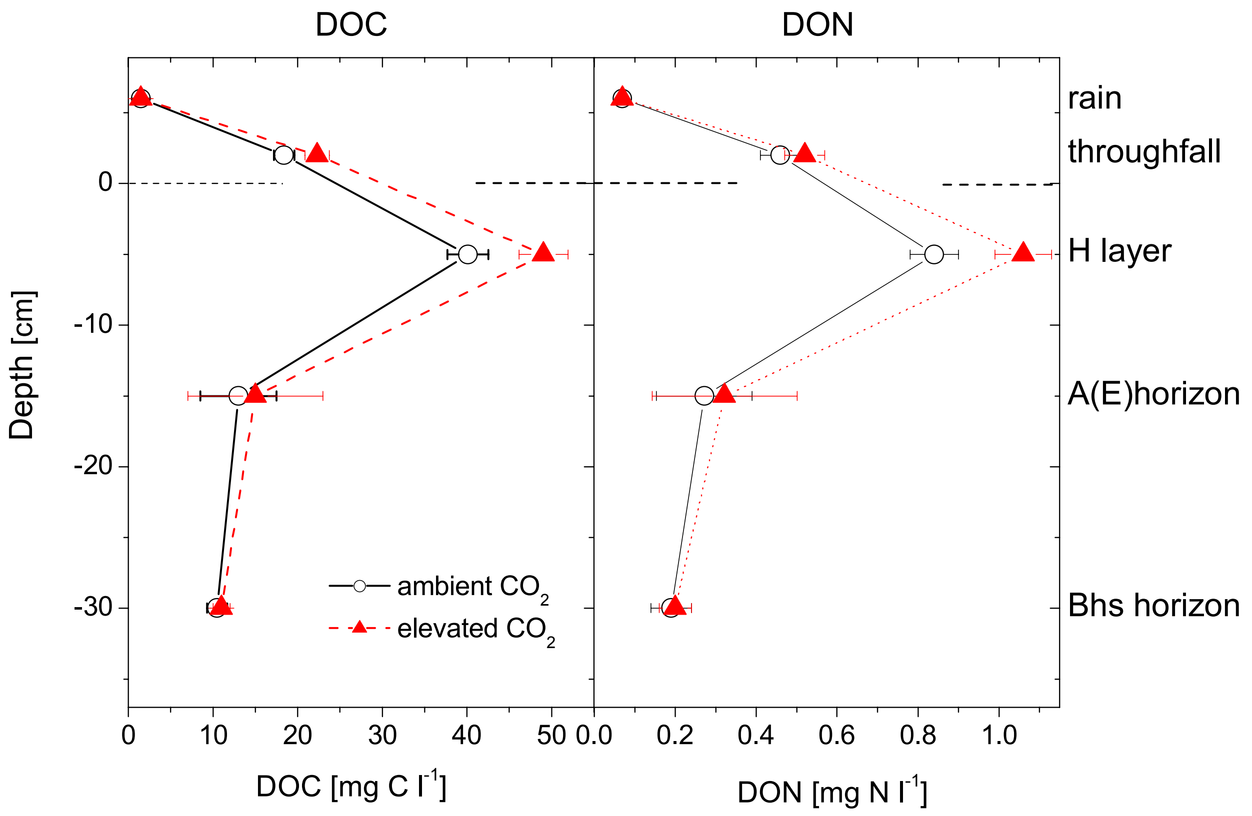

DOC
DON
rain
throughfall
H layer
A(E)horizon
Bhs horizon
Depth [cm]
0
-10
-20
-30
ambient CO_2
elevated CO_2
0
10
20
30
40
50
0.0
0.2
0.4
0.6
0.8
1.0
DOC [mg C l^{-1}]
DON [mg N l^{-1}]

Figure 1

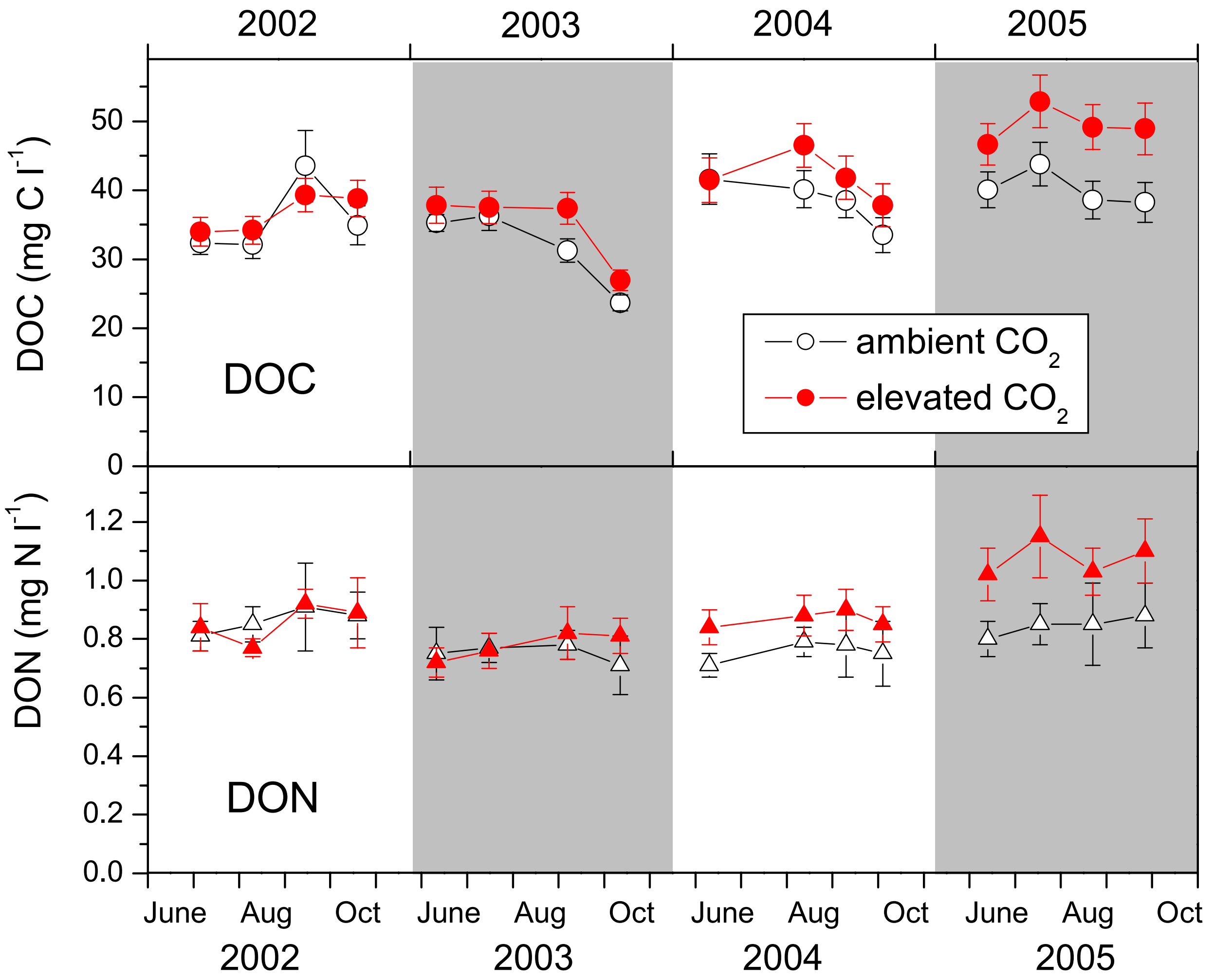

2002
2003
2004
2005
DOC (mg C l^{-1})
50
40
30
20
10
0
DOC
ambient CO$_2$
elevated CO$_2$
DON (mg N l^{-1})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
DON
June
Aug
Oct
June
Aug
Oct
June
Aug
Oct
June
Aug
Oct
2002
2003
2004
2005

Figure 2

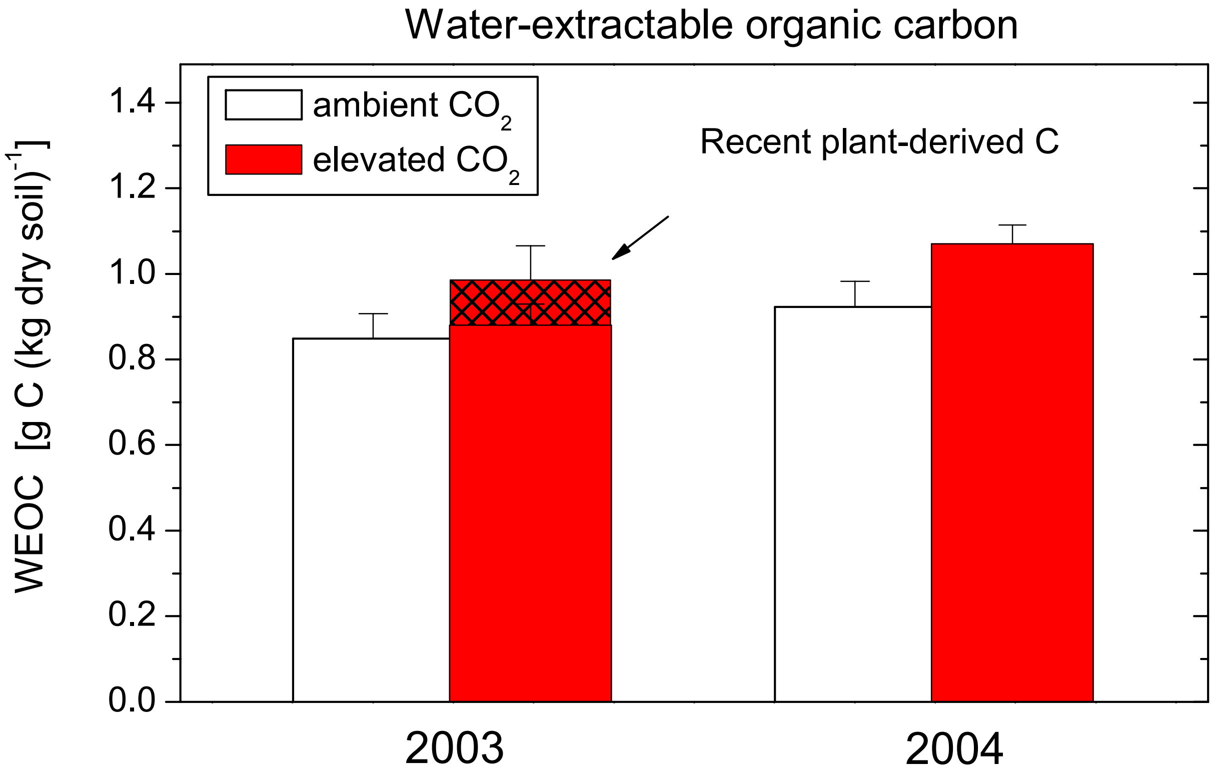

Water-extractable organic carbon
ambient CO_2
elevated CO_2
Recent plant-derived C
WEOC [g C (kg dry soil)$^{-1}$]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
2003
2004

Figure 3

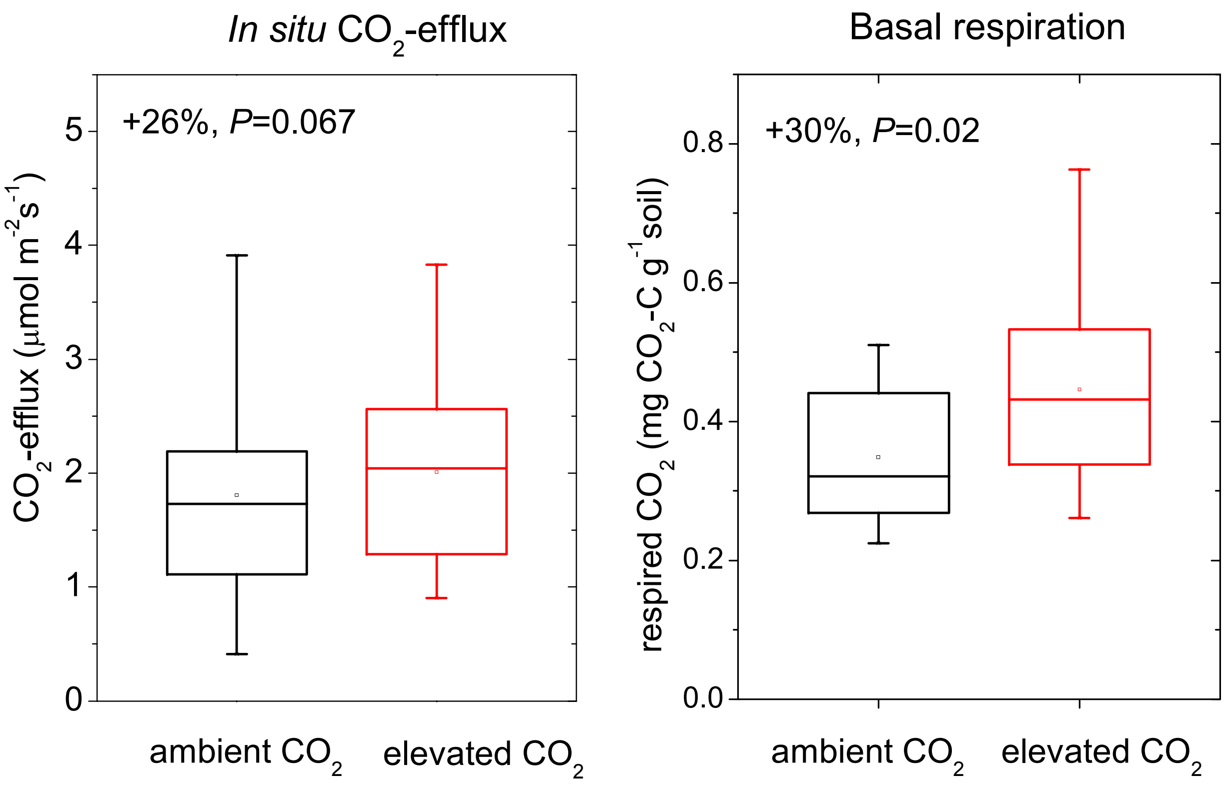
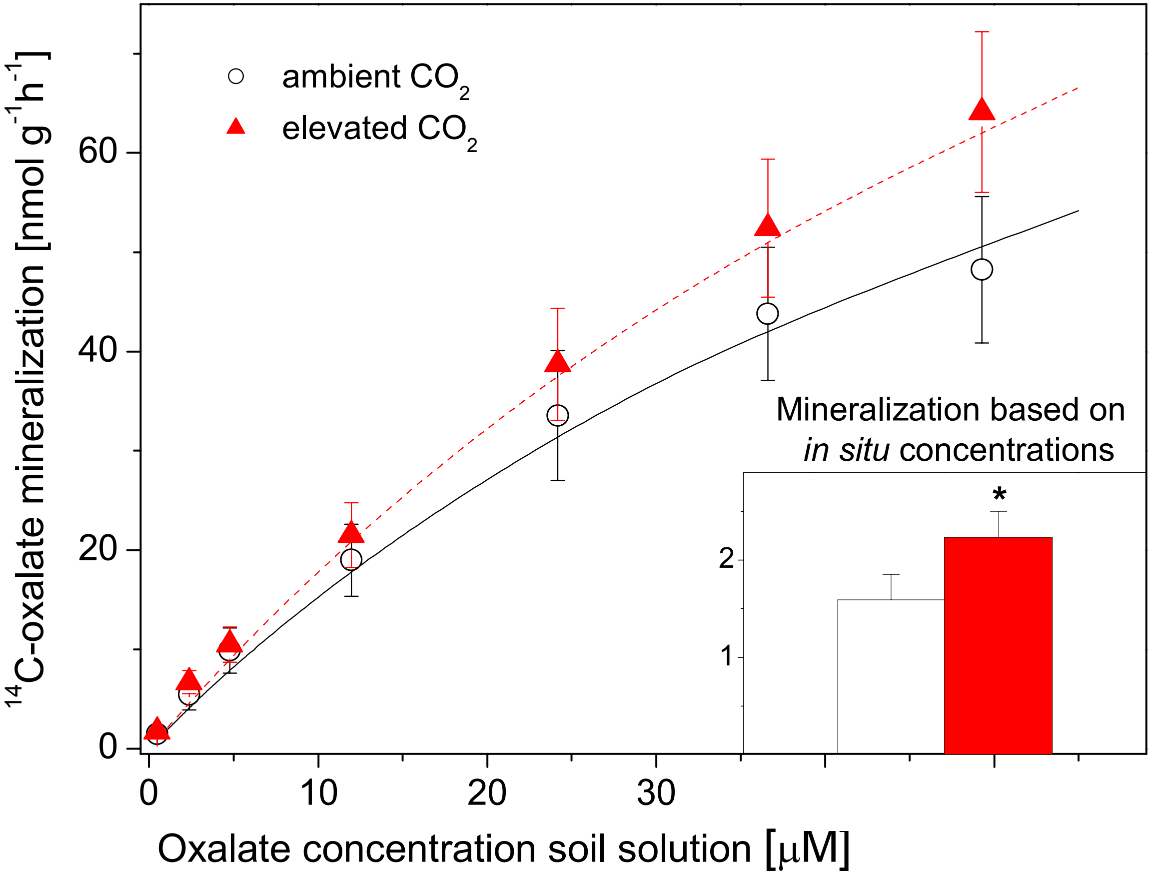
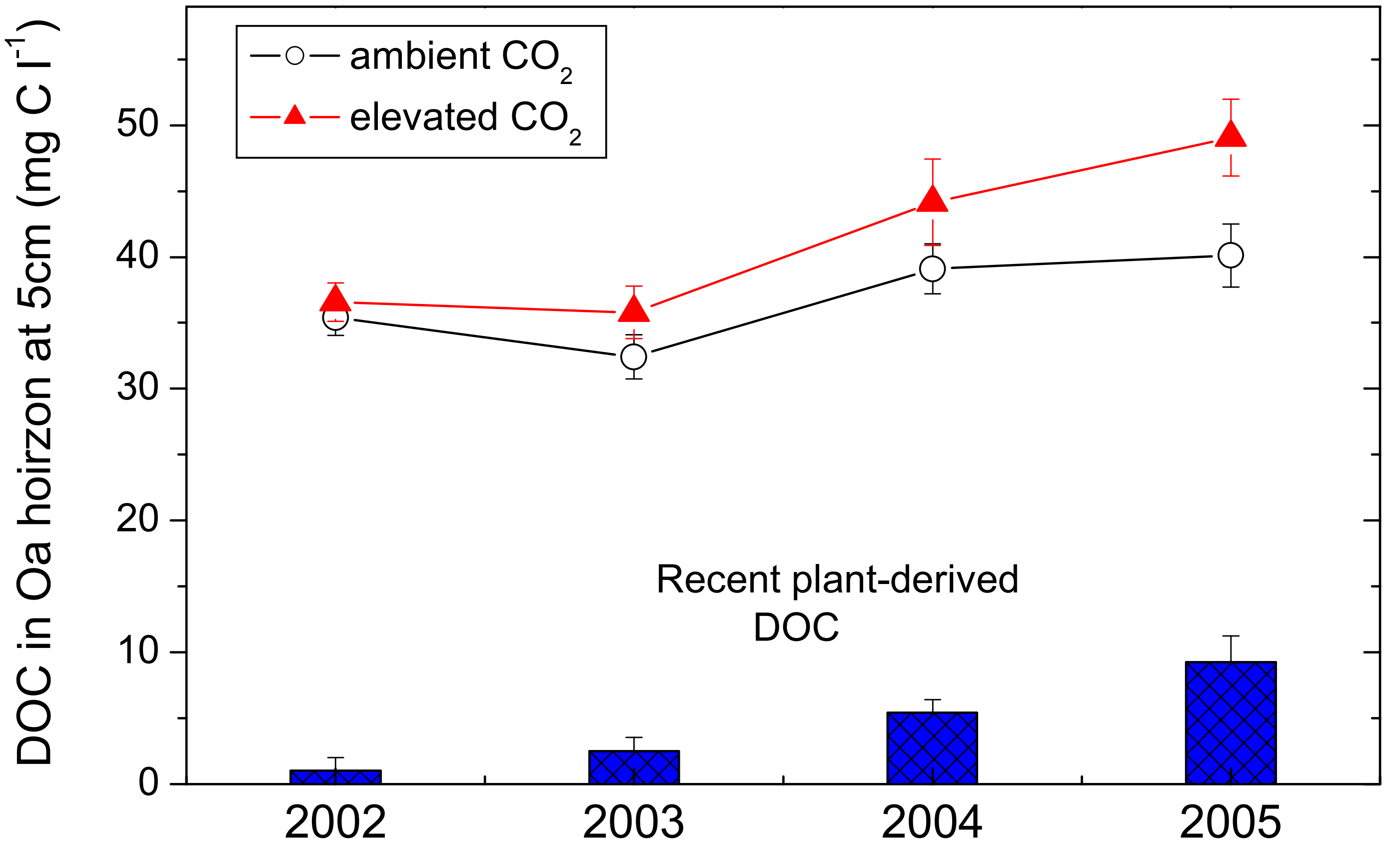

In situ CO_2-efflux
+26%, *P*=0.067
CO_2-efflux (μmol $m^{-2}s^{-1}$)
0
1
2
3
4
5
ambient CO_2
elevated CO_2
Basal respiration
+30%, *P*=0.02
respired CO_2 (mg CO_2-C g^{-1}soil)
0.0
0.2
0.4
0.6
0.8
ambient CO_2
elevated CO_2

Figure 4



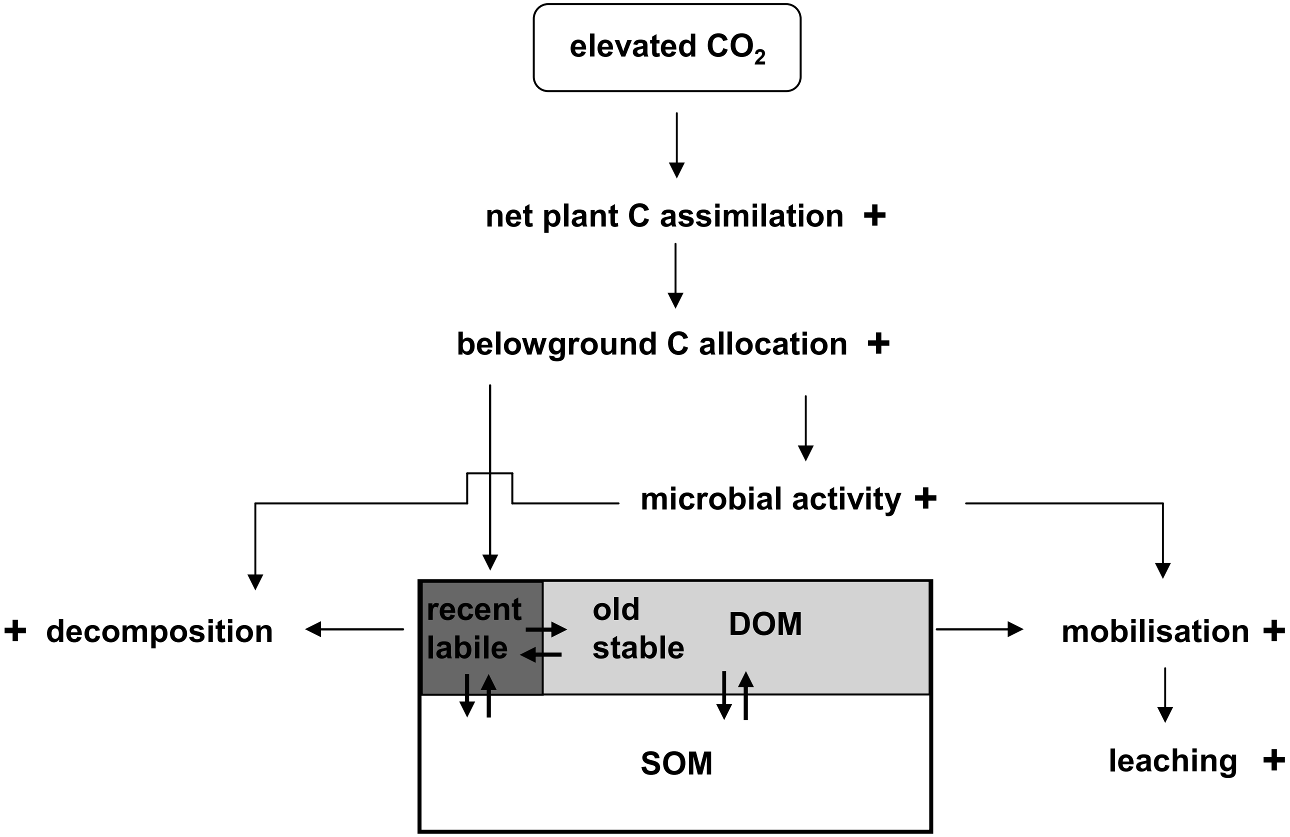
○ ambient CO_2
▲ elevated CO_2
^{14}C-oxalate mineralization [nmol g^{-1}h^{-1}]
0
20
40
60
0
10
20
30
Oxalate concentration soil solution [μM]
Mineralization based on
in situ concentrations
1
2
*

Figure 5



DOC in Oa hoirzon at 5cm (mg C l^{-1})
ambient CO_2
elevated CO_2
Recent plant-derived DOC
0
10
20
30
40
50
2002
2003
2004
2005

Figure 6


elevated CO2
net plant C assimilation +
belowground C allocation +
microbial activity +
+ decomposition
recent
labile
old
stable
DOM
mobilisation +
SOM
leaching +

7 Dissolved organic carbon leached from organic layers at the alpine treeline is dominated by older carbon

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Biogeochemistry, **in review.**

Abstract

Despite extensive knowledge about characteristics and fluxes of dissolved organic carbon (DOC), its formation and origin in soils is not fully understood. Here, we studied DOC sources by enriching treeline ecosystems on undisturbed soils with mor layers for six years with ^{13}C-depleted CO_2 and by following the fate of ^{13}C-labelled photosynthates in the plant and soil system. The ^{13}C tracing showed that significant amounts of recent assimilates were allocated to the belowground as soil-respired CO_2 consisted approximately to 50% of recent C. However, the fraction of recent plant-derived C in DOC of the Oa horizon (5 cm depth) accounted only to 7±2% of total DOC after three years and 21±4% after six years of $^{13}CO_2$-labelling. The weak ^{13}C signal, supported by ^{14}C-based mean residence times of DOC over several decades, indicates that DOC in the Oa horizon was dominated by 'old' C from soil organic matter and that sources of 'recent' C such as throughfall, fresh litter and root exudates contributed only little to DOC. The leaching of recent plant-derived DOC leached from the Oa horizon amounted only to 1-2 g $m^{-2}y^{-1}$, which was less than 1% of net primary productivity and less than 2% of the total C allocated belowground. We attribute the apparent small flux of recent DOC to (1) a rather low input of fresh organic matter as compared to the total soil organic matter (SOM) pool at the alpine treeline, (2) a rapid biodegradation of labile portions of recent DOC such as root exudates; and (3) a physico-chemical retention of recent DOC in the organic layer prolonging the time to degrade or to transform litter-derived DOC; and (4) a sustained production of ‘old’ lignin-derived DOC during decomposition of humified organic matter. We hypothesize that in mor-type organic layers, these processes result in a *biological DOC substitution* with the consumption and retention of ‘recent’ incoming DOC and the release of ‘old’ lignin-derived DOC from the solid-phase organic matter.

Keywords: carbon cycling, dissolved organic matter, elevated CO_2, radiocarbon, soil organic matter, stable isotope, tracer

Introduction

Dissolved organic matter (DOM) plays an important role in ecosystems: it represents the mobile fraction of soil organic matter (Kalbitz et al. 2000), is a key vehicle for the export of nutrients (e.g., Hagedorn et al. 2000; Michalzik et al. 2001), and determines the solubility and mobility of metals and organic compounds (Tipping 2002). Soil organic matter and plants are the main contributors to DOM in streams, rivers, and lakes (Schiff et al. 1997), and as DOC is the main carbon and energy source for limnic heterotrophic organisms (Sobek et al. 2003), it links terrestrial and aquatic ecosystems.

Although knowledge about fluxes and characteristics of DOC and its relevance for many ecological processes is extensive, only little is known about the generation processes and origin of DOC in soils and as a consequence, impacts of environmental changes on the production and fate of DOC can hardly be predicted. DOc derives from plants, litter and humus, and it is produced principally by microbial activity and root exudation (Kalbitz et al. 2000; Yano et al. 2000; Kaiser et al. 2001). The relative contribution of these components is, however, controversially discussed. Leaching experiments in the laboratory (e.g., Moore & Dalva 2001; Park et al. 2002) have highlighted that recent litter has a large potential to produce DOC, and hence it was suggested that fresh litter is a major source of DOC in soils (Qualls et al. 1991; Currie & Aber 1997). The concentration profiles of DOC in organic layers atop of mineral soils support this idea with typically highest concentrations just underneath the litter layer and no further increases in the deeper O horizons (Qualls et al. 1991; Fröberg et al. 2003). The chemical composition of DOC, however, is strongly altered during the passage through the organic layers. Decreasing fractions of simple compounds (carbohydrates, phenols) and increasing proportions of more complex, aliphatic and aromatic compounds suggest that a significant fraction of DOC leached from organic layers originates from more humified compounds (McDowell & Likens, 1988; Kalbitz et al. 2004).

Recent radiocarbon studies indicate that DOC in solutions from organic forest floor layer but also in water from piezometer wells contain a significant fraction of ‘modern’ C that had been assimilated from the atmosphere after the bomb tests in the 1960s (Schiff et al. 1997; Fröberg et al. 2003; Michalzik et al. 2003). In a Swedish spruce forest, Fröberg et al. (2003) found that the mean ^{14}C-based age of DOM leached from Oe and Oa horizons was several decades, implying that older and more humified organic matter contributes substantially to DOM. On a shorter time scale, litter manipulation experiments showed no straightforward relationship between litter inputs and DOM leaching (Fröberg et al. 2005; Kalbitz et al. 2007): (i) litter additions to forest floors increased DOC leaching from the Oa horizons suggesting that litter contributes significantly to DOC. (ii) However, exclusions of litter inputs had only little influence on DOC leached from lower forest floor layers, indicating that the major fraction of DOC is generated in Oe and Oa horizons. In a modelling study of a litter manipulation experiment in a Southern Swedish spruce forest, Tipping et al. (2005) suggested that one-third of DOC leached from the forest floor originates from fresh litter.

The contribution of roots, their exudates and associated mycorrhizae and microorganisms to DOC is also controversially debated. Yano et al. (2000) hypothesized that roots produced most of the DOC since they found no relation between DOC and freshly fallen litter, but a good correlation between DOC and fine root biomass. In line with these findings, organic C extracted with salts and by centrifugation decreased by 40 to 50% after tree girdling, which was interpreted as an indication that recent assimilates contribute significantly to DOC of organic layers (Högberg & Högberg, 2002; Giesler et al. 2007). In contrast, a ^{13}C tracer experiment in model forest ecosystems gave evidence that root exudates were a negligible source for DOC since the ‘recent’ plant-derived C contributed to less than 20% to DOC leached from minerals soils at 5-10 cm depth (Hagedorn et al. 2002, 2004).

In the present study, we make use of a 5-year CO_2 enrichment experiment in a late-successional treeline ecotone with undisturbed soils. We used the ^{13}C depleted CO_2 as a tracer within the plant and soil system, which allowed us to identify the contribution of recently assimilated CO_2 to different C pools and fluxes. Our main questions were (1) how much of the recently assimilated CO_2 is allocated to the belowground, (2) does it contribute to DOC leached from the mor-type organic layer, and finally (3) what is the origin and fate of DOC in organic layers.

Material and Methods

Study site description

The study was carried out at 2180 m.a.s.l. at Stillberg in the Central Alps near Davos, Switzerland. Trees remnant from a 1975 plantation effort with *Larix decidua*, *Pinus cembra* and *Pinus uncinata* are ca. 2 m tall and form a sparse open canopy with dense understory vegetation composed dominantly of dwarf shrubs such as *Vaccinium myrtillus*, *Vaccinium uliginosum*, *Empetrum hermaphroditum*, and common herbaceous species such as *Gentiana punctata*, *Homogyne alpina* and *Melampyrum pratense*. The terrain is steep, with slopes of 25 to 30° that are northeast exposed. Soils are sandy Ranker and weakly developed Podzols, derived from paragneiss. Soils and organic layers exhibit a high spatial variability (Bednorz et al. 2000). The organic layers have a thickness between 5 and 15 cm and are Humimors dominated by Oa horizons. Soil characteristics are given in Table 1.

Long-term average weather data at this site since the 1950s show annual precipitation of 1050 mm, mean maximum snow depth of 1.50 m, and average January and July temperatures of -5.8 °C and 9.4 °C, respectively (Schönenberger & Frey, 1988).

Experimental set-up

For the treeline free air CO_2 enrichment (FACE) experiment, a total of forty 1.1 m^2 plots comprising of 10 groups of four neighbouring plots (two each with an individual *Larix* and *Pinus* tree in the plot centre per group) were created in order to facilitate logistics of CO_2 distribution and regulation (Hättenschwiler et al. 2002). Half of these groups were randomly assigned to an elevated CO_2 treatment, while the remaining groups served as controls, resulting in a split-plot design. CO_2 enrichment was provided through 24 laser-perforated drip irrigation tubes were hung vertically around a stainless hexagonal frame (15 cm apart from each other), weighted with a 3-mm stainless steel rod (to maintain rigidity) and buried 2-4 cm into the soil, which resulted in a homogenous CO_2 ‘cloud’ around the trees and all understory vegetation in the plot. CO_2 concentrations were measured and regulated at the group level (four trees) with a control system. Seasonal mean CO_2 concentrations were 566±42, 582±35, 579±52, and 563±50 (2001-2004; ± SD) under elevated CO_2 and 370±3 ppm_v under ambient CO_2.

Treatment was provided during the growing season (ca. 15 June to 15 September) during 72-83% of the daylight hours when weather conditions were suitable; under extreme conditions, the gas release was stopped due to the enormous cost of CO_2 delivery (see experimental details in Hättenschwiler et al. 2002 and Handa et al. 2005).

Sampling and field measurements

Soil solution was collected in all plots by installing two ceramic suction cups (SoilMoisture Equipment Corp., Santa Barbara, CA, USA) per plot at 3 to 7 cm depth at an angle of 60° from the surface. All cups were located within the Oa horizon that dominated the organic layer. In addition, we installed 10 suction cups (5 per treatment) at depths of 15 cm (E horizon) and 30 cm depth (BC horizon). Suction cups were installed at fixed depths and not at defined horizons, because space within the 1.1 m^2 plots was too limited to open pits for identifying diagnostic horizons and inserting horizontal lysimeters. In order to minimize sorption of DOC to the ceramic cups, we only used suction cups that had been used in other experiments before. Prior to their installation, they were flushed first with 1 M HCl, and then with distilled water. All lysimeters at a given depth were connected to one 1-l glass bottle per plot buried in the ground. During sampling, residence times of soil waters in the sampling devices were kept as short as possible. At each sampling, we collected soil waters by evacuating suction cups with a constant 400 hPa for about 16 hours (overnight). However, during the very dry summer 2003, we had to apply the suction for a week to obtain sufficient amounts of water.

Throughfall was sampled with small polyethylene (PE)-funnels (Ø 10 cm) just above the soil surface, connected to 250-ml aluminium-foliated PE-bottles. In order to account for the spatial variability, we changed the placement of the PE-funnels after each sampling (n=4 per season) within each plot.

After collection, the samples were transported in cooling boxes to the laboratory.

CO_2 efflux from soils and its $\delta^{13}C$. Soil respiration was measured in the field with permanently installed PVC collars (10-cm ID, height 5 cm) and a LI-COR 6400-09 soil chamber connected to a LI-COR-6400 portable system for data collection LI-COR Biosciences, Lincoln, NE, USA). One PVC collar per plot (total n=40) was pressed to a depth of 2 cm into the organic layer in between dwarf shrubs. For isotopic measurements, we installed one passive air sampler at a depth of 10 cm in all 40 experimental plots. The passive air samplers were brass tubes with rubber stoppers, 3 cm in diameter and with slits cut into the lower 5 cm, allowing for sampling soil air representative for the depth between 5 and 10 cm. The tubes were pushed into holes that were made by prior coring of the soil (Ø=2 cm). Soil air was sampled by retrieving 15 ml of air with 20-ml syringe through the rubber and by injecting the air into 12-ml pre-evacuated glass vials closed with an airtight rubber septum (Exetainer gas testing vials, Labco Ltd., High Wycombe, UK).

Soils were sampled with a root corer (Ø 2 cm, *n*=6 per plot), then stored in a cooling box. Within 12 hours after the sampling, roots were removed and soils were homogenized with a 4-mm sieve. For estimation of soil microbial biomass and water-extractable DOC, soils were immediately processed. For soil C analysis, soils were dried at 40°C and sieved to <2 mm. Then, all visible roots were removed from small subsamples using a binocular and the subsamples of soils and roots were ground with a ball mill.

Analysis

All solution samples from the field were filtered through 0.45-*µ*m cellulose-acetate filters (ME2 5, Whatman–Schleicher & Schuell GmbH, Dassel, Germany) within two days after sampling and then stored at 4°C until analysis. *Concentrations* of dissolved and water-extractable organic C and of total dissolved N (TN) were determined with a TOC/TN analyser (TOC-V, Shimadzu Corp. Tokyo, Japan). Nitrate concentrations were determined by ion chromatography (DX-120, Dionex, Sunnyvale, CA), and ammonium was measured colorimetrically by automated flow injection analysis (FIAS-300, PerkinElmer, Inc., Waltham, MA, USA). Nitrate and ammonium N were summed up to give the total dissolved inorganic N (DIN); DON was estimated by subtracting concentrations of DIN from those of TN. In soil waters, the error in this indirect estimate was small since the fraction of DIN in TN was always <20%. The *molar UV absorptivity* at 285 nm of DOC was measured on a Cary 50 UV spectrophotometer (Varian, Inc., Palo Alto, CA, USA). The fractions of the XAD8-adsorbable and non-adsorbable, the *hydrophilic* DOM was analysed by acidifying soil solutions to a pH 2 with 1 *M* HCl and pumping them through large columns (200 ml) filled with XAD-8 resin. In the effluent of the XAD-8 columns, representing the hydrophilic fraction, DOC concentration was measured. Dissolved organic C and N in the XAD8-adsorbable fractions were then calculated by difference between total and hydrophilic DOC and DON. After passage of the bulk DOM samples, the columns were rinsed with water and acidified to pH 2 with HCl. For $\delta^{13}C$ measurements, the rinsed water was combined with the hydrophilic fraction and freeze-dried. The XAD8-adsorbable fraction was eluted from the columns with 0.1 M NaOH and the organic C concentration in the effluent was measured. Mass-balance calculations showed that >97% of the C in the XAD8-adsorbable fraction was recovered from the XAD-8 resin. The eluted XAD8-adsorbable DOC was neutralized with HCl and then freeze-dried for ^{13}C analysis. *Low molecular weight organic acids* (LMWOAs) were determined in soil solutions sampled in mid September 2003 and early September 2004 by capillary electrophoresis using the method of Dahlén et al. (2000), employing electrokinetic injection. To determine oxalate and citrate, EDTA (final concentration 250 *μ*M, pH 9) was added in a separate run to eliminate interference from Al and Fe ions. Sampling time for LMWOA analysis was kept as short as possible, three days in the relatively dry year 2003 and 12 hours in 2004. Unfiltered samples were immediately frozen after collection.

Water-extractable organic carbon (WEOC) from soils was determined by gently shaking 5 g of field fresh, root-free soils within 12 h after sampling with 100 ml 0.05 mM NaCl using an overhead shaker and filtering the solution (first Whatman–Schleicher & Schuell 790 ½ ; then Whatman–Schleicher & Schuell ME2 5). *Microbial biomass* was determined with the chloroform-fumigation-extraction method (Vance et al. 1987), using a soil:solution-ratio for the extraction with 0.5 M K_2SO_4 of 1:5.

Stable isotopes. The fraction of recent plant-derived C in DOC and extracts of microbial biomass was quantified by analysing $\delta^{13}C$ values of all soluble fractions on freeze-dried aliquots. In order to achieve greater and easier-to-handle amounts of dried soil waters, we added one ml of a K_2SO_4-solution containing five mg of salt to the soil solution (usually 50 ml) before freeze-drying. The C content and the $\delta^{13}C$ values of plant, soil, and freeze-dried samples were determined with an automated elemental analyser–continuous flow isotope ratio mass spectrometer (EA-1110, Carlo Erba, Milan, Italy, interfaced with a Delta-S Thermo Finnigan, Bremen, Germany). The $\delta^{13}C$ values of soil CO_2 were measured with a gasbench II linked to a mass spectrometer (Thermo Finnigan Delta Plus XL). CO_2 concentrations of gas samples were calculated from the calibration line with standard gas samples of known CO_2 concentrations (340 and 5015 ppm). Results of the C isotope analysis are expressed in δ units (‰) and referenced back to the Pee Dee Belemnite (PDB) standard.

Radiocarbon. Freeze-dried soil solutions from 12 ambient CO_2 plots (4 plots bulked to one sample) were graphitized and analyzed at the AMS facility of the ETH-PSI, Zürich.

Litter leaching and DOC biodegradation experiment

To estimate total and biodegradable DOC leaching and C mineralization from decomposing litter, we conducted a microcosm study in the laboratory during 11 weeks at 20°C. 1.25 g of dried needle litter from four individual trees per CO_2 level and tree species were placed into microcosms (250-ml filtration systems; 0.45-µm durapore membrane filters and glass wool; Stericup, Millipore, Beford, MA, USA). At each leaching cycle, 200 ml of a standard nutrient solution corresponding to throughfall were sprinkled onto the litter with a peristaltic pump for two hours and suction of 50 hPa was applied to collect solution. Solution aliquots were immediately frozen after sampling for later determining biodegradable DOC. CO_2 production was measured the next day by placing the filter units into 1.7-l PVC containers, flushing them with CO_2-free air, and measuring the increase in CO_2 with time using an infrared gas analyser (LI 6252, LI-COR Biosciences). *Biodegradable DOC* of litter leachates but also of 'fresh' soil solution was quantified by incubating leachates adjusted to 10 mg DOC l^{-1} for four weeks at 20°C (details see Hagedorn & Machwitz, 2007). A standard nutrient solution, an inoculuum and a glass fibre filter was added to 100 ml of the leachates in 250 ml flasks. Then, the flasks were closed with a folium, incubated in the dark at 20°C for four weeks and gently shaken by hand every day. Biodegraded DOC was quantified by determining the difference in DOC in 0.45-µm filtrates before and after incubation.

The *^{14}C-labeled oxalate mineralisation* was determined with bulked Oa horizon samples from the two CO_2 treatments of all blocks (n=5). A ^{14}C-radiolabelled solution (100 *µ*l, specific activity 1.7 kBq ml^{-1}, 1,2-^{14}C; ARC, Saint Lewis, MO, USA) with concentrations ranging from 10 to 1000 *µ*M (1–100 nmol; pH 4.5) was added to 1.00±0.02 g of moist soil contained in 50 ml polypropylene tubes. The soil was gently shaken and incubated at 4 ºC in sealed tubes. The $^{14}CO_2$ produced by biodegradation of the substrate was collected by placing a plastic scintillation vial containing 1.0 ml of 1 M NaOH inside the tube, on top of the soil but separated from direct contact with the soil by a spacer. $^{14}CO_2$ trapped as $NaH^{14}CO_3$ in the NaOH was determined by liquid scintillation (Wallac 1414 scintillation counter, Wallac, Tampere, Finland) using alkali compatible scintillation fluid (Wallac Optiphase 3; Wallac, Tampere, Finland). The sampling time for $^{14}CO_2$ trap removal was 1 h.

Calculations and statistics

Fluxes of DOM from the organic layer were roughly estimated by multiplying modelled drainage rates with DOM concentrations. Water fluxes were modelled with DyDOC (Michalzik et al. 2003); its hydrological subroutine is a simple ‘bucket’ model assuming that precipitation falls as snow at temperature below

0°C and snow melts above 5°C. Since we were not able to measure DOM concentrations during winter when the study site was not accessible, we roughly estimated them by assuming a Q_{10} of 2. Annual CO_2 effluxes were estimated by fitting all the mean effluxes from the 20 ambient CO_2 plots to the Arrhenius equation, which was then used to extrapolate respiration rates to the overall year. Effects of CO_2 enrichment on all measured parameters were tested by ANOVA, using a full factorial split-plot model. Concentrations of DOC, DON, water-extractable OC, and *in situ* soil respiration were log transformed before the analysis. All statistical analyses were performed with R (version 1.9.1, R Development Core Team, 2004).

The fraction of 'recent' plant-derived C was calculated from the difference in ^{13}C of the C pool and flux between ambient and elevated CO_2 and the respective difference in ^{13}C in community leaf litter between the two treatments. The net annual input of recent C into DOC was estimated by fitting the recent C fraction with a single-pool exponential decay model, calculating the fractions for one year and multiplying them with the annual DOC flux.

Results

DOM concentrations and properties

Mean concentrations of DOM during the vegetation period across five years of measurements showed the typical pattern with increasing concentrations from rainfall to the organic layer and decreasing concentrations in the mineral soils

(Figure 1). At 30 cm depth, in the E and Bhs horizons, concentrations of DOC were four times smaller than in the organic layer, but still approximately 10 mg DOC l^{-1}. Dissolved organic N comprised more than 95% of the total dissolved N and showed the same vertical pattern as DOC (data not shown). The molar UV absorptivity of DOC, an easily measurable indicator for aromaticity and the fraction of hydrophobic compounds in DOM (Dilling & Kaiser, 2003), had a similar depth distribution with maximal values in the organic layer (Figure 1).

Dissolved organic matter leached form the organic layer contained 80% of total DOC and roughly half of total DON (55%, data not shown) in the lignin-derived XAD-8-adsorbable the so-called hydrophobic fraction (Table 2). Phenolics comprised 12% of DOC, while low-molecular weight organic acids (LMWOAs: acetic, butyric, citric, formic, amlonic, lactic and oxalic acids) made only a minor contribution to total DOC (0.42 ± 0.04%). Consistently with the small hydrophilic fraction and the low contents of LMWOAs, only 6% of the DOM leached from the organic layer was biodegradable under optimal conditions during one month.

Enrichment of CO_2 increased DOC concentrations significantly (+25% after 5 years; $P<0.05$; Hagedorn et al. 2008) but did not affect DOM properties such as molar UV absorptivity, C/N ratios, and the distribution of the hydrophobic and hydrophilic fraction (Table 2).

DOM and CO_2 fluxes

The estimates of DOM export from the organic layer are based on modelled drainage rates. Measured precipitation in 2002, 2003, and 2004 was 1380, 910, and 1200 mm. We estimated that the annual drainage from the organic layer was 1160, 680, and 1000 mm, respectively, with the major portion being drained during the vegetation period. Thus, annual evapotranspiration was estimated to be 200 to 230 mm, which agrees with measured evapotranspiration rates in lysimeter experiments with dwarf shrub communities at the study site (200 to 300 mm; de Jong et al. 2002). Assuming that DOC and DON concentrations during winter months (when we did not sample soil water) followed a Q_{10} of 2 (Michalzik et al. 2003), we estimated that DOC exports from the organic layer ranged between 20 and 35 g C $m^{-2}y^{-1}$; DON export was about 5 to 7 g N $m^{-2}y^{-1}$. The potential error due to this extrapolation for the winter months seems small as 78 to 98% of the modelled drainage occurred during the sampling period.

The close correlation of soil respiration rates with soil temperature (r^2=0.90, n=31) enabled us to roughly estimate annual CO_2 effluxes from continuously monitored soil temperatures. The total annual CO_2 effluxes were between 240 and 320 g CO_2-$Cm^{-2}y^{-1}$, with 70 to 85% being respired from June to October.

^{13}C isotope tracing – fluxes of recent C

Plant biomass. The change in ^{13}C of plant material as a result of the addition of ^{13}C-labelled CO_2 ($\Delta^{13}C=\delta^{13}C_{elevated} - \delta^{13}C_{ambient}$) is the basis to calculate fractions of plant-derived C in soil pools and of fluxes, and hence to estimate turnover rates.

Fumigation with CO_2 depleted in ^{13}C significantly decreased $\delta^{13}C$ values of plant tissues as compared to those grown under ambient CO_2. Cress (*Lepidium sativum* L.) grown as an annual control plant showed a difference of approximately -6.6±0.6‰ (von Felten et al. 2007). In trees and dwarf shrubs, the decline in ^{13}C was smaller and not uniformly distributed across plant species and plant compartments ($\Delta^{13}C$=-4.1 to -7.2‰). Fine root biomass showed a much smaller $\Delta^{13}C$ of -2.7‰ after four years of CO_2 enrichment compared with aboveground tissues (Handa et al. 2008). This indicates that fine roots (living and dead) contained substantial fractions of C taken up before the CO_2 enrichment started and suggests slow root turnover rates of several years. The large range in $\Delta^{13}C$ made it difficult to define a common and precise $\Delta^{13}C$ as the plant input signal. For the calculations of fractions of recent plant-derived C in soil C pools and fluxes, we used the approximate fractions in litter fall from trees (65%) and dwarf shrubs (35%), which resulted in a $\Delta^{13}C$ of -5.5‰. This value corresponded also to the $\Delta^{13}C$ in cellulose and lignin of the trees (von Felten et al. 2007). On the basis of the $\Delta^{13}C$ in roots we estimated that belowground litter production was 45±15 g C m^{-2} y^{-1}. Leaf litterfall was 55±5 g C m^{-2} y^{-1} (T. Handa, unpublished data) and thus, the total litter input was approximately 100 g C m^{-2} y^{-1}. This is a rather conservative estimate as total litter inputs should equal the measured heterotrophic soil respiration of 130 to 170 g C m^{-2} y^{-1} under steady-state conditions.

Soil organic matter showed a small but significant shift in ^{13}C of -0.25‰ after three years of CO_2 addition, which signifies that the fraction of recent C was below 5% (Figure 2). We cannot rule out that some of the ^{13}C input originated from a 'contamination' with remaining small root fragments although roots were picked out of the Oa material before analysis, using a binocular. Given that roots had a Δ^{13}C of less than -3‰ and assuming that remaining roots were 10% of the total fine root biomass, the resulting Δ^{13}C in SOM would have been no more than -0.05‰, or less than 20% of the observed shift.

Soil respired CO_2 collected at 5 to 10 cm depth was dominated by recent plant C, implying that CO_2 assimilated by plants was rapidly respired from soils (Figure 2). The Δ^{13}C of soil CO_2 ranged between -1.2 and -3.7‰ without any tendency to increase with time. In order to correct the δ^{13}C values for contamination with experimentally added atmospheric CO_2 (with a much smaller δ^{13}C), we regressed them with the reciprocal of the CO_2 concentration to estimate the δ^{13}C of the soil air (Keeling, 1958). The intercept of the regressions under elevated and ambient CO_2 differed between -2 and -4.2‰ with a mean Δ^{13}C of soil CO_2 of -2.5‰. The resulting fraction of recent plant-derived C to soil respired CO_2 was 50±9%.

Dissolved organic carbon. In Oa layer DOC collected at 3 to 7 cm depth, the Δ^{13}C was much smaller than in fine roots, microbial biomass, and CO_2 in soil air, indicating that the input of 'recent' plant-derived C was small (Figure 2). However, the change in ^{13}C due to the CO_2 addition increased with time (Figure 3) and was significant after the third year of CO_2 enrichment (P<0.01). The difference was not an artefact related to a different composition of DOM with the hydrophobic fraction being more depleted in ^{13}C as the hydrophilic one (Figure 4; Kaiser et al. 2001). Firstly, there was no significant CO_2-effect on the fraction of hydrophobic/hydrophilic DOM. Secondly, we predicted the 'natural' $\delta^{13}C$ value of DOM without any tracer input based on the molar UV absorptivity at 285 nm which correlated with the $\delta^{13}C$ values under ambient CO_2 (r^2=0.68, n=60). This showed that the 'natural' difference between ambient and elevated CO_2 differed by less than 0.10‰ during the six years and, thus, was much smaller than the measured shifts in ^{13}C due to the CO_2 addition (-1.0‰ after 6 years; Figure 3). In the mineral soils at 15 and 30 cm depths, the ^{13}C decline was not detectable. The fraction of 'recent plant-derived C (f_{recent}) in Oa-DOC was 7±2% and 21±4% of total DOC after three and six years of CO_2 enrichment (Figure 3). Tree species significantly influenced the ^{13}C label in DOC. The fraction of new C in DOC was 30 to 90% greater under larch than under pine trees ($P<0.05$), which very likely resulted from the annual litter renewal and the greater litter production by larch. The net annual input of recent DOC (f_{recent} x total DOC flux) amounted to 1-2 g recent DOC $m^{-2}y^{-1}$, which corresponds only to 1-2% of the recent C respired from soils (Figure 7).

Fractions of DOC responded differently to the addition of ^{13}C-depleted CO_2. Hydrophilic DOC leached from the Oa horizon contained larger fractions of recent plant-derived than XAD8-adsorbable DOC (23 vs. 10%; Figure 4). On a flux basis, 60% of recent DOC, but 80% of bulk DOC leached was in the lignin-derived XAD8-adsorbable fraction.

Radiocarbon. The ^{14}C content of Oa horizon DOC was 105 ± 3 % of absolute modern (Table 2), which suggests that DOC contained significant fractions of pre-bomb C (before 1955). Estimated mean residence times calculated by the method of Harkness et al. (1986) were 4 or 155 years. The small ^{13}C signal from recent photosynthates in DOC suggests that a longer residence time is more likely and that DOC is a mixture of recent and old components.

Litter leaching and DOC turnover

During the 11 week mineralization-leaching study in the laboratory, the total loss of C was 22% for the *Pinus* and 30% of the *Larix* litter, which equals roughly the approximate annual mass loss in the field (Larix: 45%, Handa, unpublished data; dwarf shrubs: 22% during winter; Wipf, 2006). The contributions of DOC leaching to the total C losses from litter were 13% for the pine and 18% for the larch litter (Figure 6). The DOC biodegradability decreased from the first leaching cycles to the end of the experiment with a mean one-month degradation of 43 and 30% of DOC leached from pine and larch litter.

The mineralization of ^{14}C-labelled oxalate at 4°C was fast with 20 to 60% being mineralized within 1 h for the highest and the lowest addition, respectively. It conformed a hyperbolic (Michaelis-Menten) type of kinetics over the concentration interval studied (r^2>0.8; Table 3). Relating the mineralization kinetics to field measured oxalate concentrations and *in situ* soil temperatures yields an annual oxalate turnover of approximately 3 to 4 g C $m^{-2}y^{-1}$. This corresponds to 10 to 20% of the annual DOC export from the organic layer although the concentrations of the fast-turning oxalate were only 0.03% of bulk DOC.

Discussion

1. DOM fluxes in treeline ecosystems

Our estimates of DOC and DON fluxes in alpine treeline ecosystems show that they are apparently quantitatively more important as compared with other C and N fluxes than in more productive forests at lower altitudes. The DOC export from the organic layer was between 20 to 35 g C m^{-2} y^{-1} which corresponds roughly to 50% of the C from aboveground litterfall and 10% of the overall CO_2 effluxes from soils including hetero- and autotrophic respiration. Since DOC fluxes are in the range of temperate and boreal forests (Michalzik et al. 2001) but aboveground litterfall and soil respiration rates are much smaller (Raich & Schlesinger, 1992), relative larger amounts of DOC were exported from organic layer than at lower altitudes. This implies that either roots represent a more significant direct or indirect source for DOC or that litter is processed differently in such a harsh alpine environment. Soils of the study site have thick Oa horizon-dominated organic layers which are typical for sub- and alpine ecotones (e.g., Holtmeier & Broll, 1992; Bednorz et al. 2000). The thick Oa horizons indicate that humified components accumulate and remain unmixed with the mineral soil and, thus, relative more 'substrate' for DOM is formed in the long-term. Dissolved organic matter is also the major export pathway for N from the organic layers. More than 95% of total N was leached as DON (0.6 g N m^{-2} y^{-1}), which almost equals the total N deposition in the Alps at these altitudes, ranging between 0.5 and 0.9 g N m^{-2} y^{-1} (Schmitt et al. 2005). The dominance of organic N in the soil water suggests that either little mineralization of N takes place at this high altitude site or that the uptake of inorganic N by the plant community is highly efficient, probably as result of an adaptation to the low N status of the ecosystem.

2. Sources of DOC

The pathways of DOC formation in organic layers are controversially discussed: organic layer DOC could either be leached *directly* from litter or roots or *indirectly* from above- and belowground litter that has first been transformed into a substrate from which DOC is produced and leached (Currie & Aber, 1997; Michalzik et al. 2003). Our ^{13}C tracer-based field experiment on undisturbed soils gives some insight into the sources and sinks of DOC. The ^{13}C signal from the addition of ^{13}C-depleted CO_2 was small in DOC (Figures 2 and 3), which indicates that the contribution of recent, less than six-year old photosynthates to DOC leached from Oa horizons was <25%. Thus, root exudates, fresh litter and throughfall add little to DOC and consequently, older soil C is the most important source of DOC in Oa horizons.

Our estimates of DOC sources contain, however, some uncertainty due to the inhomogeneous ^{13}C labelling of different plant species and plant compartments in this ‘old’ treeline ecosystem. For instance, the fractions of recent assimilates in fine roots were only 20 to 50% after three years of CO_2 addition, which shows that fine roots – living and dead – consisted also of a considerable fraction of relative ‘old’ C. This implies that the turnover of fine roots was relatively slow, at least in the order of some years, which agrees with slow inputs of ^{13}C labels into fine roots of a lobolly pine plantation (Matamala et al. 2003) and high radiocarbon ages of fine roots in temperate forests (Gaudinski et al. 2001). The different ^{13}C signatures in plant materials induce a great range in the estimates of plant-derived C in DOM: when we assume that the ^{13}C of fine roots represents the ^{13}C input signal, the fraction of ‘recent’ plant C would be 50%, but below 20% when the isotopic shift in needles and leaves is taken into account. We think, however, that recycling of older plant C might ‘dilute’ the ^{13}C label in fine roots (Strand et al. 2008) and thus, the ^{13}C signatures in leaves are representative for ‘recent’ photosynthates.

The ^{13}C tracing evidenced that significant amounts of recent assimilates were rapidly allocated to roots and soils (Figure 7). Approximately 50% of soil respiration consisted of recent photosynthates and soil microbial biomass was substantially made up from ‘recent’ plant-derived substrates (Figure 2). The mass balance of recent photosynthates indicated, however, that less than 1% of net primary productivity and less than 2% of the C allocated into the soil was leached as recent DOC from the organic layer (Figure 7). This does not imply that root exudation contributes little to the C flux through the soluble phase. Published turnover rates of low-molecular weight organic acids in organic layers are one to six hours (van Hees et al. 2005). The ^{14}C-oxalate mineralization experiment showed that although the concentrations of low-molecular weight organic acids were low (<0.5% of bulk DOC), the total flux was rather large (Table 3). Assuming constant oxalate concentrations and mineralization kinetics (obtained at 4° C) in soil solution, oxalate turnover would result in a CO_2 efflux of 3 to 4 g C m^{-2} y^{-1}, which corresponds approximately to the annual DOC leaching from fresh litter. As a consequence, we conclude that root exudation and leaching of labile low-molecular-weight compounds will contribute significantly to the CO_2 efflux from soils but not to the DOC exported from the organic layer.

These results apparently contradict the idea that fresh organic material is the primary source of DOC (Qualls et al. 1991; Currie & Aber, 1997), which is based on leaching experiments showing that fresh litter has the highest potential to produce DOM (Moore & Dalva 2001; Park et al. 2002). The significantly greater fraction of recent plant-derived C in water extracts from root-free, field-fresh organic layer material than in SOM - its parent material (Figure 2) - indicates that recent organic matter is indeed much more readily soluble than the older one. Nevertheless, water-extractable OC was, as the *in situ* DOC, clearly dominated by older C. The radiocarbon measurements support this conclusion (Table 2). They show that the DOC leached from the Oa horizon contained significant amounts of pre-bomb C and, thus, had a mean residence time of several decades. Consequently, DOC leached from the Oa horizon consisted of a small fraction of rapidly cycling compounds and a large fraction of slowly turning components. We think that the main reasons for the dominance of ‘old’ C in DOC in our study are: (1) a sustained DOC production from old SOM, which has a two magnitudes greater C pool than recent SOM (Figure 2). (2) a rapid mineralization of root exudates and of fresh litter-derived DOC, and (3) the low productivity and, hence, low litter inputs in these treeline ecosystem. Here, above-ground litter inputs amounted only to 55 g C m^{-2} y^{-1}, which is small as compared with the total organic layer C stock of 3500 g C m^{-2}. In lowland forests, the absolute and relative C inputs are usually much greater. For instance, in southern Sweden, where Tipping et al. (2005) estimated that litter-derived DOC contributed to one-third of DOC exported from forest floors, litterfall was 130 g C $m^{-2}y^{-1}$ and the C pool in the organic layer was 4300 g C m^{-2}.

Our ^{13}C-based result showing that older C dominates in DOC leached from organic layers contrasts with the conclusion from a boreal forest in Northern Sweden where a decline in extractable organic C after tree girdling suggested that recent photosynthates drive DOC production in organic layers probably via ectomycorrhizal fungi (Högberg & Högberg, 2002; Giesler et al. 2007). The likely reason for the contrasting conclusion might be due to the only 2-cm thin organic layer in the girdling study (5-15 cm at this treeline site) and the different method of DOC sampling: extraction by salts and centrifugation in the Swedish study and *in situ* sampling with suction devices in this ^{13}C-study. In a previous CO_2 enrichment experiment, Hagedorn et al. (2004) have shown that water-extractable OC contains much higher fractions of 'hydrophilic' and younger DOC than DOC sampled with suction devices.

3. Production of DOC

Our field study gives some evidence on DOC generation: from the slow but steady increase of the ^{13}C signal in DOC reflecting recent photosynthates (Figures 3 and 5) we can infer that recent DOC was rather generated from older litter in later stages of decomposition than from root exudates, throughfall DOC or very fresh litter leachates. If these labile fresh plant-derived DOC sources would have been the main sources for recent DOC, then the ^{13}C signal would have responded much sharper as it was the case for soil-respired CO_2 which was dominated by recent photosynthates. Our conclusion that decomposed litter contributes more significantly to DOC leaching agrees with a leaching study by Don & Kalbitz (2005) indicating that DOC leaching from coniferous litter increased with advancing decomposition. Moreover, DOC biodegradability decreases with increasing litter decomposition (Don & Kalbitz, 2005; Hagedorn & Machwitz, 2007), and thus, the fraction of 'refractory' DOC increases with time.

Our ^{13}C tracer field experiment also suggests that a large fraction of the dominating 'older' DOC leached from the Oa horizon was produced during the degradation of lignin from older SOM: firstly, the molar UV absorbance being indicative for aromatic compounds increased from throughfall to the leachates from the Oa horizon (Figure 1). And secondly, the XAD-8 adsorbable lignin-derived DOC, making up 80% of the DOC leached from the Oa horizon contained smaller fractions of recent plant-derived C than the hydrophilic one (Figure 4). This implies that the older DOC generated in the organic layer was mainly composed of lignin-degradation products. Our results are corroborated by the repeated leaching of decomposing litter by Kalbitz et al. (2006), showing that lignin degradation and the generation of lignin-derived DOM increased with advancing stages of decomposition. Additional support comes from the analysis of DOM leached from Oa horizons of forest floors using ^{13}C NMR and lignin-monomeres (Guggenberger et al. 1994). These analyses indicated that organic solutes are mainly lignocellulose-degradation products that have strongly been microbially altered in the course of ligninolyse. Our DOC biodegradation experiment showed that Oa horizon DOC was hardly bioavailable - only 6% were degraded during one month under optimal conditions. This suggests that the lignin-derived DOC generated in Oa horizons is rather the left-over from incomplete SOM decomposition than an easily-available substrate as it is the case for throughfall or litter-derived DOM.

4. Sinks of recent plant-derived DOC

Mass balance calculations indicate that the small contribution of recent plant-derived C to DOC in the Oa horizon is not only caused by the low litter input rates but also by the removal of recent plant-derived DOC during passage through the organic layer (Figures 5 and 7). The mineralization and leaching study in the laboratory showed that DOC leaching contributed to 13 to 18% to the total mass loss (CO_2+ leaching) from decomposing litter (Figure 6). Given that the *in situ* litter mass loss was 45% for Larix leafs and 15% for roots during the first year (T. Handa, unpublished data), suggests that annual DOC leaching from fresh litter amounted to 3 to 5 g DOC m^{-2} y^{-1}, which is substantially more than the *in situ*, ^{13}C-based estimate of 'recent' DOC leaching from the organic layer (1-2 g DOC m^{-2} y^{-1}; Figure 7). Since throughfall (4-7 g DOC $m^{-2}y^{-1}$) and roots exudates are additional sources for recent DOC, the production of 'recent' plant-derived DOC clearly exceeded the measured recent DOC leaching from the Oa horizon (Figure 5 and 7). This implies that a substantial fraction of DOC was removed during its transport through the organic layer. Biodegradation seems the most obvious pathway of DOC removal but the DOC laboratory decay study indicated that biodegradation alone could not account for the removal of recent DOC in the organic layer. We estimated that between 20 to 50% of DOC leached from decomposing litter was degraded during one month under optimal conditions. However, even when we assume that all of the 'bio-available' litter-derived DOC would be mineralized, then the leaching of the remaining 'refractory' litter-derived DOC (2-3 g C m^{-2} y^{-1}) would still exceed the measured export of recent DOC (Figure 7). In the field, solute transport through organic layers occurs on the time scales of hours and days (Feyen et al. 1999). We therefore conclude that because of the short time, biodegradation alone cannot explain the removal of recent DOC during passage through the organic layer. Other, probably, physico-chemical processes seem to contribute to the strong sink for DOC.

There is extensive evidence for DOC retention through rapid sorption to mineral surfaces in mineral soils (e.g., Kalbitz & Kaiser, 2007), but much less is known for the interaction of DOC with SOM (Qualls et al. 2000). Based on a strong loss of ^{13}C-labelled DOC leached from coniferous litter during the passage through the forest floor going beyond a simple biodegradation, Fröberg et al. (2007) hypothesized that ‘young’ DOM is removed by sorption through electrostatic attraction or binding, hydrogen bonding and/or van der Waals forces (see e.g., Tipping & Woof, 1991). Hagedorn & Machwitz (2007) observed that the molar UV absorptivity of DOC increases during biodegradation and, thus, remaining DOC becomes more enriched in aromatic moieties and higher in molecular weight. These structural changes may favor hydrophobic interactions of remaining recent DOC with solid SOM. Such interactions could at least prolong the residence time of recent DOC in organic layers, which could in turn increase the decomposition and/or the microbially-driven transformation of DOC.

5. The *biological DOC substitution* in organic layers: a conceptual model

Our results show that DOC leached from the organic layer consisted mainly of lignin-derived ‘older’ C from the Oa horizon and that recent plant-derived DOC became removed whilst passing through the organic layer. This suggests a *biological DOC substitution* in the 'reactor' organic layer: incoming recent DOC is biologically 'replaced' by DOC originating from older SOM (Figure 8). Recent DOC particularly the labile 'hydrophilic' components are removed from organic layer solution by biodegradation. Hydrophobic interactions with more humified solid organic matter may prolong the time needed for the degradation or transformation of more recalcitrant recent DOC compounds. Preferential flow could, however, lead to a bypassing of recent incoming DOM (Hagedorn et al. 2000; Kaiser & Guggenberger, 2005). The removal of recent DOC is accompanied by a sustained release of stable lignin-derived DOC from older and more humified SOM. Soluble organic C is generated through microbial activity during decomposition, litter removal experiments indicate the formation of significant amounts of DOC in Oa horizons (Fröberg et al. 2005; Kalbitz et al. 2007), very likely during lignin degradation (Kalbitz et al. 2006; Michel et al. 2006).

The occurrence of a *biological DOC substitution* is supported by other radiocarbon 'bomb' based studies. In agreement with our results, they show that DOC collected under forest floors has a mean age of some decades (Michalzik et al. 2003; Fröberg et al. 2003), which implies that only a small fraction of 'recent' plant-derived C passes directly through forest floors and that the major fraction of litter C has first to be transformed into a DOC *substrate* before it is exported as DOC from the organic layer. The *biological DOC substitution* indicates that the *reactor* organic layer is more active, with more ‘fresh’ DOC being consumed and more ‘older’ DOC being generated than suggested by the small changes in DOC concentrations across organic layers.

The consequences of a *biological DOC substitution* in organic layers are longer travel times for incoming ‘recent’ litter-derived DOC and a greater retention of DOC-associated nutrients and pollutants in organic layers than under the assumption that litter is the main direct DOC-source. A *biological DOC substitution* in organic layers also implies that the greatest fraction of DOC leached from Oa horizons is rather the recalcitrant left-over from decomposition than the fuel for microbial activity in the mineral soil.

Conclusions

Our ^{13}C-tracer and radiocarbon-based study in treeline ecosystems gives evidence that DOC leached from Oa horizons consisted of continuum of rapidly to slowly cycling components. The small fractions of recent plant-derived C in DOC leached from the Oa horizon indicate that DOC originated from the thick Oa horizon rather than from recent primary productivity. Although significant amounts of recent assimilates were rapidly allocated to roots and respired back from soils, DOC from the Oa horizon was dominated by ‘old’ C. We attribute the small leaching of recent DOC from the organic layer to: (1) a small input of new organic C as compared to the indigenous SOM pool in the organic layer; (2) a rapid degradation of labile DOC such as root exudates and litter-derived low-molecular-weight compounds; (3) a physico-chemical retention of recent DOC in the organic layer prolonging the residence time and allowing to degrade or to transform litter-derived DOC; and (4) a small but sustained production of lignin-derived DOC during decomposition of humified organic matter. We hypothesize that these processes result in a *biological DOC substitution* in organic layers with a removal of incoming labile DOC and a release of stable DOC from indigenous SOM.

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Figure 6: Carbon mineralization and leaching of total and refractory DOC from litter during 11 weeks in the laboratory. Refractory DOC represents DOC resistant to a one-month decay under optimal conditions. Means and standard errors of 4 replicates.

Figure 7: Partitioning of recent photosynthates in soils and DOC fluxes.

Figure 8: The *biological DOC substitution* – a conceptual model: incoming new DOC is ‘exchanged’ with DOC leached from indigenous SOC. Biodegradation and physico-chemical interaction with more humified organic matter are removing new DOC from organic layer solution. The removal of new DOC is accompanied by a sustained release of DOC from older and more humified SOC. Preferential flow could lead to a bypassing of new incoming DOC.

Table 1: Soil properties of the treeline ecosystem at 2200 m a.s.l., Stillberg, Switzerland

Horizon Depth		Soil organic C %	C/N Mass ratio	pH ($CaCl_2$)	CEC eff $\text{mmol}_C\ \text{kg}^{-1}$	BS %
Oa	Ambient CO_2	40.8 ± 2.5	27.2 ± 0.7	4.2 ± 0.1	169 ± 8	83 ± 3
5-0 cm	Elevated CO_2	40.3 ± 2.4	27.2 ± 0.5	4.2 ± 0.1	167 ± 13	80 ± 3
Aeh	Ambient CO_2	10.3 ± 1.5	27.8 ± 0.7	3.8 ± 0.1	71 ± 5	37 ± 2
0-5 cm	Elevated CO_2	11.5 ± 1.8	27.9 ± 0.5	3.8 ± 0.1	72 ± 6	36 ± 2

CEC: cation exchange capacity

BS: base saturation

Table 2: Characteristics and radiocarbon content of DOM sampled in the Oa horizon (3 to 7 cm from the surface). Means and standard errors of n=16 to 20.

	DOC/DON	XAD-8 absorbable DOC (% of DOC)	Phenolics (% of DOC)	Biodegradable DOC (% of DOC)	C14-content[a] (% of modern)
Ambient CO_2	47.0 ± 2.4	80.6 ± 1.0	12.6 ± 1.7	6.0 ± 0.7	105 ± 2.9
Elevated CO_2	45.4 ± 1.8	80.5 ± 1.4	12.1 ± 0.7	5.9 ± 0.7	n.d.

[a] n=3.

Table 3: Concentrations of low-molecular weight organic acids (LMWOAs), ^{14}C-labeled oxalate mineralization and extrapolated turnover rates in the organic layer.

	∑LMWOAs µM	Oxalate µM	Ox. mineralization [a] nmol $g^{-1}h^{-1}$	Ox. turnover in organic layer [b] mg C $day^{-1}5cm^{-1}m^{-2}$	 g C $y^{-1}5cm^{-1}m^{-2}$
ambient CO_2	6.4 ± 1.0	1.9 ± 0.3	119 ± 33	7.4 ± 0.6	2.7 ± 0.2
elevated CO_2	7.8 ± 1.6	2.3 ± 0.4	170 ± 13	10.4 ± 0.6	3.8 ± 0.2

[a] is the maximum respiration rate (Vmax) of ^{14}C labeled oxalate at 4°C obtained by fitting mineralization rates at to a Michaelis-Menten-type kinetic and correcting for adsorption (van Hees et al., 2005)

[b] extrapolated using in situ concentrations of oxalate, soil temperatures, and a soil bulk density of 0.16 g cm^{-3}.

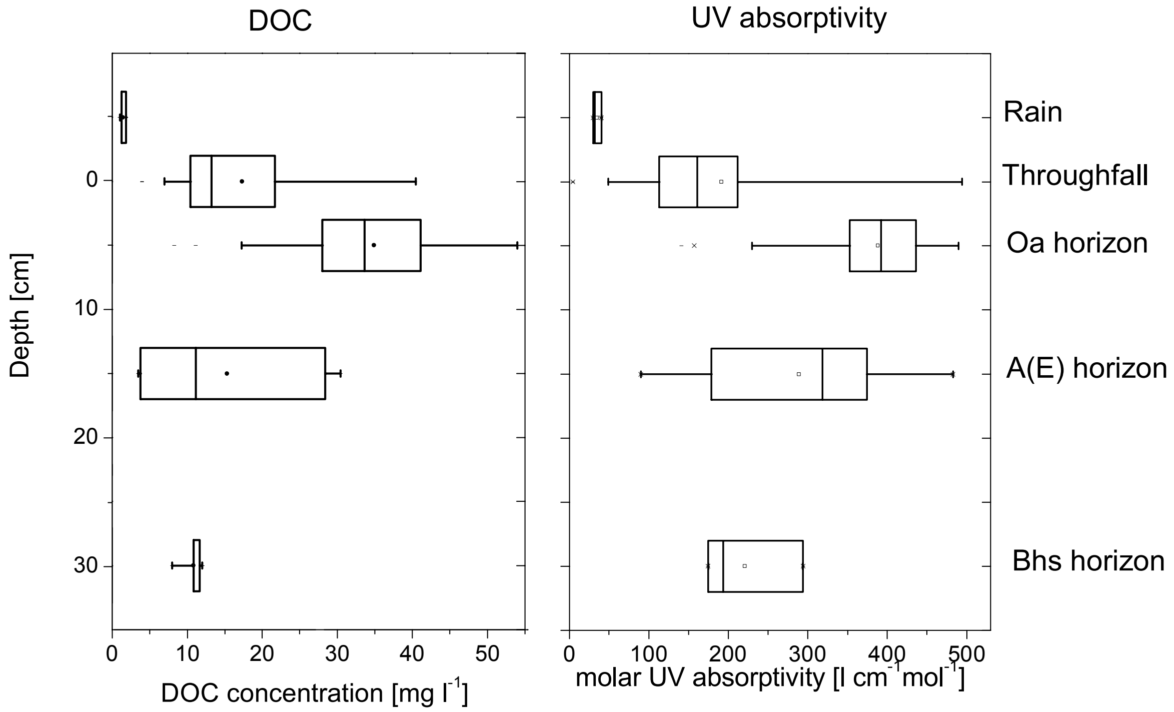


Figure 1: DOC concentrations and molar UV absorptivity at 285 nm in alpine treeline soils at Stillberg (2200 m a.s.l.) under ambient CO_2 (n=20).

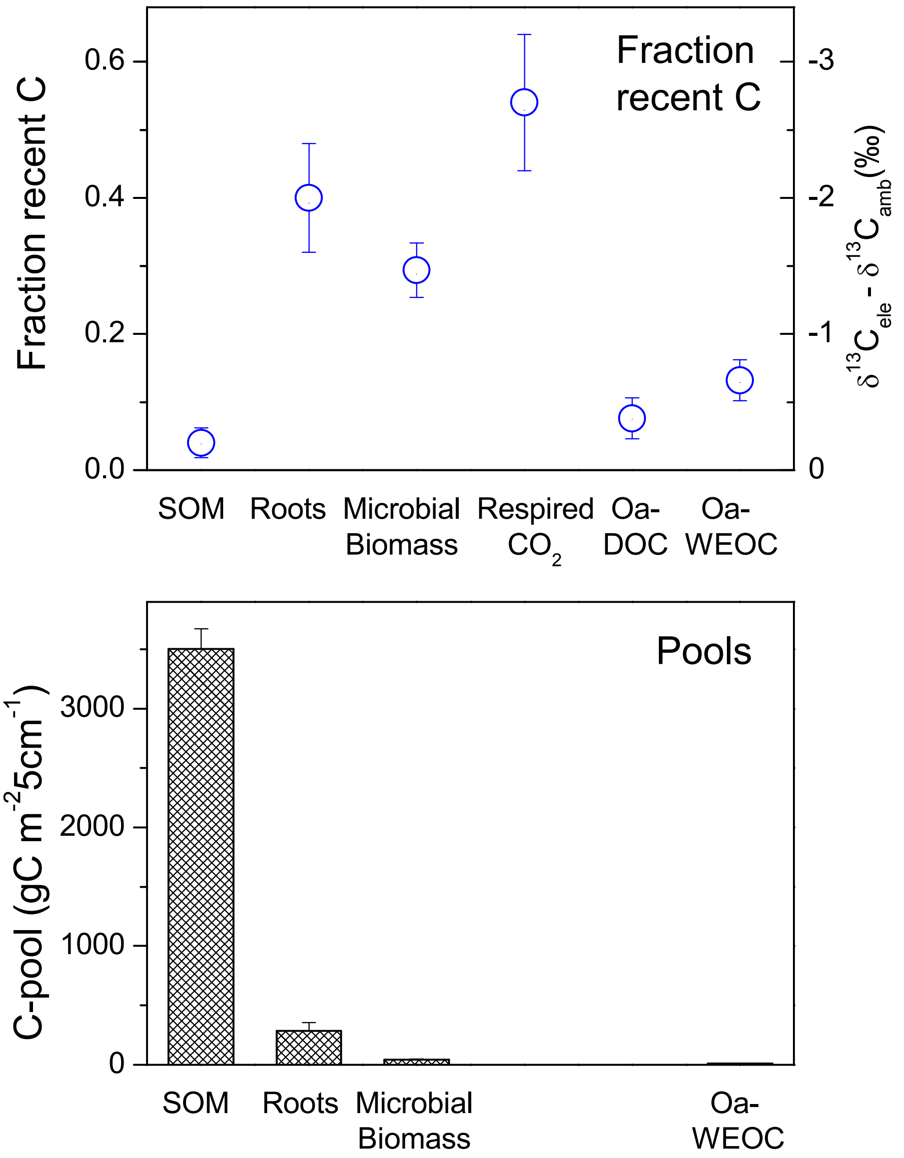


Figure 2 Carbon pools and $\Delta^{13}C$, the difference in ^{13}C between C-fluxes and pools after three years of adding ^{13}C depleted CO_2. Means and standard errors of 20 plots.

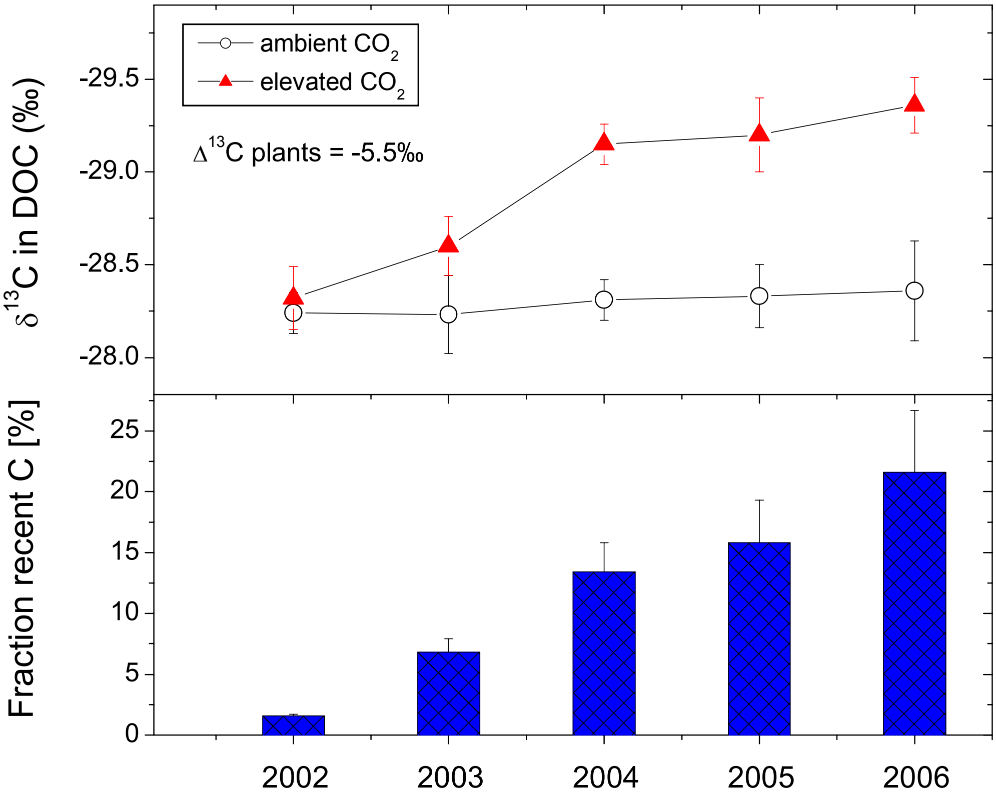


Figure 3: The change in δ^{13}C in Oa layer DOC (Δ13C) through enriching treeline ecosystems with ^{13}C-depleted CO_2. The fraction of recent plant-derived C in DOC. Means and standard errors of 20 plots.

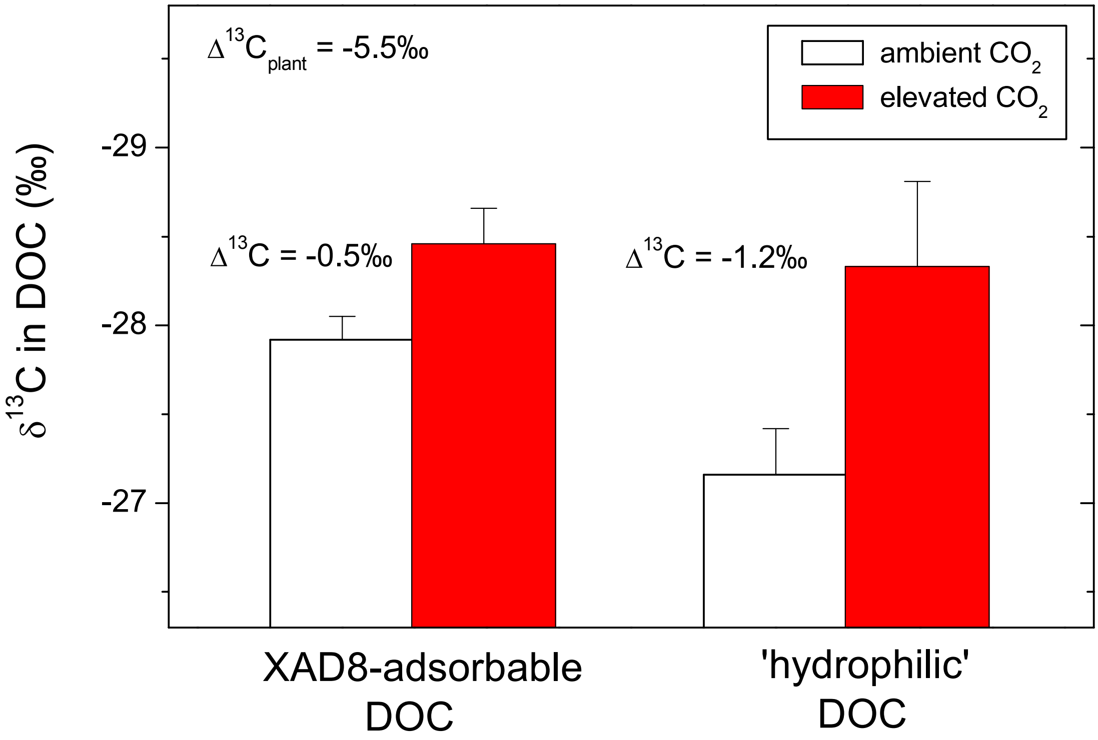


Figure 4: The $\delta^{13}C$ in the XAD-8 adsorbable lignin-derived DOC after the fourth year of adding ^{13}C-depleted CO_2. Means and standard errors of 16 plots.

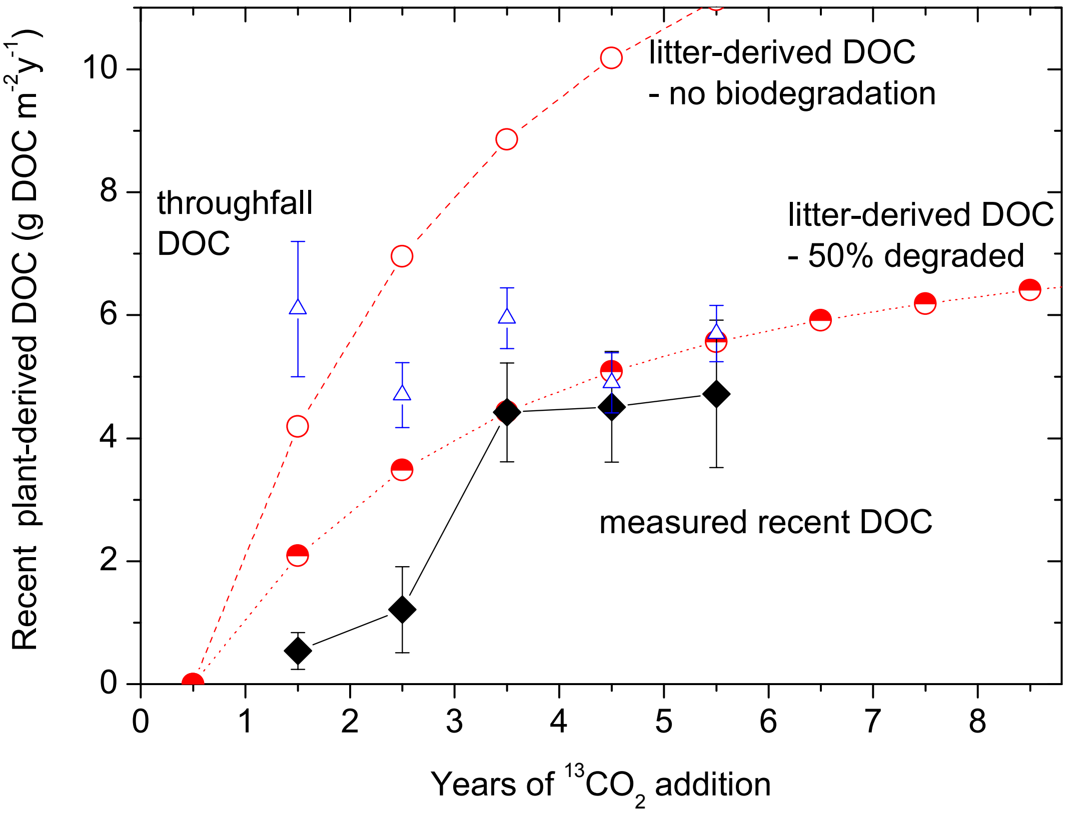
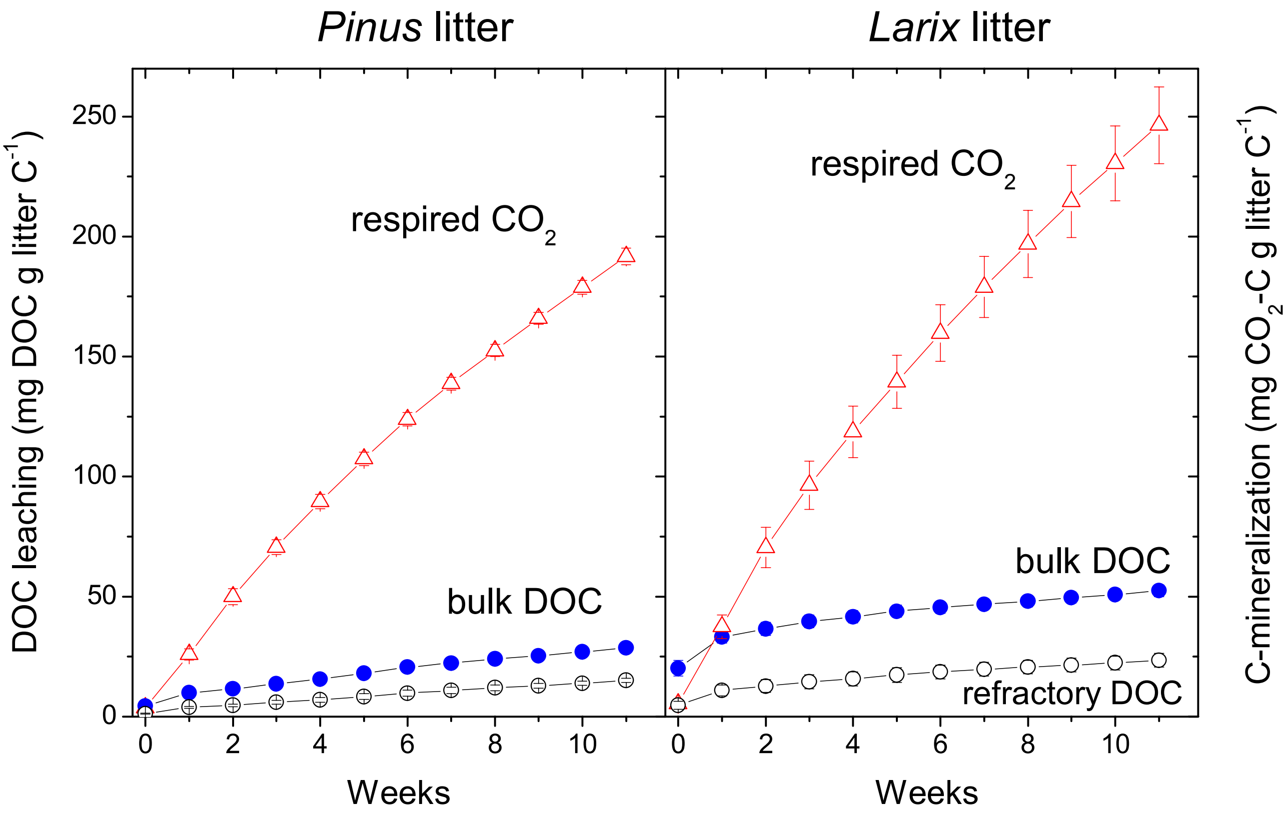


Figure 5: Modeled and measured fluxes of recent plant-derived DOC. Litter-derived DOC was estimated from an annual litter production of 55 g C m^{-2}y^{-1} leafs and 45 g C m^{-2}y^{-1} fine roots and by assuming that (a) litter mass loss followed a single-pool exponential decay kinetics with k=0.5y^{-1} for leafs and 0.15 y^{-1} for roots (based on litter bag studies) and (b) that 15% of lost litter were leached as DOC (leaching experiment). The DOC biodegradation study indicated that between 30 and 50% of DOC leached from pine and larch litter were degraded within one month. Measured new DOC leaching was estimated by multiplying DOC fluxes with the fraction of recent plant-derived DOC.



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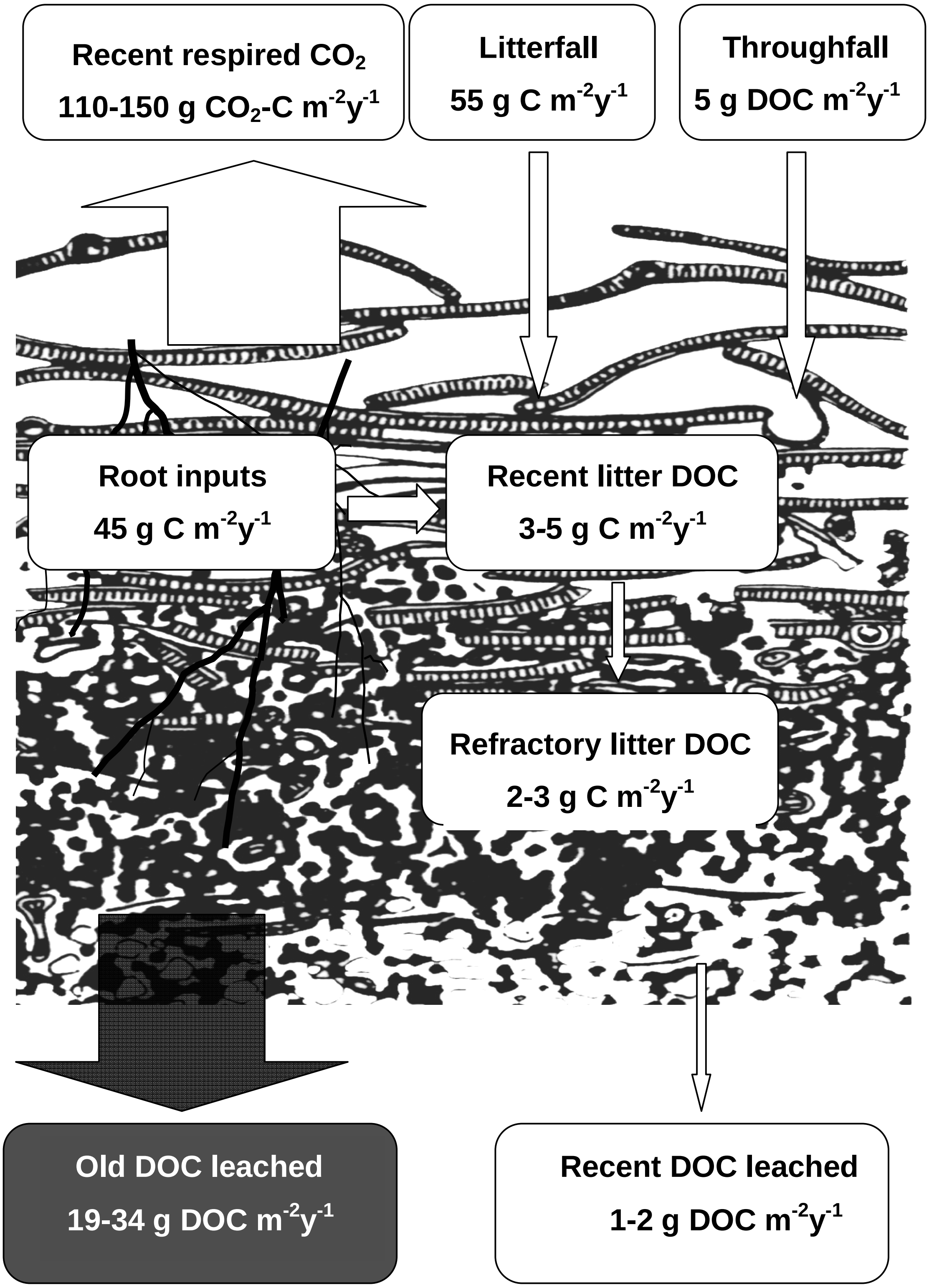
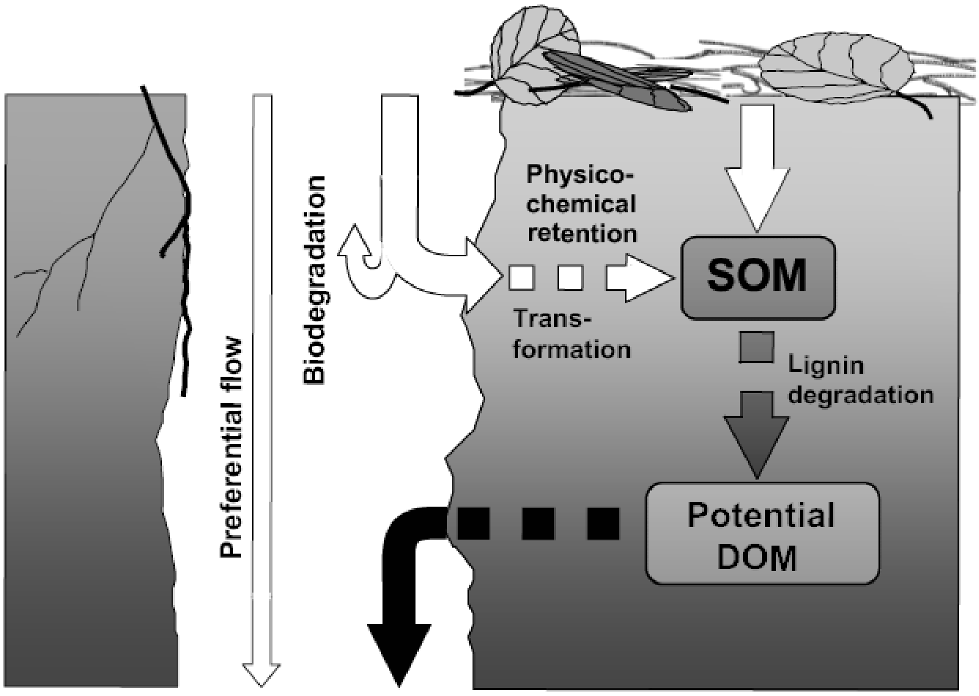


Figure 7: Partitioning of recent photosynthates in soils and DOC fluxes.



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8 Ecosystem consequences of elevated atmospheric CO_2 concentration at the alpine treeline

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Abstract

Global environmental changes, among which rising atmospheric CO_2 concentration is considered to be one of the more drastic ones, profoundly affect the structure and functioning of ecosystems with important implications for ecosystem goods and services provided to human societies. Research on direct effects of elevated CO_2, as opposed to its indirect activity as a greenhouse gas, mostly looked at responses in plant growth and production, often interpreted in the context of potential changes in carbon sequestration. Ecosystem oriented research, essentially developing in parallel to the important technological advance of Free Air CO_2 Enrichment (FACE), however provides evidence for a multitude of CO_2-induced ecosystem consequences beyond plant biomass and production. After a five- year experiment at the Swiss treeline FACE site (2180 m asl, Davos, Switzerland), we offer a synthesis of results obtained at different levels of organization and put them explicitly in an ecosystem context. In response to elevated CO_2 concentration, we observed: (1) a sustained stimulation of photosynthesis and no change in stomatal conductance in either of the dominant conifer species, *Larix decidua* and *Pinus uncinata*; (2) species specific growth responses evident in the shoot and stem increment records of the dominant perennial plants. Among the trees, *L. decidua* showed a clear positive growth response while *P. uncinata* showed none. Among the dwarf shrubs, all *Vaccinium* species showed a trend towards stimulated growth, while in contrast, *Empetrum hermaphroditum* did not; (3) an increase in seed abundance of the annual hemiparasites *Melampyrum sylvaticum* and *Melampyrum pratense*; (4) changes in leaf quality, evident particularly as higher non-structural carbohydrates, in the dominant

perennials; (5) reduced relative growth of herbivores in the ecosystem resulting from changes in forage quality studied on the Alpine Grasshopper, *Miramella alpina* and the Larch Bud Moth, *Zeiraphera diniana*; (6) higher frost damage of *Vaccinium* buds in light of a late spring frost; (7) no evidence of any changes in root production, root standing crop or root decomposition; (8) a trend towards increased mycorrhization of seedling root tips; (9) slightly later litter fall of *Larix* and *Vaccinium* but no change in *Larix* litter decomposition rates; (10) higher soil organic matter, particularly evident in seasonal measurements of dissolved organic carbon, and higher rates of soil respiration and measured microbial activity. In addition, the stable isotope $\delta^{13}C$ permitted us to trace C allocation in leaves, branches, roots, soil aqueous solution and soil air in order to understand the system's carbon cycling pattern. Collectively, these results show that the consequences of elevated CO_2 concentration extend beyond any potential carbon sequestration through accelerated plant growth and can have significant impacts on ecosystem processes with feedbacks through trophic levels and with implications for carbon and nutrient cycling.

9 Summary

Following four years of CO_2 enrichment (2001-2004) of trees and their understory dwarf heath, and a one-time tree defoliation treatment in the spring of the second year at the Swiss treeline FACE site on Stillberg (Davos, Switzerland), this dissertation summarizes responses from both the above- and below-ground components of this treeline ecosystem.

At the tree physiological level (Handa et al. 2005, chapter 2), we found that elevated CO_2 enhanced photosynthesis in both *Larix decidua* and *Pinus uncinata* by ca. 40% and led to increased nonstructural carbohydrate concentrations in the needles of both species, but to no significant decrease in stomatal conductance. There was no evidence for photosynthetic downregulation in either of the conifer species. Irrespective of CO_2 concentration, defoliation in both species stimulated photosynthesis (*Larix*, +7 %; *Pinus* +52%) and increased stomatal conductance (*Larix*, +42%; *Pinus*, +108%) in remaining current-year needles in the treatment year and reduced leaf nitrogen concentration (-11% in *Larix* only) in the year following defoliation. These results are by and large consistent with what has been observed in multiple other CO_2 enrichment experiments to date (Ceulemans et al. 1999, Norby et al. 1999, Nowak et al. 2004, Zotz et al. 2005) and the strong physiological effects on the trees from the carbon source removal treatment highlight how an extreme disturbance can impact the tree's carbon budget.

Despite the c. 40% stimulation of photosynthesis in response to CO_2 enrichment, this did not translate into carbon that is purely available for growth regardless of whether one looks at the shoot or stem increment growth records for either of the studied tree species. In response to elevated CO_2, we observed a consistent positive growth response in *Larix* evident both in the annual shoot increment record (c. +20-30%; Handa et al. 2005, chapters 2&3) and the stem increment record (+41%; when cumulatively integrated over four years and measured relative to four years of pre-treatment measurements; Handa et al. 2006, chapter 3). The increase in radial stem wood growth was the result of more latewood production, in particular, the formation of larger tracheids, rather than a greater number of cells. In contrast, both of these lines of evidence (shoot and stem increment record) showed no positive growth response of *Pinus* trees, with the exception of the very first year of shoot increment data (Hättenschwiler et al. 2002, chapter 5). Our studies underline, yet again, how CO_2 effects on plants show strong species specificity (Loehle 1995), and how any meaningful study attempting to address ecosystem responses, must consider all its key players and account for species diversity (Körner et al. 2005). Defoliation led to a pronounced decrease in annual ring width of both species, marked in particular by less latewood production in the treatment as well as subsequent year, underlining again the importance of how a biotic interaction within the system might completely modify ecosystem responses in a changing global environment (Zvereva & Kozlov 2006).

Plants are frequently observed to increase carbon allocation to below-ground sinks and particularly, to accelerate fine root turnover in response to elevated CO_2 concentration. Our study shows that in this natural system, no change in response to elevated CO_2 exposure occurred. There was no difference in total root standing crop after four years, in new root production measured over three years and also no effect on root decomposition measured over 26 months (Handa et al. 2008, chapter 4). The lack of positive growth response below-ground contrasts with the sustained four year above-ground growth response of *Larix decidua*, but is in line with the lack of positive above-ground growth response of the later successional *Pinus uncinata* trees and that of some of the understory dwarf shrubs (Zumbrunn 2004). Multiple studies have reported positive root growth responses to elevated CO_2 concentrations, although very few have been conducted in the field, have exceeded a study duration >1 year or have used mature trees (Norby & Jackson 2000, Tingey et al. 2000). Root quality measurements indicated that elevated CO_2 significantly increased starch concentration, but there was no change in N concentration or in dehydrogenase activity. Other studies have also shown higher starch concentration (Janssens et al. 1998), but also lower N content in roots under elevated CO_2 (Janssens et al. 1998, Pregitzer et al. 2000, Wan et al. 2004). However, this result is certainly not ubiquitous (Tingey et al. 2003, King et al. 2005). Finally, our stable isotope data indicate that only ca. 30% of the new carbon was incorporated into new roots indicating a rather slow root turnover in this system.

9.1 Conclusions

The development of this study site was prompted as part of a general initiative of complementary projects at the University of Basel to test the “carbon limitation” versus “growth limitation” hypotheses of treeline formation. Körner and Paulsen’s (2004) worldwide assessment of high altitude rooting zone temperatures support the existence of a common threshold for forest growth at high elevation raising the question of a common mechanistic explanation. To date, little evidence has accumulated in favour of the carbon limitation hypothesis. The temperature response of photosynthesis of trees at these high altitudes is well tuned such that a shortage of assimilates seems unlikely (Tranquillini 1979, Häsler 1982, Körner 1998) and their carbon charging (measured as non-structural carbohydrates of various *Pinus* species) is not less but equal or even greater with increasing proximity to treeline (Hoch et al. 2002, Hoch & Körner 2003). This body of evidence has tipped the scale in favour of a growth limitation driven mechanism where tissue formation, especially for roots and their functioning, may be limited by cold treeline temperatures. Our first-ever *in situ* direct test offers support for and against carbon limitation. As observed by Hoch & Körner (2003), *Pinus* trees are not carbon limited, whereas the sustained positive growth response suggests that *Larix* trees are. The continued observation of species-specific CO_2 responses in various studies prompted Loehle (1995) to suggest that the life history strategies of certain plants make them more responsive than others. Plants with a determined growth pattern like *Pinus* have much less leeway to profit from CO_2 enrichment and any compound interest effects that might accumulate than fast-growing, indeterminate plants like *Larix*. The treeline ecosystem that we study represents a self-supporting ‘steady state’ system which is typically not carbon limited (Körner et al. 2005), as evident by the response of the later successional elements of the system (*Pinus uncinata* and the below-ground ‘closed canopy’ of the rooting systems). The early successional element of the system, *Larix decidua*, a typical pioneer tree, however, was able to take advantage of the sparse canopy above-ground and utilize the extra carbon for canopy expansion, suggesting that the debate regarding “carbon limitation” versus “growth limitation” is not yet completely closed. Even if the CO_2 response in *Larix* is restricted to a juvenile phase, we conclude that the structure of the treeline ecotone is likely to change in a CO_2 enriched world with an altered competitive balance of these two species in favour of *Larix*.

10 Literature

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Zvereva EL, Kozlov MV (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: a metaanalysis. Global Change Biology, **12**, 27-41.

Curriculum vitae

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Born on 10 April, 1975 in Longueuil, Canada

Nationality: Canada and Switzerland
Civil status: Single
Languages: English, French, German
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Education

2001-2006	Ph.D. in Botany, University of Basel, Switzerland „Tree and ecosystem responses to four years of in situ CO_2 enrichment at the Swiss treeline“ Supervisors: Prof. Christian. Körner, PD Dr. Stephan Hattenschwiler, Defended May 2006 with *Summa cum laude*
1996-1998	Master of Science in Botany, University of Toronto, Canada „Revegetation trials in degraded coastal marshes of the Hudson Bay lowlands“ Supervisor: Prof. RL Jefferies
1993-1996	Bachelor of Science in Biology, McGill University, Canada
1991-1993	Diploma of Collegial Studies, Marianopolis College, Montreal, Canada

Scientific Professional Experience

2007-	Post-doctoral associate reasearcher, Centre of Functional Ecology and Evolution (CNRS), Montpellier, France Research: (1) Biodiversity and biogeochemical cycles: a search for mechanisms across a latitudinal gradient from the subarctic to the tropics (a EURODIVERSITY European Science Foundation project, http://biocycle.cefe.cnrs.fr), (2) The effect of elevated CO_2 and temperature on decomposition processes at the alpine treeline.
2006	Post-doctoral researcher, University of Basel, Switzerland Research: Treeline trees in a CO_2 enriched world
2006	*Oecologia* Editorial Assistant to Editor-in-Chief, Prof. Ch. Körner
2004-2005	Coordinator of logistics and research at the Swiss treeline Free Air CO_2 Enrichment (FACE) site (Stillberg, Davos)
2000-	Referee for a number of scientific journals including *Acta Oecologica, Applied Soil Ecology, Canadian Journal of Botany, Canadian Journal of Forestry Research, Ecology Letters, Global Ecology and Biogeography, Journal of Ecology, Oikos*

2000 Scientific research technician, Institute of Arctic Biology, University of Alaska Fairbanks
Research: (1) Plant herbivore relations in the Yukon-Kuskokwim Delta, Profs. J Sedinger & R Ruess, (2) Treeline dynamics across a latitudinal gradient, Profs. R Ruess, B Sveinbjornsson

1994-1996 Field and research assistant at the Mont St. Hilaire UNESCO Man and Biosphere Reserve, McGill University
Research: Phenotypic plasticity in *Carex*, Profs. MJ Lechowicz, G Bell & M Waterway

1993 Research assistant in environmental geology, McGill University
Research: The role of aqueous complexation in explaining secondary porosity in sedimentary basins, Dr. J. Fein

Teaching Experience

2008 Supervision of Master student (T. De Oliveira)

2007 Co-supervision of Master students (B. Jackson and S. Rouifed), Centre d'écologie fonctionelle et évolutive (CNRS), Montpellier

2001-2006 Plant ecology lectures and field courses directed by Prof. Ch. Körner, University of Basel

1996-1998 Teaching assistant in forest ecology to Prof. T. Carleton, University of Toronto

Scientific Awards and Scholarships

2006 Best Poster Award International Young Scientists' Global Change Conference (Beijing, China)
2006 Invitation (100 from 1000 applicants) to the International Young Scientists' Global Change Conference (Beijing, China)
2005 Swiss Global Change Day Best Poster Award (Bern, Switzerland)
2004 Freiwillige Akademische Gesellschaft Scholarship
2001-2003 FQRST (Fonds québecois de recherche sur la nature et technologie), Doctoral Research Scholarship
Tenure: Botanical Institute, University of Basel
1998 Ontario Graduate Scholarship
Tenure: Department of Botany, University of Toronto
1997 FCAR (Fonds des chercheurs et aide à la recherche du Québec) M. Sc. Research Scholarship
Tenure: Department of Botany, University of Toronto
1994 Canada Scholarship
Tenure: Department of Biology, McGill University
1993 National Science and Engineering Research Council of Canada Summer Undergraduate Research Award,
Tenure: Department of Earth and Planetary Science, McGill University

International Scientific Meetings (published abstracts & oral presentations)

2007	Ecological Society of America Annual Meeting (San Jose, USA)
2006	Bristish Ecological Society Annual Meeting (Oxford, UK)
2006	Young Scientists Global Change Conference & Earth Science Systems Partnership Open Science Meeting (Beijing, China)
2005	Ecological Society of America Annual Meeting (Montreal, Canada)
2004	Ecological Society of America Annual Meeting (Portland, USA)
2003	Ecological Society of Germany, Switzerland and Austria Annual Meeting (Halle, Germany)
2002	Ecological Society of America Annual Meeting (Tucson, USA)
2001	Ecological Society of Germany, Switzerland and Austria Annual Meeting (Basel, Switzerland)
1998	North American Arctic Goose Conference (Victoria, Canada)
1998	Society for Ecological Restoration Annual Meeting (Groningen, Netherlands)

Scientific Publications

Handa IT, Jefferies RL (2008) Interactions between lesser snow geese and Arctic coastal vegetation leading to alternative stable states. In *New Models for Ecosystem Dynamics and Restoration* (eds. R. Hobbs and K Suding), Island Press, in press.

Hagedorn F, van Hees P, **Handa IT**, Hättenschwiler S (2008) Elevated atmospheric CO_2 fuels leaching of old dissolved organic matter at the alpine treeline, *Global Biogeochemical Cycles*, in press.

Handa IT, Hagedorn F, Körner C, Hättenschwiler S (2008) No stimulation in root production in response to four years of *in situ* CO_2 enrichment at the Swiss alpine treeline, *Functional Ecology*, in press.

Handa IT, Körner C, Hättenschwiler S (2006) Conifer stem growth at the altitudinal treeline in response to four years of CO_2 enrichment, *Global Change Biology*, **12**, 2417-2430.

Handa IT, Körner C, Hättenschwiler S (2005) Carbon economy in treeline conifers: a test of the carbon limitation hypothesis through *in situ* CO_2 enrichment and defoliation. *Ecology*, **86**, 1288-1300.

Hättenschwiler S, **Handa IT**, Egli L, Asshoff R, Ammann W, Körner Ch (2002) Atmospheric CO_2 enrichment of alpine treeline conifers. *New Phytologist*, **156**, 363-375.

Handa IT, Harmsen R, & Jefferies RL (2002) Patterns of vegetation change and the recovery potential of degraded areas in a coastal marsh system of the Hudson Bay lowlands. *Journal of Ecology*, **90**, 86-99.

Handa IT & Jefferies RL (2000) Assisted revegetation trials in degraded salt-marshes. *Journal of Applied Ecology*, **37**, 944-958.

Fein JB, Yane L, Jyoti A, **Handa T** (1995) Experimental study of aluminium and calcium malonate complexation at 25, 35 and 80° C. *Geochimica et Cosmochimica Acta*, **59**, 1053-1062.

Fein JB, Yane L, **Handa T** (1994) The effect of aqueous complexation on the decarboxylation rate of oxalate. *Geochimica et Cosmochimica Acta*, **58**, 3975-3981.

In review:

Hagedorn F, **Handa IT**, Kaiser K, van Hees P, Saurer M, Hättenschwiler S (2008) Sources and sinks of DOC in organic layers- insights from $^{13}CO_2$ *in situ* tracing shows a cascade dynamic of dissolved organic matter at the alpine treeline. *Biogeochemistry*, in review.

In preparation:

Handa IT, Hättenschwiler S, Hagedorn F, Asshoff R, Egli S, Körner C, Rixen C, Serio D, Siegwolf R, von Felten S, Wipf S, Zumbrunn T. Ecosystem consequences of elevated atmospheric CO_2 concentration at the alpine treeline, in preparation for *Ecosystems.*

Jackson B, David JF, **Handa IT**, Coq S, Hättenschwiler S. Macrofaunal diversity by litter diversity interactions in a Mediterrenean field microcosm study. In preparation.

Rouifed S, David JF, **Handa IT**, Hättenschwiler S. Elevated temperature enhances the decomposition of rapidly decomposing but not recalcitrant CO_2 enriched temperate forest leaves. In preparation.

Zumbrunn T, **Handa IT**, Hättenschwiler S. Response of multiple dwarf shrub species to four years of CO_2 enrichment at the alpine treeline. In preparation.

During the PhD dissertation study period (2001-2006), I attended lectures and field course from: Prof. Dr. Christian Körner, PD Dr. Jurg Stöcklin, PD Dr. Stephan Hättenschwiler, PD Dr. Pascal Niklaus, PD Dr. Fritz Schweingruber, PD Dr. Jan Esper, PD Dr. Markus Fischer, Dr. Heinz Schneider, Dr. Rolf Siegwolf, Dr. David Dupuy.