

Plant responses to long-term *in situ* CO₂ enrichment and soil warming at treeline in the Swiss Alps

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* These two papers were published under my maiden name, Melissa A. Martin.

Chapter 1.

General Introduction

1.1 *Plant responses to rising atmospheric CO₂ and a warmer climate*

The atmospheric CO₂ concentration is now higher than it has ever been during the last 25 million years (Petit *et al.* 1999, Pearson and Palmer 2000, Siegenthaler *et al.* 2005), and models using coupled carbon-climate cycle simulations predict concentrations to reach between 730 and 1,020 $\mu\text{mol mol}^{-1}$ by the year 2100 (Meehl *et al.* 2007). This change has been researched extensively from a number of perspectives, including the large role of anthropogenic activities in causing the rise, direct effects on organisms, particularly plants, and indirect effects through associated changes in climate. CO₂ is a basic resource for plant growth, and over the last 25 years the number of studies devoted to determining if higher concentrations of atmospheric CO₂ directly stimulate plant productivity in agroecosystems and natural ecosystems has grown exponentially (see reviews by Bazzaz 1990, Mooney *et al.* 1991, Nowak *et al.* 2004, Ainsworth and Long 2005, Körner 2006). CO₂ enrichment studies have evolved and diversified immensely since the first greenhouse experiments using individually grown, often young, plants with an optimal supply of other resources (see review by Lawton 1995). In contrast, many recent studies have applied manipulations in conditions as close as possible to natural environments, with competition among individual plants and limited nutrient availability. In particular, the development of free air CO₂ enrichment (FACE) technology has enabled *in situ* studies of non-seedling plants growing in a broad range of environments. These experiments have revealed that stimulation of plant growth under elevated CO₂ depends heavily on the availability of non-carbon resources (Körner 2006, McCarthy *et al.* 2010) and that co-occurring plant species often respond differently (Schäppi and Körner 1996, Handa *et al.* 2005, Körner *et al.* 2005). *In situ* studies have also revealed that physiological changes in plants grown under elevated CO₂ can lead to disadvantages in certain climatic conditions, such as increased susceptibility to damage from freezing events during the active season (e.g. Beerling *et al.* 2001, Obrist *et al.* 2001).

Indirect effects of rising levels of atmospheric CO₂ and other greenhouse gases include numerous changes in climate, such as altered precipitation patterns, increased frequency of extreme events, and overall global warming (Meehl *et al.* 2007). Temperature is a key factor that regulates plant activity, including growth, phenology, and reproduction, and the predicted increase in global temperatures (1.8-4 K by 2100; Meehl *et al.* 2007) will undoubtedly have strong impacts on plants. A meta-analysis of 20 experimental warming studies (0.3-6 K increase, 2-9 year duration) in diverse environments indicated that above-ground plant productivity was significantly enhanced by increased temperatures (Rustad *et al.* 2001). Temperature manipulation studies have

also contributed to evidence that climate warming can cause changes in the timing of key phenological events (see review by Aerts *et al.* 2006). In mountain environments, for example, advanced tissue dehardening and phenological development during spring means that newly formed tissues are vulnerable to freezing damage at an earlier date when temperatures below zero are more frequent. Freezing damage to leaves and flowers can have severe consequences for plant growth and reproductive success, as well as indirect impacts on pollinators and herbivores (Inouye 2000, 2008). Many terrestrial biogeochemical processes are also strongly influenced by temperature, and experimental warming has been found to increase rates of soil respiration and net N mineralization in several environments (meta-analysis by Rustad *et al.* 2001). Overall, like the direct effects of increased CO₂ concentration on plants, the magnitude and even direction of plant and ecosystem responses to warming varies across study sites and species (Rustad *et al.* 2001, Dormann and Woodin 2002, Walker *et al.* 2006).

An appropriate experimental duration is essential for determining the effects of elevated CO₂ and warming on plants, particularly for long-lived species like trees, and on ecosystem processes (Millard *et al.* 2007). Negative feedbacks of elevated CO₂ concentration on plant growth can result from greater nutrient uptake with accelerated growth and therefore lower nutrient availability (Luo *et al.* 2004, Reich *et al.* 2006), from stimulated microbial immobilization of nutrients through an increased input of labile carbon (Hagedorn *et al.* 2000), or from increased interspecific competition when other species have a stronger direct growth response to CO₂ enrichment. Positive feedbacks are also possible because elevated CO₂ can enhance plant nutrient use efficiency and increase nutrient acquisition by stimulating mycorrhizal and root growth (Norby *et al.* 2004). Similarly, increased temperature impacts many interacting physical, biological, and chemical processes operating at various time scales, and several years of observation are necessary to understand the temporal dynamics of plant and ecosystem responses (Rustad *et al.* 2001, Cornelissen *et al.* 2007). For example, it is uncertain if warming only affects the relatively labile soil carbon pool, which could become depleted within a few years, or if the recalcitrant soil carbon pool is also sensitive, and if there are longer-term increases in soil N availability (Melillo *et al.* 2002). For both experimental CO₂ enrichment and warming, long-term studies are additionally important because natural variation in climate can strongly influence treatment effects, and these patterns only become apparent over several years.

1.2 *The alpine treeline environment*

High elevation and high latitude environments are expected to be particularly sensitive to shifts in environmental conditions, serving as a “bellwether” for changes in other regions (Smith *et al.* 2009). Atmospheric pressure, and therefore CO₂ partial pressure, is lower at high elevations, so plants in these environments might be particularly responsive to rising CO₂ concentrations (Körner 2003). Further, ongoing global warming is already documented to have particularly strong ecological impacts on high latitude and high elevation regions where plants grow close to their low temperature limit (Theurillat and

Guisan 2001, Walther 2003, Dorrepaal *et al.* 2009). There are many studies of how atmospheric and climate change affect arctic ecosystems (Arft *et al.* 1999, Dormann and Woodin 2002, Aerts *et al.* 2006), but relatively few on temperate high elevation environments. Only two other experiments have applied CO₂ enrichment in this setting: a late-successional sedge community (1991-1995; Körner *et al.* 1997) and a mix of glacier forefield pioneer species (2006-2008; N. Inauen *et al.*, unpublished data) were studied at high elevation sites in the Central Swiss Alps. The Stillberg FACE experiment described in this thesis, located near Davos, Switzerland, is the only existing CO₂ study involving trees growing near their upper elevational limit. There is a somewhat longer and more widespread history of warming studies in mid-latitude alpine environments, for example in a Rocky Mountain dry montane meadow (Harte and Shaw 1995) and in an alpine fellfield in northern Japan (Kudo and Suzuki 2003). However, most *in situ* warming experiments in cold environments still occur in subarctic and arctic ecosystems.

Regarding the ecology of trees in particular, treeline, defined as “the abrupt transition in life-form dominance beyond which plants with massive single stems and tall crowns either cannot be developed, become unaffordable or are disadvantageous” (Körner 2003), is an exciting environment for research about atmospheric and climate change (see reviews by Grace *et al.* 2002, Smith *et al.* 2009, Wieser *et al.* 2009). There is evidence of enhanced tree ring width of high-elevation conifers during the past 150 years (e.g. Lamarche *et al.* 1984, Nicolussi *et al.* 1995, Rolland *et al.* 1998) and treelines in many locations are advancing to higher elevations (Harsch *et al.* 2009). Reasons for these changes are unclear, due to confounding factors (e.g. temperature, CO₂ levels, land use change) and because a physiological explanation for the global pattern of treeline formation has not been fully resolved. Several competing hypotheses for high elevation treeline formation exist relating to climatic stress, repeated disturbance, low regeneration success, nutrient limitation, thermal limitation of carbon investment into growth processes (sink limitation), and insufficient carbon to maintain a positive carbon balance (source limitation). The first three of these hypotheses are thought to be important only in specific local and regional situations and cannot explain the pattern that high elevation treelines follow a global isotherm of $6.7 \pm 0.8^{\circ}\text{C}$ soil temperature during the growing season (Körner and Paulsen 2004). Thus, the source and sink limitation hypotheses are the most plausible explanations for treeline formation from a global perspective (Körner 1998).

The sink limitation hypothesis states that low temperature restricts the rate at which carbon can be used in biosynthetic processes more than it limits the rate of net photosynthesis (Körner 1998). Whereas smaller plants benefit from radiant canopy warming during the day and stored warmth in the topsoil during the night, trees experience tight atmospheric coupling and self-shading that prevents radiative warming of their own root zone (Grace *et al.* 2002, Körner 2003). This hypothesis has been supported by evidence that wood, shoot and root growth cease at temperatures slightly above 0°C (Häsler *et al.* 1999, Alvarez-Uria and Körner 2007, Rossi *et al.* 2007). The opposing source limitation hypothesis states that low temperatures, short vegetation

periods (in extratropical areas), and frequent damage and disturbance cause a shortage of photo-assimilates and a negative carbon balance over the long term in trees, which have a large proportion of non-productive tissues. Photosynthetic rate in treeline trees is relatively insensitive to temperature, which casts doubt on assimilation limitation at the level of leaf gas exchange (Pisek and Winkler 1958, Häsler 1982). Similarly, concentrations of non-structural mobile carbon in trees have consistently been found to increase with increasing elevation at locations across the globe, suggesting restricted carbon investment rather than acquisition (e.g. Hoch and Körner 2003, Shi *et al.* 2006). The strongest support for the carbon limitation hypothesis perhaps lies at the whole tree level, where recurring tissue damage and loss can substantially reduce net carbon acquisition over the long term and lead to impaired tree growth (Sveinbjörnsson 2000). Experimental manipulation of the atmospheric CO₂ concentration at the high elevation treeline can provide unique insight into the unresolved global treeline phenomenon while also helping to improve predictions about shifts in tree growth, distribution, and species composition in treeline ecotones under environmental change.

1.3 This doctoral thesis

Study site and experimental design

My PhD research was conducted in the context of an existing environmental change experiment, where free air CO₂ enrichment (spring 2001 to autumn 2009) and soil warming (spring 2007, ongoing) were applied at the temperate high elevation treeline at Stillberg, Davos in the Central Alps, Switzerland (9° 52' E, 46° 46' N). The study site and experimental design have been described in detail in previous publications (e.g. Hättenschwiler *et al.* 2002, Handa *et al.* 2006, Hagedorn *et al.* 2010) and are summarized in each of the chapters of this thesis. Briefly, the experiment covers an area of 2500 m² and is situated on a NE-exposed 25 to 30° slope at 2180 m a.s.l., corresponding to or slightly above the natural climatic treeline. The site is located within a 5 ha long-term afforestation research area (2080-2230 m a.s.l.) where high elevation provenances of three treeline tree species, *Larix decidua* (European larch), *Pinus cembra* (Swiss stone pine), and *Pinus mugo* ssp. *uncinata* (mountain pine) were planted into the intact dwarf shrub community in 1975 (Schönenberger and Frey 1988). A long-term meteorological station located within the plantation at 2090 m a.s.l. provided climate information for before and during the experimental period. At the beginning of this experiment in 2001, *L. decidua* was the dominant survivor in the plantation, followed by *P. uncinata*, while few individuals of *P. cembra* remained. Both *Pinus* species were subject to high mortality due to fungal attacks (Senn and Schönenberger 2001). The experiment was thus designed with *L. decidua* and *P. uncinata* as the two target tree species.

The experiment consists of 40 hexagonal 1.1 m² plots, 20 with a pine individual in the centre and 20 with a larch individual in the centre. The trees are sparsely distributed and do not form a closed canopy, with a dense cover of understorey plants in each

experimental plot surrounding the tree base. Each plot therefore consists of a single tree and the typical understorey vegetation of dwarf shrubs (*Vaccinium myrtillus*, *V. gaultherioides*, *V. vitis-idaea*, *Empetrum hermaphroditum*) and herbaceous species (e.g. *Avenella flexuosa*, *Gentiana punctata*, *Homogyne alpina*, *Leontodon helveticus*, *Melampyrum pratense*). The long-term CO₂ enrichment experiment started in early June 2001, at which point the 40 plots were assigned to 10 groups of four neighbouring plots (two larch and two pine trees per group) in order to facilitate the logistics of CO₂ distribution and regulation. Half of these groups received supplementary CO₂ (+200 μmol mol⁻¹) during the photosynthetically active daylight hours for the snow-free growing seasons, while the remaining groups served as controls and received no additional CO₂ (c. 380 μmol mol⁻¹). A one-time 80% defoliation treatment was applied to the trees in June 2002 to simulate a severe insect outbreak event (Handa *et al.* 2005, 2006). Tree responses to this earlier treatment are not discussed further in the present work except when explaining how it was accounted for in statistical analyses. In spring 2007, one plot of each tree species identity was randomly selected from each of the 10 CO₂ treatment groups and assigned a soil warming treatment (+4 K), where heating cables laid on the ground surface underneath the dwarf shrub layer were used to warm the soil and near-ground air during the snow-free period (Hagedorn *et al.* 2010). From 2007-2009, therefore, we had a balanced factorial experimental design with a replication of five individual plots for each combination of CO₂ concentration, warming treatment and tree species.

Overall study aims

The field measurements and data analysis I completed during my PhD studies built upon the knowledge and data gathered by other colleagues involved in the project, primarily Dr. Tanya Handa, who worked on the experiment during the earlier years of the experiment for her doctoral work at the University of Basel. With this foundation of previous research activity, the aim of my dissertation work was to understand the magnitude and temporal dynamics of plant responses to nine years of CO₂ enrichment and three years of soil warming. Continuation of measurements conducted throughout the entire CO₂ enrichment period yielded several data sets documenting long-term plant growth responses to this treatment. The warming aspect of the experiment was initiated the summer I joined the project, allowing me three growing seasons of observation. Rather than focusing primarily on the two tree species and the fundamental question of source vs. sink limitation at treeline, I chose a broader investigation of how trees and the dwarf shrub heath understorey layer respond to atmospheric and climate change. In assessing responses over multiple years, I could also study how the treatments interacted with natural interannual variation in climatic conditions and with extreme temperature events. In particular, field observations that the treatments altered plant susceptibility to damage from freezing events prompted a closer look at this topic with an experimental approach. Finally, in collaboration with other researchers, I related my findings about plant growth responses to the broader question of how the carbon

balance of the system was affected by soil warming. Specific research questions for each of these topics are listed in the individual chapter summaries below.

Chapter 2.

Species-specific tree growth responses to nine years of CO₂ enrichment at the alpine treeline.

In this chapter, I present new tree growth data from 2005 to 2009, the final five years of the long-term CO₂ enrichment experiment, and re-analyze results from earlier years of the study in the context of the full nine-year treatment period. For analyses in this chapter, it was possible to pool across soil warming treatments in order to focus on tree responses to long-term exposure to elevated CO₂. Specifically, we aimed to understand: (1) whether trees show sustained growth stimulation under elevated CO₂ over several years; (2) if co-occurring *Larix decidua* and *Pinus uncinata* respond differently to long-term CO₂ enrichment; and (3) how interannual variability in climatic conditions influences the growth response to CO₂ enrichment.

Chapter 3.

Growth and community responses of alpine dwarf shrubs to in situ CO₂ enrichment and soil warming

Dwarf shrubs are a major component of alpine plant communities, and changes in growth, abundance and distribution of these species under environmental change are likely to have important ecological consequences. This chapter focuses on growth responses of three dominant dwarf shrub species, *Vaccinium myrtillus*, *Vaccinium gaultherioides* (group *V. uliginosum* agg.) and *Empetrum nigrum* ssp. *hermaphroditum*, to CO₂ enrichment and soil warming and how the treatments influenced changes in understory species richness over time. Main hypotheses included: (1) the co-occurring dwarf shrubs would vary in their above-ground growth response to long-term CO₂ enrichment, and any initial stimulation would decline over time due to increasing nutrient limitation; (2) soil warming would lead to enhanced dwarf shrub growth due to direct stimulation and/or increased soil nutrient availability; (3) soil warming would alleviate non-carbon constraints on the growth response to elevated CO₂, yielding the largest response when the two experimental treatments were applied together; and (4) the experimental treatments would lead to shifts in species composition and richness in the experimental plots.

Chapter 4.

Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO₂

Freezing conditions can occur year-round in high elevation environments and are particularly common in temperate regions during the early part of the alpine growing season and in early autumn. Physiologically active (dehardened) plants, especially newly

developed tissue, are comparatively sensitive to freezing temperatures and often suffer damage from episodic freezing events during the early growing season. This chapter describes an experimental study of how CO₂ enrichment and soil warming impact the freezing resistance of newly formed leaf tissue from 10 plant species during the early growing season. We hypothesized that (1) CO₂ enrichment would negatively affect early growing season freezing resistance but that this effect would not be associated with a phenological shift induced by the treatment and (2) soil warming would advance phenology, leading to more mature leaf tissue in the early growing season and, therefore, reduced freezing sensitivity of fully expanded leaves. We also predicted that (3) plants of different functional type (PFT) and growth form (PGF) groups would have distinct freezing damage thresholds under current conditions, and plants within the same group would respond similarly to the experimental treatments.

Chapter 5.

Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline (co-authorship)

This chapter is a contribution from the overall research team in the Stillberg FACE and soil warming project. Objectives of this chapter about short-term warming responses were to (1) estimate the *in situ* temperature dependency of soil respiration and dissolved organic carbon (DOC) leaching (2) determine if soil warming induces a moisture limitation for soil carbon fluxes and plant growth; and (3) quantify how the one-year warming treatment affects the carbon balance at the alpine treeline. In addition, this chapter contains a detailed description of the soil heating system and its performance during 2007, the first growing season of treatment. I contributed to this work primarily by measuring plant growth responses to warming and by estimating vegetation contributions to the carbon budget of the system.

Chapter 2.

Species-specific tree growth responses to nine years of CO₂ enrichment at the alpine treeline

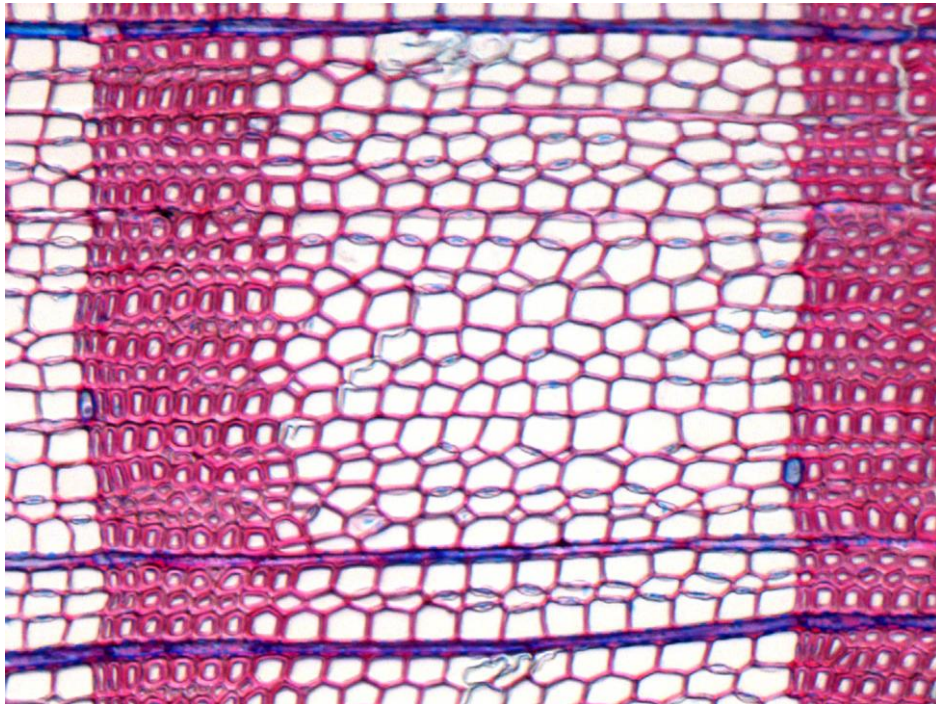
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Abstract

1. Using experimental atmospheric CO₂ enrichment, we tested for tree growth stimulation at the high elevation treeline, where there is overwhelming evidence that low temperature inhibits growth despite an adequate carbon supply. We exposed *Larix decidua* (European larch) and *Pinus mugo* ssp. *uncinata* (mountain pine) to nine years of free air CO₂ enrichment (FACE) in an *in situ* experiment at treeline in the Swiss Alps (2180 m a.s.l.).
2. Accounting for pre-treatment vigour of individual trees, tree ring increments throughout the experimental period were larger in *Larix* growing under elevated CO₂ but not in *Pinus*. The magnitude of the CO₂ response in *Larix* ring width varied over time, with a significant stimulation occurring in treatment years three to seven (marginal in year six).
3. After nine years of treatment, leaf canopy cover, stem basal area, and total new shoot production were overall greater in *Larix* trees growing under elevated CO₂, whereas *Pinus* showed no such cumulative growth response. The *Larix* ring width response in years three to seven could have caused the cumulative CO₂ effect on tree size even if no further stimulation occurred, so it remains unclear if responsiveness was sustained over the longer term.
4. *Larix* ring width was stimulated more by elevated CO₂ in years with relatively high spring temperatures and an early snowmelt date, suggesting that temperatures were less limiting in these years and greater benefit was gained from extra carbon assimilated under elevated CO₂. The magnitude of CO₂ stimulation was also larger after relatively high temperatures and high solar radiation in the preceding growing season, perhaps reflecting gains due to larger carbon reserves.
5. *Synthesis*. Contrasting above-ground growth responses of two treeline tree species to elevated CO₂ concentrations suggest that *Larix* will have a competitive advantage over less responsive species, such as co-occurring *Pinus*, under future CO₂ concentrations. Stimulation of *Larix* growth might be especially pronounced in a future warmer climate.

Introduction

Atmospheric CO₂ concentration is now higher than it has ever been during the last 25 million years (e.g. Pearson and Palmer 2000), and models using coupled carbon-climate cycle simulations predict CO₂ levels to reach between 730 and 1,020 μmol mol⁻¹ by the year 2100 (IPCC 2007). Over the last 25 years, researchers have experimentally manipulated the atmospheric CO₂ concentration to study the effects on plant growth and productivity from the genetic level to the whole ecosystem scale. Tree responses have been studied with particular interest because forests are major terrestrial biomass carbon stores and hence play an important role in the global carbon cycle (Schimel 1995, Jarvis 1998, Huang *et al.* 2007). Results from studies of older trees in systems with complete plant-soil coupling indicate high interspecific differences in growth responses and overall lower responsiveness in biomass production than initially found in chamber experiments with young trees and otherwise optimal growth conditions (Nowak *et al.* 2004, Norby *et*

al. 2005, Körner 2006). Further, it has become apparent that the availability of resources other than carbon plays a large role in the CO₂ response (Spinnler *et al.* 2002, Finzi *et al.* 2006, Körner 2006). Although the field is developing rapidly, long-term experimental studies about growth responses of pole stage and mature trees in natural environments are still rare and tend to focus on systems dominated by a single species.

It has become clear from long-term CO₂ enrichment studies that an appropriate experimental duration is essential for understanding the dynamics of plant responses to elevated CO₂, particularly for long-lived plants like trees (Körner 2006). Long-term CO₂ enrichment can have a negative feedback on plant growth if greater nutrient sequestration into organic matter during CO₂-induced growth enhancement is not met with sufficient replenishment of nutrients via mineralization (Luo *et al.* 2004, Reich *et al.* 2006, Millard *et al.* 2007, Pepper *et al.* 2007). Early studies of young trees also attributed a decline in the CO₂ response to downward adjustment of photosynthetic capacity under longer-term exposure to elevated CO₂ (e.g. Medlyn *et al.* 1999); however, studies of mature trees growing in near-natural conditions have not provided much evidence for such an effect (Nowak *et al.* 2004, Zotz *et al.* 2005). Positive feedbacks are possible over the longer term because elevated CO₂ can enhance plant nutrient use efficiency and increase nutrient acquisition by stimulating mycorrhizal and root growth (Norby *et al.* 2004, Treseder 2004). Long-term CO₂ manipulation experiments are particularly important because temporal variation in climate or resources might influence treatment effects, and these patterns are only observable over several years. Changes in the responses to elevated CO₂ might also occur as trees become older because CO₂ responsiveness might be greatest in young trees, when growth is vigorous and nutrient supply is relatively high (Wang 2007), but few CO₂ manipulation experiments have been long enough in duration to investigate this question adequately. In natural ecosystems, shifts in the responses of trees might additionally result from CO₂-mediated biotic interactions between trees and understorey species or herbivores (Zvereva and Kozlov 2006).

The current understanding of how trees will respond to increasing atmospheric CO₂ concentrations is based almost entirely on low-elevation forest sites, where direct competition for light, space, water, and nutrients potentially regulates the CO₂ response under conditions of a steady-state leaf area index (Körner 2006). In contrast, no previous *in situ* CO₂ enrichment experiments on trees have involved conditions where low temperature is thought to be a major limiting factor for growth. Several dendrochronological studies of high elevation conifers have led to the argument that, along with climate warming, rising atmospheric CO₂ concentrations might have contributed to increasing tree ring width over the last 150 years (Graybill and Idso 1993, Nicolussi *et al.* 1995). However, the confounding and possibly interacting effects of these two factors, along with several other biotic and abiotic variables, complicates interpretation of growth trends from dendrochronological records (Graumlich 1991, Huang *et al.* 2007). Experimental studies aimed at understanding how trees growing at treeline respond to rising CO₂ concentrations and how that response depends on

temperature can provide a valuable complement to dendrochronological studies for predicting future changes in these ecosystems.

High elevation treelines follow a global isotherm of $6.7 \pm 0.8^\circ\text{C}$ (mean growing season soil temperature; Körner and Paulsen 2004), and the explanation for this existential limit has been debated extensively by the scientific community (e.g. Tranquillini 1979, Körner 2003, Smith *et al.* 2009). The most plausible explanations for treeline formation from a global perspective are the sink and source limitation hypotheses (Körner 1998). The former states that low temperature restricts the rate at which carbon can be used for structural growth more than it limits the rate of net photosynthesis. The latter proposes that low temperatures and frequent damage and disturbance (at high latitudes) cause a shortage of photo-assimilates and a negative carbon balance over the long term. Photosynthetic rates in treeline trees are relatively insensitive to temperature, which casts doubt on assimilation limitation at the leaf level (Pisek and Winkler 1958, Häsler 1982). Further, concentrations of non-structural carbon reserves in trees have consistently been found to increase with increasing elevation at locations across the globe, suggesting that restricted carbon investment, rather than acquisition, limits tree growth (e.g. Hoch and Körner 2003, Shi *et al.* 2008). The sink limitation hypothesis has also been supported by evidence that wood formation was only active when the minimum daily temperature was above $2\text{-}4^\circ\text{C}$ in *Larix decidua*, *Picea abies* and *Pinus cembra* growing at treeline in the Italian Alps (Rossi *et al.* 2007). Likewise, root growth in seedlings of the three conifer species *Picea abies*, *Pinus cembra* and *Pinus sylvestris*, given optimal levels of other resources, ceased at temperatures below $4\text{-}5^\circ\text{C}$ (Alvarez-Uria and Körner 2007). For *Larix decidua* saplings growing at our own treeline research site in the Swiss Alps, the rates of root and shoot elongation were exponentially related to temperature, with a distinct reduction in above- and below-ground growth below $5\text{-}7^\circ\text{C}$ (Häsler *et al.* 1999).

Experimental manipulation of atmospheric CO_2 concentration at the high elevation treeline provides the unique opportunity to directly test whether CO_2 enrichment stimulates tree growth in an environment where there is overwhelming evidence that low temperature inhibits growth despite an adequate carbon supply. We exposed *Larix decidua* (European larch) and *Pinus mugo* ssp. *uncinata* (mountain pine), growing at the alpine treeline for more than 30 years, to elevated CO_2 and studied tree growth over nine years. During the first four years of CO_2 enrichment, *Pinus* showed low responsiveness to the enhanced carbon supply whereas *Larix* showed sustained above-ground growth stimulation under elevated CO_2 , contrary to predictions based on the sink limitation hypothesis (Hättenschwiler *et al.* 2002, Handa *et al.* 2005, 2006). In this paper, we present new growth data from 2005 to 2009, the final five years of the long-term experiment, and re-analyze results from earlier years of the study in the context of the full experimental period. We aimed to understand 1) whether trees show sustained growth stimulation under elevated CO_2 over several years; 2) if co-occurring *Larix* and *Pinus* respond differently to long-term CO_2 enrichment; and 3) how interannual variability in climatic conditions influences the growth response to CO_2 enrichment.

Materials and methods

Study site and experimental setup

The study site is located at Stillberg, Davos in the Central Alps, Switzerland (9° 52' E, 46° 46' N). The free air CO₂ enrichment (FACE) experiment covers an area of 2500 m² and is situated at or slightly above the natural climatic treeline (2180 m a.s.l.) on a NE-exposed 25 to 30° slope (Hättenschwiler *et al.* 2002, Handa *et al.* 2006). The FACE site is located within a 5 ha long-term afforestation research area where seedlings of three treeline species, *Larix decidua* L., *Pinus cembra* L. and *Pinus mugo* ssp. *uncinata* Ramond, of high elevation provenances were planted into the intact dwarf shrub community in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). Long-term annual precipitation at this location is 1050 mm and average temperature is -5.8°C in January and 9.4°C in July (Schönenberger and Frey 1988). Soil types are sandy Ranker and Podzols (Lithic Cryumbrepts and Typic Cryorthods), derived from siliceous Paragneis parent material and dominated by an organic Humimor layer of 5-20 cm (Schönenberger and Frey 1988, Bednorz *et al.* 2000).

For the FACE experiment, 40 hexagonal 1.1 m² plots were established in early June 2001, 20 with a *Pinus uncinata* individual in the centre and 20 with a *Larix decidua* individual in the centre. Trees selected for the experiment were separated by a distance of at least 2 m and fit the following additional criteria: one dominant stem, no serious signs of disease or herbivory, not more than one neighbouring tree within 80 cm, and total tree height of 0.8 to 1.5 m (Hättenschwiler *et al.* 2002). These trees are now 38 (*Pinus*) and 36 (*Larix*) years old, with average heights of 1.4 m (*Pinus*) and 2.3 m (*Larix*) and a stem basal diameter of 5 to 10 cm. The trees are sparsely distributed and do not form a closed canopy, with a dense cover of understorey plants in each experimental plot surrounding the tree base. Each plot therefore consists of a single tree and the typical understorey vegetation of dwarf shrubs (deciduous *Vaccinium myrtillus* and *V. gaultherioides*, evergreen *V. vitis-idaea* and *Empetrum hermaphroditum*) and herbaceous species (e.g. *Gentiana punctata*, *Homogyne alpina*, *Leontodon helveticus*, *Melampyrum pratense*). The 40 plots were assigned to 10 groups of four neighbouring plots (two *Larix* and two *Pinus* trees per group) in order to facilitate the logistics of CO₂ distribution and regulation. Half of these groups were randomly assigned to an elevated CO₂ treatment (target concentration 550 μmol mol⁻¹) while the remaining groups served as controls and received no additional CO₂ (c. 380 μmol mol⁻¹). The resulting experimental setup for multi-year analysis was a split-split-plot design with (a) 40 “split-split-plots” (individual tree, unit upon which repeated measures were made) nested within (b) 20 “split-plots” (containing either two *Pinus* or two *Larix* individuals) nested within (c) 10 “whole plots” with either ambient or elevated CO₂ concentration (four trees each).

The free air CO₂ enrichment system released pure CO₂ through laser-punched drip irrigation tubes during daytime hours only (10 to 14 hours per day). From 2001 to 2006,

the tubes hung vertically from a hexagonal frame surrounding each plot. From 2007 to 2009, tubes were woven into the tree crown in a manner similar to the web-FACE approach (Pepin and Körner 2002) in addition to a ring of vertical tubes from ground level to 50 cm height. This modified system provided more efficient CO₂ delivery to the entire canopy as trees continued to grow both taller and wider and helped avoid structural problems with larger frames in the steep, rocky terrain. Detailed descriptions of the original and revised setup and of the temporal and spatial variability of the CO₂ enrichment facility are given in Hättenschwiler *et al.* (2002) and in Hagedorn *et al.* (2010). Over the nine years of the experiment, the seasonal mean CO₂ concentrations in enriched plots during the CO₂ dispensing period, ± 1 standard deviation, ranged from 545 ± 45 to 595 ± 62 $\mu\text{mol mol}^{-1}$ (based on daily averages). Accounting for interruptions in CO₂ delivery due to adverse weather conditions and technical failures, trees received CO₂ enrichment for 73 to 87% of the potential treatment period each season (daytime only). The carbon isotope composition of the supplied pure CO₂ gas came from fossil fuel sources depleted in ¹³C compared to CO₂ in ambient air (-29.9‰ vs. -10.1‰). Therefore, the isotopic ratio of needle tissue provided evidence of how much supplementary CO₂ trees received. The nine-year mean isotopic ratio in needles of trees exposed to elevated CO₂ was shifted by -5.71‰ (averaged across the two species), indicating a mean canopy CO₂ concentration of $535 \mu\text{mol mol}^{-1}$. The adapted CO₂ supply system used in 2006 to 2009 did not change the needle isotopic ratio compared to the earlier years, demonstrating that enrichment was effective even as the trees grew larger. Further, isotope values from the ambient CO₂ plots corresponded to the natural ¹³C abundance, verifying that the enrichment system did not alter the CO₂ concentration in these control plots (von Felten *et al.* 2007). CO₂ concentration and $\delta^{13}\text{C}$ values for individual years are presented in Table 1.

Climate

A long-term meteorological station maintained by the Swiss Federal Institute for Forest, Snow and Landscape Research is located at 2090 m a.s.l., approximately 100 m below the FACE site, and provided climate information for the Stillberg research site both before and during the experimental period. Daily (24 h) mean soil (10 cm depth) and air (2 m height) temperature and solar radiation (310-2800 nm) were used to calculate means for each individual month of the year (temperature only) and over the entire growing season (approximately late May to early October). The beginning and end of the growing season were defined as the first date when daily mean soil temperature rose above 3.2°C in spring and fell below 3.2°C for more than two contiguous days in autumn, respectively (Körner and Paulsen 2004). Cumulative precipitation was calculated over the entire growing season. Maximum snow depth was determined for each winter, and snowmelt date was defined as the date in spring when the ground surface temperature rose sharply from values near zero during periods with snow cover.

Table 1. Summary of seasonal CO₂ enrichment

(a) Total number of days in the CO₂-enrichment period (first day in spring to last day in autumn), corresponding to the approximate snow-free season. (b) Seasonal mean CO₂ concentrations in CO₂-enriched plots during the dispensing period each year, ± 1 standard deviation (calculations based on daily averages). (c) Percentage of each of the nine seasonal treatment periods (daytime only) during which plots were exposed to elevated CO₂, accounting for interruptions in CO₂ delivery due to adverse weather conditions (e.g. photon flux density $<100 \text{ mmol m}^{-2} \text{ s}^{-1}$, wind speeds $>75 \text{ km h}^{-1}$, temperatures $<5^\circ\text{C}$, snow or sleet), technical failures, and interruptions for soil respiration measurements. (d) Shift in the carbon isotopic ratio ($\delta^{13}\text{C}$) of needles from trees exposed to elevated CO₂ compared to trees exposed to ambient air. Values represent means pooled across the two tree species, ± 1 standard error. $n = 20$ for 2001 and 2002 (data from von Felten *et al.* 2007), $n = 6$ for 2003-2005, and $n = 18$ for ambient CO₂ trees and 19 for elevated CO₂ trees for 2006-2009. The average isotopic shift was -5.72‰ for 2001-2005 (original CO₂ supply system) and -5.70‰ for 2006-2009 (adapted system), yielding an overall nine-year average of -5.71‰ .

Year	(a) Days in season	(b) Mean [CO ₂]	(c) Percent on	(d) Shift in needle $\delta^{13}\text{C}$ (‰)
2001	86	566 \pm 42	75	-4.94 \pm 0.58
2002	101	582 \pm 35	81	-5.76 \pm 0.88
2003	103	579 \pm 52	73	-5.46 \pm 1.92
2004	91	578 \pm 41	78	-6.53 \pm 1.21
2005	90	565 \pm 63	73	-5.91 \pm 1.51
2006	100	599 \pm 62	82	-6.08 \pm 0.56
2007	124	575 \pm 55	87	-5.87 \pm 0.99
2008	107	591 \pm 56	82	-5.29 \pm 0.79
2009	119	545 \pm 51	87	-5.55 \pm 0.89

Tree ring width

We collected microcores of each tree stem between 21 October and 21 November 2009. Trees were sampled at the base of the crown (approximate height 50 cm in *Larix* and 30 cm in *Pinus*), avoiding the (often) curved section at the base of the tree. A 2 mm-diameter increment puncher with a cutting length of 35 mm (TREP HOR, Università degli Studi di Padova, S. Vito di Cadore, Italy; Rossi *et al.* 2006) was used to extract two microcores, one from each side of the tree facing perpendicular to the slope. With this minimally invasive tool, we were able to collect at least one microcore from each tree that extended back to 1997, thus including four years before CO₂ enrichment started. The width of each tree ring was measured using an MS5 stereomicroscope at x40 magnification (Leica Microsystems GmbH, Wetzlar, Germany). Individual rings showing reaction wood (round, highly lignified cells throughout the ring) were excluded from the analysis because ring width tends to be inflated in these rings (1.2% of all measured rings). Three pines died during the study after becoming infected with the fungus *Gremmeniella abietina* in the early years of the experiment, reducing the experimental replication for this species to $n = 8$ for ambient and $n = 9$ for elevated CO₂ treatment.

Annual lateral shoot length increment

The terminal shoot on five mid-canopy lateral branches was measured after the first growing season of CO₂ enrichment (2001), along with the length of the terminal shoot produced in the preceding pre-treatment year (2000). In each of the four subsequent years (2002-2005), five lateral branches were again randomly selected and the terminal shoot was measured at the end of the growing season (Handa *et al.* 2005, 2006). The length of the terminal shoot and all new lateral shoots was measured on 10 to 15 tagged mid-canopy lateral branches in 2006 to 2009 to obtain a more representative mean length measurement, as trees had grown substantially during the experiment. The three pines that died during the study were removed from all years of the shoot length analysis because they were already visibly unhealthy in the early years of the experiment.

Tree size

We used multiple measurements from the final two years of the experiment to estimate the cumulative effects of several years of CO₂ enrichment on overall tree size and shoot production. (1) Total tree height was measured in autumn 2009, following the method used for the regular census of trees at Stillberg since they were planted in 1975 (Schönenberger and Frey 1988). (2) Percentage leaf canopy cover was estimated using hemispherical photographs taken in August 2008 during the seasonal peak of leaf area (Nikon Coolpix camera with a fisheye lens attachment; Memphis, TN, USA). One photo was taken in each plot, with the camera positioned approximately 20 cm down slope of the tree trunk and 20 cm above the ground. The tree canopy excluding the main trunk was isolated in the image, and the percentage of a standard image size covered by canopy was calculated using image processing software (ImageJ version 1.43k; Rasband 1997-2008). (3) The number and length of all new shoots on each tree was measured at the end of the season in 2008 and 2009. The resulting sum of all new shoots (averaged over the two years) represented gross annual shoot production per tree. (4) *Larix* stem diameter was measured in autumn 2009 (mean of 40 and 80 cm above ground) and used to calculate basal area at the end of the experiment. Many of the *Pinus* individuals branched close to the ground and did not have one main stem; consequently, stem basal area was not considered representative of tree size in this species, leaving canopy cover and shoot production as the best available proxies for cumulative CO₂ effects on above-ground growth.

Statistical analysis

Ring width and lateral shoot length were tested with Type I analysis of covariance, using repeated measures linear mixed effects models to incorporate data from all treatment years. We first fitted a full model: CO₂ treatment (ambient, elevated), tree species (*Larix*, *Pinus*), and their interaction were between-subject fixed factors; treatment year (categorical variable, 1 to 9) and all two- and three-way interactions with year were within-subject fixed factors. Mean shoot length measured in the pre-treatment year 2000

was included as a covariable in the shoot length model and the mean ring width of the four pre-treatment years 1997-2000 was used as a covariable in the ring width model, thus accounting for any differences between treatment groups that existed prior to the experiment in both species. All two-way interactions between the covariable and the three main effects were also tested.

Based on sub-models of each individual treatment year, we determined that the random effects associated with split-plot- and whole-plot-specific intercepts could be omitted for both ring width and shoot length models (West *et al.* 2007, Zuur *et al.* 2009). Consequently, we used repeated measures models that only included random effects for each individual tree. Full shoot length and ring width models both indicated heterogeneity of residual variance across the nine years, which we addressed by applying a heterogeneous residual variance structure. We accounted for violation of independence of residuals from different treatment years by applying a residual auto-correlation structure (auto-regressive model of order 1 (corAR1); Pinheiro *et al.* 2008). We then applied backward selection using maximum likelihood (ML) to remove any interactions between fixed factors that did not contribute significantly to the model fit. We refit the reduced final models using the restricted maximum likelihood (REML) estimation method (Zuur *et al.* 2009). The full models showed a strong tree species effect on both ring width and shoot length, and we completed repeated-measures tests for *Larix* and *Pinus* separately to compare the CO₂ effect in the two species. A strong year effect and marginally significant CO₂ x year interaction in *Larix* ring width prompted us to test the CO₂ effect on that species in individual treatment years.

The effect of elevated CO₂ on total tree height, leaf canopy cover, total shoot production, and basal area (*Larix* only), each measured during the final two years of the experiment, was tested separately for the two tree species using a statistical approach parallel to that used for ring width and shoot length. Tree height in 1995 was used as a covariable for testing the effect of elevated CO₂ on final tree height. Stem diameter and leaf canopy cover were not measured before the experiment started but total tree height, ring width and lateral shoot length all indicated that, on average, the vigour of *Larix* trees was very similar in the two treatment groups prior to CO₂ enrichment. We could thus confidently test CO₂ effects on *Larix* final basal area and leaf canopy cover without relating these measurements to pre-treatment values. *Pinus* mean tree height was also similar in the two CO₂ treatment groups prior to the experiment, but pre-treatment ring width and shoot length were somewhat greater in the elevated CO₂ group. The test of the CO₂ effect on the leaf canopy cover in *Pinus* was therefore interpreted with caution, as any differences between CO₂ treatment groups might have been at least partially due to pre-treatment differences in vigour.

A one-time defoliation event applied in June 2002 influenced the CO₂ effect to some extent in both species during 2002 and 2003 (Handa *et al.* 2005, 2006), and we included only undefoliated trees for tests of the CO₂ effect on shoot length and ring width for these two years (n = 5 for *Larix* and 4 for *Pinus*). The defoliation treatment had no influence on

the CO₂ effect in subsequent years, and we therefore included all trees. Further, a soil warming treatment was applied to half of the plots during the snow-free period in 2007 to 2009, in a crossed manner with both the CO₂ treatment and the previous defoliation treatment. Heating cables arranged on the ground surface of the plots increased the growing season mean soil temperature by 4 K (5 cm depth) and near-ground air temperature by 1 K (20 cm above ground), but the treatment had no effect on temperatures in the tree canopy (Hagedorn *et al.* 2010). Important distinctions between the warming treatment and naturally warm growing seasons were that air temperature in the tree canopy remained at control levels, the heated soil volume (area 1.1 m², depth < 20 cm) was smaller than tree rooting zones found previously at the Stillberg site (Bernoulli and Körner 1999), and snowmelt date was not altered. Analysis of variance tests for parameters measured in the last three years of the experiment revealed no significant interaction between CO₂ and soil warming treatments for either species. We therefore pooled warmed and unwarmed trees for the statistical analysis of the long-term CO₂ effects presented in this paper in order to maintain the replication used in 2004 to 2006 (n = 10 for *Larix*, n = 8 for ambient CO₂ *Pinus* and 9 for elevated CO₂ *Pinus*). Soil warming had a slight positive effect on *Pinus* (but no effect on *Larix*) growth in 2008 and 2009, which influenced the mean ring width and shoot length values in those years but did not alter the CO₂ effect.

We used ordinary least squares regression to determine if interannual differences observed in the magnitude of the CO₂ effect on *Larix* ring width (mean of all elevated CO₂ trees standardized to pre-treatment ring width / mean of all ambient CO₂ trees standardized to pre-treatment ring width) could be explained by climatic conditions in the current or preceding year. As *Pinus* ring width showed no significant response to elevated CO₂ in the multi-year analysis or in any individual year, an investigation of how climatic conditions influenced the CO₂ effect was not relevant for this species.

Assumptions of normality and homoscedasticity of the residuals in all final models were verified visually using diagnostic plots and, when necessary, response variables were log transformed to improve homoscedasticity. For all statistical tests, effects were considered significant at P < 0.05. Due to relatively low replication and therefore statistical power, we also designated P values ≥ 0.05 but < 0.10 as marginally significant. All analyses were performed using R version 2.8.1, and mixed models were run using the nlme package (Pinheiro *et al.* 2008, R Development Core Team 2008-2010).

Results

Tree ring width and elevated CO₂

Ring width in both tree species and both CO₂ treatment groups increased substantially over the 13 years measured in the stem microcores sampled in 2009 (Figs 1a and b), apparently an age-dependent pattern because no clear trends were visible in the climate

data (Figs 1c and d). Repeated measures analysis including both species and all years of the experiment showed an overall significant positive CO₂ effect on tree ring width ($F_{1,33} = 7.7$, $P = 0.009$; Fig. 1). Rings were wider in *Larix* than in *Pinus* ($F_{1,33} = 10.0$, $P = 0.003$), and ring width varied across the nine years of the experiment ($F_{8,244} = 15.5$, $P < 0.0001$). The pre-treatment covariable did not have a significant effect on ring width during the experiment ($F_{1,33} = 1.8$, $P = 0.193$), and none of the two- or three-way interactions between the main effects or between the main effects and the covariable contributed significantly to the model fit. Separate repeated measures analyses of the two tree species revealed that CO₂ treatment ($F_{1,17} = 4.4$, $P = 0.052$) and the pre-treatment covariable ($F_{1,17} = 4.3$, $P = 0.054$) had a marginally significant effect on *Larix* ring width (35% median stimulation under elevated CO₂, standardized to four years of pre-treatment growth; Fig. 1a). In contrast, elevated CO₂ did not have a significant effect on *Pinus* ring width ($F_{1,14} = 1.3$, $P = 0.268$) and pre-treatment ring width was not significant as a covariable ($F_{1,14} = 1.5$, $P = 0.242$; Fig. 1b). The interaction between the covariable and CO₂ treatment was not significant in models of either species, indicating that the strength of the relationship between ring width and pre-treatment growth did not change under elevated CO₂. Ring width varied significantly across treatment years in both *Larix* ($F_{8,120} = 21.3$, $P < 0.0001$) and *Pinus* ($F_{8,108} = 5.6$, $P < 0.0001$). The CO₂ x year interaction was marginally significant in *Larix* only ($F_{8,120} = 1.9$, $P = 0.068$), and analysis of individual years revealed that the CO₂ treatment effect in *Larix* was significant in years 2003, 2004, 2005 and 2007, marginally significant in 2006, and not significant in the first two or last two years of the experiment (see Tables 2 and 3 for ANCOVA results).

Lateral shoot length and elevated CO₂

Mean annual lateral shoot length in *Larix* showed a temporal pattern similar to ring width, with an overall increase over the experimental period and reduced growth in the final treatment year (Fig. 2a). *Pinus* lateral shoot length did not follow this pattern and instead decreased somewhat over the years (Fig. 2b). Lateral shoot length did not show a significant CO₂ effect when fit after the pre-treatment growth covariable in a Type I analysis of covariance model including both species ($F_{1,33} = 0.1$, $P = 0.813$; Fig. 2). Pre-treatment shoot length influenced growth in individual trees throughout the experiment in both species ($F_{1,33} = 221.9$, $P < 0.0001$) and showed a significant interaction with treatment year ($F_{8,234} = 7.4$, $P < 0.0001$). Treatment year and the treatment year x tree species interaction were both highly significant ($P < 0.0001$), whereas none of the other two- or three-way interactions contributed significantly to the model fit. There was a strong tree species effect ($F_{1,33} = 278.5$, $P < 0.0001$), with longer lateral shoots in *Larix* than in *Pinus*, but separate tests of the two species confirmed that CO₂ did not significantly affect lateral shoot length in either species (*Larix*: $F_{1,17} = 1.8$, $P = 0.194$; *Pinus*: $F_{1,14} = 1.0$, $P = 0.343$). Although not significant, there was a small but consistent trend of longer lateral shoots under elevated CO₂ relative to pre-treatment shoot length in *Larix* only (median stimulation of 11% over the nine years; Fig. 2). The interaction between CO₂ treatment and treatment year did not contribute significantly to the model fit for either species (see Table 2 for ANCOVA results).

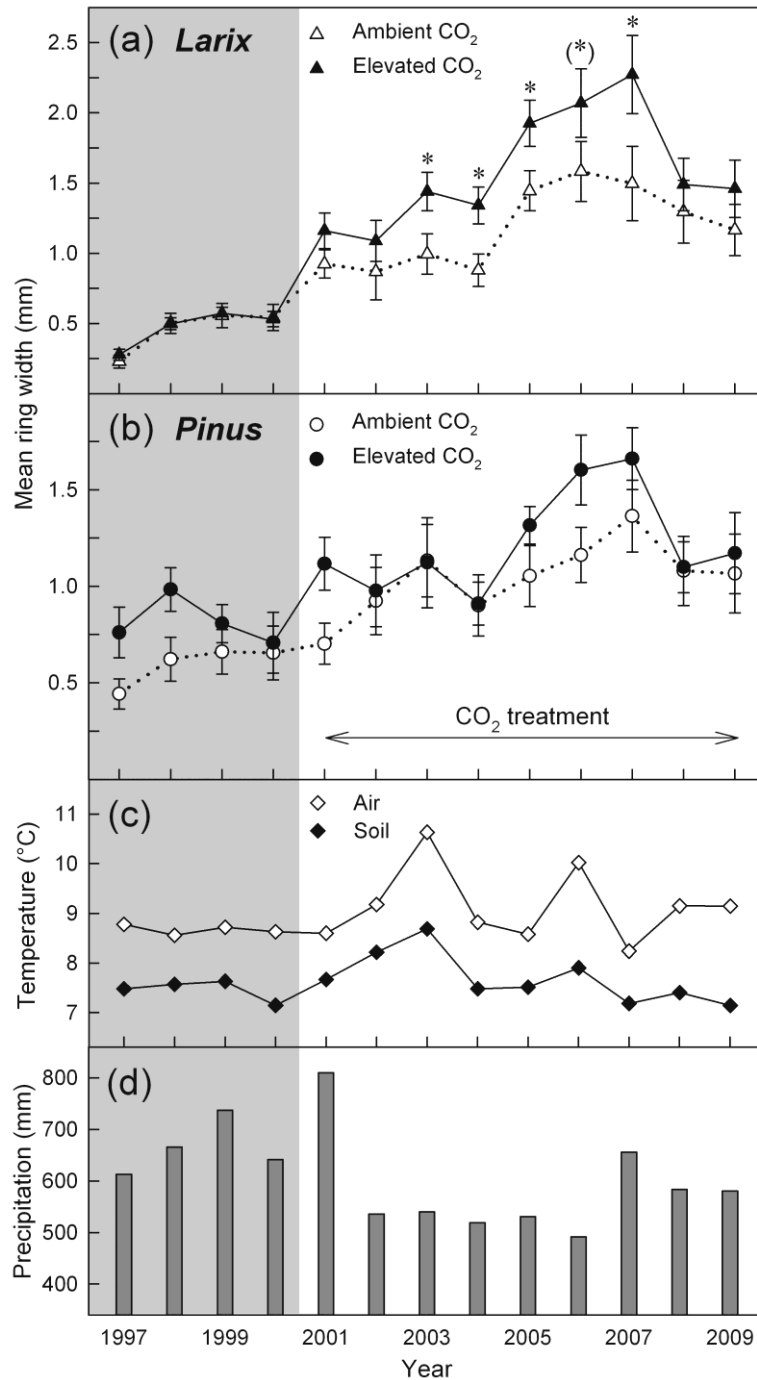


Figure 1. Mean ring width in *Larix* (a) and *Pinus* (b), ± 1 standard error (2002 and 2003: n = 5 for *Larix*, n = 4 for *Pinus*; all other years: n = 10 for *Larix*, n = 8 for ambient CO₂ *Pinus* and n = 9 for elevated CO₂ *Pinus*). Years 1997 to 2000 show pre-treatment differences in vigour, and the mean ring width of these years was used as a covariable in statistical tests. For *Larix*, * indicates a significant CO₂ effect (P < 0.05) and (*) indicates a marginally significant effect (0.05 ≤ P < 0.10). The bottom two panels show mean air and soil temperature (c) and cumulative precipitation (d) for each growing season.

Table 2. ANCOVA results for repeated measures tests of lateral shoot length and ring width

Summary of repeated measures linear mixed effects models for treatment effects on the growth parameters annual lateral shoot length increment and tree ring width. Models were fit for (a) both tree species, (b) *Larix decidua*, and (c) *Pinus uncinata* exposed to elevated CO₂ concentrations for nine years. Shoot length in 2000 and mean 1997-2000 ring width were used as pre-treatment covariables in statistical models for shoot length and ring width, respectively. Treatment year was included as a categorical variable (1 - 9). The response variable was log-transformed where necessary to further improve homoscedasticity (all models except *Pinus* ring width). Interactions that did not contribute significantly to the model fit were removed during the model selection process.

		Lateral shoot length increment			Ring width		
		DF	F	P	DF	F	P
<i>Full model</i>	Covariable	1, 33	221.9	<.0001	1, 33	1.8	0.193
	CO ₂	1, 33	0.1	0.813	1, 33	7.7	0.009
	Tree species	1, 33	278.5	<.0001	1, 33	10.0	0.003
	Year	8, 234	28.7	<.0001	8, 244	15.5	<.0001
	Tree species x Year	8, 234	14.5	<.0001	-	-	-
	Covariable x Year	8, 234	7.4	<.0001	-	-	-
<i>Larix</i>	Covariable	1, 17	9.0	0.008	1, 17	4.3	0.054
	CO ₂	1, 17	1.8	0.194	1, 17	4.4	0.052
	Year	8, 124	35.9	<.0001	1, 120	21.3	<.0001
	CO ₂ x Year	-	-	-	1, 120	1.9	0.068
	Covariable x Year	8, 124	2.3	0.025	-	-	-
	<i>Pinus</i>	Covariable	1, 14	20.4	0.0005	1, 14	1.5
CO ₂		1, 14	1.0	0.343	1, 14	1.3	0.268
Year		8, 102	20.4	<.0001	8, 108	5.6	<.0001
Covariable x Year		8, 102	2.7	0.010	-	-	-

Table 3. ANCOVA results for *Larix* ring width in individual years

Summary of analysis of covariance tests of the effect of elevated CO₂ on *Larix decidua* ring width in the nine individual treatment years. Models were fit with generalized least squares using the restricted maximum likelihood estimation method (REML). Mean 1997-2000 ring width was used as a covariable in statistical tests for each year. The response variable was log-transformed in all years to improve homoscedasticity. Trees given a one-time defoliation treatment shortly after snow melt in 2002 were excluded from the analysis in 2002 and 2003. In 2006 and 2007, one tree from each CO₂ treatment group was excluded because reaction wood inflated the ring width.

Year	DF	Covariable		CO ₂	
		F	P	F	P
2001	1, 17	2.61	0.124	1.34	0.263
2002	1, 7	1.51	0.258	1.22	0.305
2003	1, 7	3.11	0.121	6.86	0.035
2004	1, 17	5.09	0.037	7.51	0.014
2005	1, 17	5.12	0.037	4.93	0.040
2006	1, 15	8.04	0.013	3.81	0.070
2007	1, 15	8.84	0.010	6.82	0.020
2008	1, 17	5.80	0.028	1.17	0.294
2009	1, 17	3.37	0.084	1.48	0.240

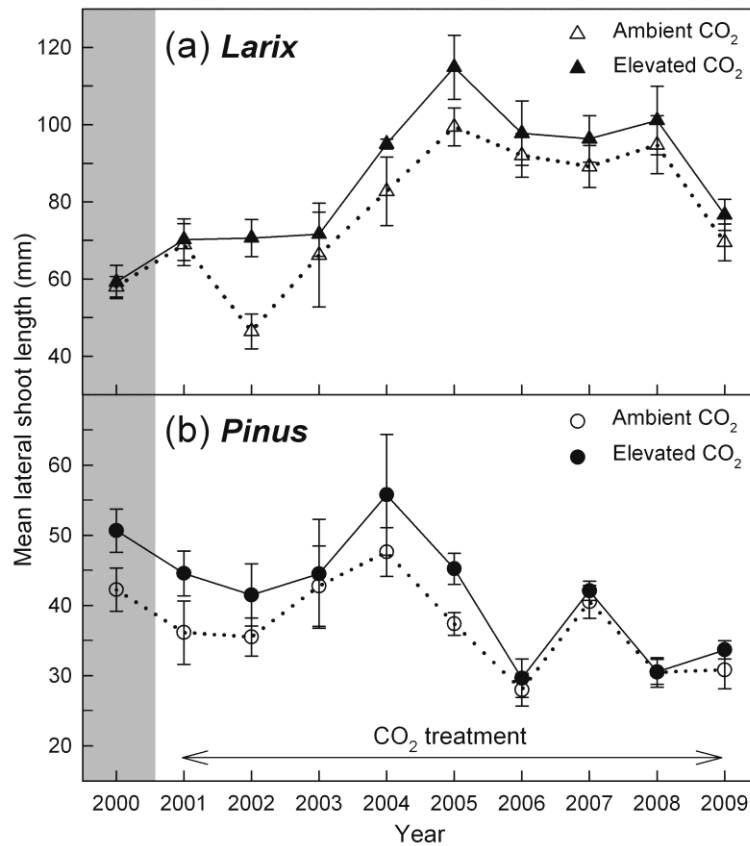


Figure 2. Mean annual growth of shoots on randomly selected mid-canopy lateral branches in *Larix* (a) and *Pinus* (b), ± 1 standard error (2002 and 2003: $n = 5$ for *Larix*, $n = 4$ for *Pinus*; all other years: $n = 10$ for *Larix*, $n = 8$ for ambient CO_2 *Pinus* and $n = 9$ for elevated CO_2 *Pinus*). Year 2000 shows pre-treatment differences in vigour and was included as a covariable in statistical tests.

Cumulative CO_2 effect on tree size after nine years of enrichment

Nine years of exposure to elevated CO_2 led to a cumulative effect of increased tree size and shoot production in *Larix* but no such response in *Pinus* (Fig. 3). In the final two years of the experiment, *Larix* trees growing under elevated CO_2 were not significantly different in height compared to trees growing under ambient conditions (2009; $F_{1,17} = 1.7$, $P = 0.216$; Fig. 3) but had a marginally significantly greater leaf canopy cover ($27 \pm 13\%$ difference in 2008; $F_{1,18} = 3.4$, $P = 0.082$; Fig. 3). The lack of response in tree height probably reflects damage to leader shoots from snow and animals that occurs frequently at the treeline and the resulting nonlinear canopy structure of many trees. There was no CO_2 effect on either tree height or leaf canopy cover in *Pinus* ($P > 0.80$; Fig. 3). In 2008 and 2009, *Larix* trees growing under elevated CO_2 produced on average $42 \pm 18\%$ more new shoots than trees experiencing ambient conditions ($F_{1,18} = 5.5$, $P = 0.031$), leading to a stimulation in the total length of all new shoots produced per tree of $62 \pm 25\%$ ($F_{1,18} = 5.1$, $P = 0.037$; Fig. 3). During these last two years of the experiment, *Pinus* did not produce a significantly different number of shoots ($F_{1,15} = 0.9$, $P = 0.361$) or total shoot length per tree ($F_{1,15} = 1.4$, $P = 0.257$) when grown under elevated CO_2 . The stem basal

area of *Larix*, calculated from 2009 diameter measurements, indicated a marginally significant stimulation of $49 \pm 19\%$ ($F_{1,18} = 4.2$, $P = 0.056$; Fig. 3), largely a result of the combined stimulation of radial stem growth in years three to seven of the nine treatment years (see ring width results above). Results of these statistical tests are summarized in Table 4.

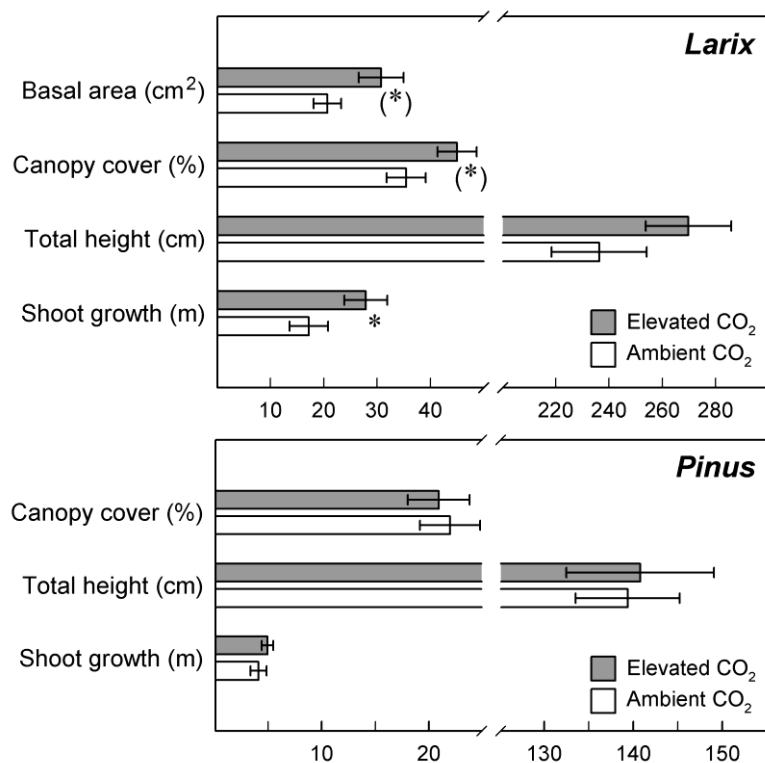


Figure 3. Summary of measurements of tree size at the end of the experiment. Mean values for ambient and elevated CO₂ treatment groups, ± 1 standard error ($n = 10$ in *Larix*, $n = 8$ in ambient CO₂ *Pinus* and $n = 9$ in elevated CO₂ *Pinus*). Basal area was calculated for *Larix* only, based on diameter measurements at approx. 50 cm above ground in autumn 2009; percent canopy cover was measured using hemispherical photographs during 2008; total tree height was measured along the main stem in autumn 2009; and shoot growth is the sum length of all new shoots at the end of the season, averaged over 2008 and 2009. * indicates a significant CO₂ effect ($P < 0.05$) and (*) indicates a marginally significant effect ($0.05 \leq P < 0.10$). Effects on *Larix* basal area were largely due to stimulation during years three to seven of the nine-year treatment.

Table 4. AN(C)OVA results for measurements of tree size and shoot production

Summary of analysis of (co)variance tests of the effect of elevated CO₂ on measurements of tree size and shoot production in the final two treatment years. Tests were completed separately for (a) *Larix decidua* and (b) *Pinus uncinata*. Tree height in autumn 1995 was used as a pre-treatment covariable in the test of total tree height. Log-transformation was applied to the total shoot growth response variable (sum length of all new shoots per tree).

		DF	CO ₂		Pre-treatment covariable	
			F	P	F	P
(a) <i>Larix</i>	Total height	1, 17	1.65	0.216	0.23	0.636
	Leaf canopy cover	1, 18	3.39	0.082		
	Shoot count	1, 18	5.47	0.031		
	Total shoot growth	1, 18	5.05	0.037		
	Basal area	1, 18	4.16	0.056		
(b) <i>Pinus</i>	Total height	1, 14	0.01	0.930	3.83	0.071
	Leaf canopy cover	1, 15	0.07	0.800		
	Shoot count	1, 15	0.89	0.361		
	Total shoot growth	1, 15	1.39	0.257		

Effects of climate on the magnitude of the CO₂ response

Growing season climatic conditions varied during the four-year pre-treatment period and the nine years of experimental CO₂ enrichment (Fig. 1c and d). Over these 13 years, the seasonal average of daily mean soil (10 cm depth) and air temperature ranged from 7.1 to 8.7°C and from 8.3 to 10.6°C, respectively (Fig. 1c). The widespread European heat wave in 2003 (e.g. Rebetez *et al.* 2006) resulted in the highest temperatures during the experimental period. The 2006 growing season was also one of the warmest, with particularly high temperatures during July (13.6°C mean daily air temperature). Cumulative precipitation during the snow-free season showed substantial interannual variation, with a range of approximately 300 mm, largely due to the exceptionally wet season in 2001 (Fig. 1d). Growing season solar radiation showed a strong negative correlation with precipitation, and only regressions relating ring width and precipitation are presented below (correlations between climate variables are presented in Table 5). Maximum snow depth varied from 107 to 197 cm during the experimental period, and snowmelt date ranged from 7 May to 6 June.

The magnitude of the CO₂ effect on *Larix* ring width in each year was influenced by some of the measured climate variables. The mean CO₂ effect was significantly greater in years with less accumulated snow in winter ($r^2 = 0.58$, $P = 0.018$). Similarly, the CO₂ effect was larger in years with an early snowmelt date (i.e. high June soil temperatures), but only when 2004 was excluded (unusually late snowmelt date despite a low to moderate snow pack; $r^2 = 0.70$, $P = 0.010$; Fig. 4a). The CO₂ effect was also larger when the preceding growing season was characterized by relatively high soil temperatures ($r^2 = 0.66$, $P = 0.008$; Fig. 4b), with a particularly strong relationship when the unusually high mean soil temperature in 2003 was excluded ($r^2 = 0.83$, $P = 0.002$; Fig. 4b). The effect of

air temperature during the same period was also statistically significant but weaker, possibly due to the slightly greater interannual fluctuation of this parameter (Table 5). When daily mean temperatures averaged over individual months were tested, soil and air temperature in July of the preceding year (soil: $r^2 = 0.71$, $P = 0.004$) and air temperature in November ($r^2 = 0.47$, $P = 0.042$) of the preceding year both had a positive influence on the CO_2 effect size. Cumulative precipitation during the growing season did not (negatively) co-vary significantly with temperature during the same period, and yet the magnitude of the *Larix* CO_2 effect was also positively influenced by low precipitation in the preceding year, but only when the exceptionally wet year 2001 was excluded ($r^2 = 0.74$, $P = 0.006$; see Table 5 for all regression results).

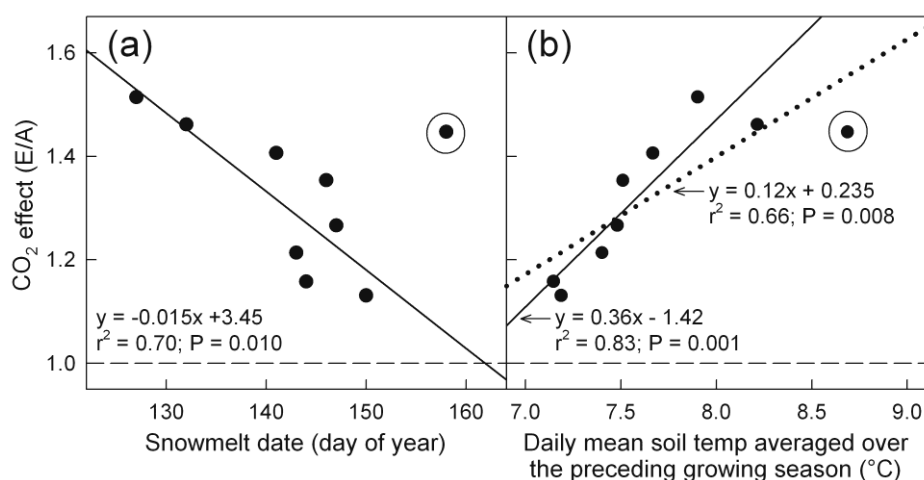


Figure 4. Relationship between the CO_2 effect size on *Larix* ring width in each year and (a) snowmelt date in the current year and (b) daily mean soil temperature (10 cm depth) averaged over the preceding growing season. The CO_2 effect was calculated as the ratio of the standardized means of all elevated CO_2 trees ($n = 10$) to the standardized means of all ambient CO_2 trees ($n = 10$). For each of the nine treatment years (represented by a single point), ring width in an individual tree was standardized to the mean 1997-2000 pre-treatment ring width. The linear regression equation, coefficient of determination (r^2), and error probability of the regression slope (P value) are shown for each relationship. Snowmelt date in 2004 was exceptionally late despite a low to moderate accumulation of snow, and this year was removed from the linear regression (circled point in Figure a). Regressions are shown both including (dotted line) and excluding (solid line) soil temperature during the exceptionally warm year 2003 (circled point in Figure b).

Table 5. Linear regressions testing the effect of climate variables on the CO₂ effect size for *Larix* ring width

(a) Summary of single linear regressions relating climate variables to the CO₂ effect size for *Larix* ring width. CO₂ effect size is defined as: mean of all elevated CO₂ trees relative to pre-treatment ring width / mean of all ambient CO₂ trees relative to pre-treatment ring width. * and (*) indicate significant ($P < 0.05$) and marginally significant ($0.05 \leq P < 0.10$) results, respectively. For regressions where air and soil temperature yielded similar and non-significant results, the variables have been combined on one line and values represent whichever temperature parameter yielded a higher R-squared value. Only regressions with air temperature are shown for months with snow cover when soil temperature was approximately 0°C (October - May). (b) Correlations between various climate variables used for the regressions in (a). *Abbreviations:* AT = mean daily air temperature, ST = mean daily soil temperature, GS = growing season, SR = daily mean solar radiation, precip = cumulative precipitation, prec. = during the preceding growing season, and excl. = excluding.

(a) Climate variables and the CO₂ effect size for Larix ring width

Climate variable	residDF	F	P	R-squared
Max snow height	7	9.6	0.018 *	0.58
Snowmelt date	7	1.7	0.239	0.19
Snowmelt date (excl. 2004)	6	13.8	0.010 *	0.70
June AT and ST (prec.)	7	0.5	0.489	0.07
July AT (prec.)	7	8.5	0.023 *	0.55
July ST (prec.)	7	17.5	0.004 *	0.71
August AT and ST (prec.)	7	0.2	0.638	0.03
September AT and ST (prec.)	7	1.2	0.318	0.14
October AT (prec.)	7	0.3	0.608	0.04
October AT (prec.; excl. 2003)	6	3.8	0.098 (*)	0.39
November AT (prec.)	7	6.2	0.042 *	0.47
December AT (prec.)	7	0.0	0.915	0.00
January - March AT	7	0.6	0.481	0.07
April AT	7	3.2	0.117	0.31
May AT	7	1.2	0.303	0.15
June AT and ST	7	2.5	0.155	0.27
June ST (excl. 2004)	6	7.3	0.036 *	0.55
July AT and ST	7	0.9	0.376	0.11
August AT and ST	7	0.0	0.830	0.01
September AT and ST	7	0.2	0.654	0.03
October AT	7	1.2	0.313	0.14
GS AT (prec.)	7	5.4	0.053 (*)	0.44
GS ST (prec.)	7	13.6	0.008 *	0.66
GS ST (prec.; excl. 2003)	6	28.9	0.002 *	0.83
GS SR (prec.)	7	6.2	0.042 *	0.47
GS precip (prec.; excl. 2001)	6	17.5	0.006 *	0.74

(b) Correlations between climate variables

Climate variable 1	Climate variable 2	R-squared
Max snow height	Snowmelt date	0.21
Max snow height	June ST	0.31
Snowmelt date	June ST	0.52
GS ST	GS SR	0.07
GS ST	GS precip (excl. 2001)	0.13
GS SR	GS precip (excl. 2001)	0.37

Discussion

Species-specific responses during nine years of CO₂ enrichment

Nine years of free air CO₂ enrichment at the treeline in the Swiss Alps led to an overall stimulation of above-ground growth in *Larix* but no significant growth response in *Pinus*. Tree ring width measurements confirmed the species-specific responsiveness to elevated CO₂ observed in an independent set of tree cores during the first four treatment years (Handa *et al.* 2006) and indicated that this pattern continued in the following five years. Our longer-term results for mean lateral shoot length were also consistent with earlier results (Handa *et al.* 2005, 2006), with a consistent but non-significant trend of longer shoots in *Larix* exposed to elevated CO₂ but not in *Pinus*. At the whole tree level, species-specific responses were apparent in leaf canopy cover, total new shoot growth and basal area, indicating a cumulative effect of increased tree size in *Larix* after nine years of exposure to elevated CO₂ but no effect in *Pinus*.

Our FACE study was unique in that it included two co-occurring tree species in a well-replicated experimental design, thus allowing us to conclude that the different responsiveness of *Larix* and *Pinus* to elevated CO₂ was related to species identity rather than site conditions. Most previous CO₂ enrichment experiments have included only one tree species, making it difficult to distinguish between species and site effects. A FACE experiment at the Swiss Canopy Crane experimental site included several deciduous species and similarly observed species-specific responses, but the ability to compare growth responses among species was limited by the low (or no) replication of individual species (Asshoff *et al.* 2006). In our study, different responsiveness for the two species could not be explained by the photosynthetic response, as both *Larix* and *Pinus* experienced strong photosynthetic stimulation in the first three treatment years (Handa *et al.* 2005) that persisted in the final year of CO₂ enrichment (K. Streit, unpublished data). Further, *Larix* and *Pinus* growing at our study site had a similar relative growth rate and *Pinus* actually had greater total biomass than *Larix* three years before the CO₂ experiment began (Bernoulli and Körner 1999). Therefore, the contrasting CO₂ responses in *Larix* and *Pinus* observed here cannot be explained by the previously proposed argument that faster growing species are more responsive to elevated CO₂ (Tangle 2001, Poorter and Navas 2003). Various differences between *Larix* and *Pinus* that might have contributed to their distinct CO₂ responses have been described in detail previously (Handa *et al.* 2005, 2006). Briefly, responsiveness in *Larix* but not *Pinus* might be explained by: deciduous leaves and the associated higher rate of assimilation return per unit carbon investment in *Larix* than in evergreen *Pinus*; and potentially larger sink capacity in *Larix*, due to production of long shoots with indeterminate growth, compared to *Pinus* which displays only determinate growth.

Previous research on the CO₂ responsiveness of non-seedling trees in cold environments has largely been conducted on a single species using growth chambers, and results from these experiments have varied widely. Long-term stimulation of radial

stem growth was observed in a six-year closed chamber study of young *Pinus sylvestris* (approximately 14 years old at the start of the experiment) growing in nitrogen-poor sandy soil in Finland (Kilpelainen *et al.* 2005). In contrast, a model ecosystem study simulating climatic conditions at higher montane elevations typically characterized by conifer tree species dominance revealed no stimulation of stem growth in *Picea abies* saplings over three years, regardless of nitrogen availability (Hättenschwiler *et al.* 1996). In a broader comparison, the observed growth stimulation of *Larix* is consistent with results for young, closed-canopy stands of *Pinus taeda* growing in a lowland temperate location (Duke Forest FACE), where basal area increment was enhanced during eight years of CO₂ enrichment (Moore *et al.* 2006). This growth response contributed to a sustained higher rate of standing biomass accumulation, with soil N availability largely explaining spatial variability in the magnitude of the stimulation in productivity (McCarthy *et al.* 2010). Woody biomass was also enhanced in a high-density coppice plantation including clones of three *Populus* species (POPFACE; Liberloo *et al.* 2006) and in a young *Populus tremuloides* plantation initiated with seedlings (Aspen FACE; Kubiske *et al.* 2006), both experiments with a high availability of non-carbon resources. In contrast to the above studies, initial enhancement of above-ground stem growth declined substantially after the first year of exposure to elevated CO₂ in a *Liquidambar styraciflua* forest, and extra carbon was instead allocated to leaf and fine root production (Oak Ridge FACE; Norby *et al.* 2002, Norby *et al.* 2004). Transient increases in stem growth were similarly observed in only one out of five deciduous species growing under elevated CO₂ in a mature temperate forest, and in that case no long-term growth stimulation was observed above or below ground (Asshoff *et al.* 2006, Bader *et al.* 2009).

Non-carbon resource availability and the long-term CO₂ response

It is difficult to compare the positive CO₂ effect on *Larix* growth with results from other studies because the treeline environment, and our study site in particular, does not easily fit into either an expanding or steady-state category of growth conditions (Körner 2006). The late successional, essentially undisturbed natural dwarf shrub heath provides steady-state consumption and recycling of resources below ground and an overall steady-state leaf area index of the understorey and tree canopy as a whole. On the other hand, trees are isolated and crown expansion is largely unconstrained with respect to light or space (Handa *et al.* 2005). Height and ring width measurements of *Larix* trees in our experiment, and more broadly throughout the same elevation zone of the Stillberg plantation, indicated an increasing growth rate in years leading up to and during the experiment (Fig. 1a; Rammig *et al.* 2005, Amos 2007). Similar age-related patterns of increasing radial growth were observed at treeline in 20-30 year-old *Larix decidua* and *Picea abies* in the Austrian Alps (Li *et al.* 2003) and for the first 50-70 years of growth in *Pinus cembra* in the Swiss Alps (Esper *et al.* 2008). Due to the expanding tree canopy, the cumulative CO₂ effect observed in *Larix* tree size after nine years of treatment could have resulted from stimulation during a few years that was carried over due to compound interest or from a continuous positive CO₂ effect on annual growth (Körner 2006). However, the cumulative CO₂ effect on above-ground growth (50% increase in stem

basal area after nine years) was small compared to the exponentially increasing effect on biomass (50 to >250% difference after only three to four years) observed previously for seedlings in unrestrictive growing conditions (Körner 2006). The substantially older age of trees at the start of our experiment undoubtedly played a role in the smaller magnitude of the long-term cumulative effect of CO₂ enrichment, but below-ground competition and low growth temperatures might have imposed additional constraints on the response.

The reduced stimulation of *Larix* ring width in the last two years of the experiment raises the question of whether CO₂-induced growth stimulation in *Larix* disturbed the current balance between growth and soil nutrient supply, thereby leading to a constrained long-term growth response due to nutrient limitation. Increased allocation to roots and higher fine root production are frequently observed responses to elevated CO₂ (Norby *et al.* 2004, Tingey *et al.* 2005) and are commonly interpreted as a sign of increased nutrient limitation, nitrogen in particular (Luo *et al.* 2004). However, trees in our study did not show enhanced fine root production in response to four years of exposure to elevated CO₂ (Handa *et al.* 2008), and bulk root samples from the end of the study also showed no indication of greater biomass allocation to roots (F. Hagedorn, unpublished data). Likewise, needles of trees exposed to elevated CO₂ had a somewhat enhanced C/N ratio in treatment years one to three (significant only in 2001; Hättenschwiler *et al.* 2002, Handa *et al.* 2005, Asshoff and Hättenschwiler 2006), but this response did not affect the C/N ratio of the organic layer or cause a decrease in extractable N in the soil in any year of the study (F. Hagedorn, unpublished data). Collectively, these observations suggest that the CO₂-induced growth stimulation in *Larix* was not large enough to cause a progressive reduction in soil N availability under elevated CO₂.

Climate influence on the CO₂ effect in Larix

The observed correlation between the general climatic conditions at our site and the CO₂ effect size in *Larix* ring width provides a possible explanation for the interannual variability in the CO₂ effect. Tree ring increments showed a stronger CO₂ response in years with a relatively small maximum snow depth and high early-season temperatures, which in combination led to an early snowmelt date. *Larix* radial stem growth starts in early to mid June at our site (M. Martin, unpublished dendrometer data), confirming previous observations of *Larix* at treeline in the Alps (Rossi *et al.* 2007, Moser *et al.* 2010). Therefore, it is possible that the extent to which trees were able to use extra carbon assimilated under elevated CO₂ for early-season growth depended on whether temperatures were high enough for those growth processes to be functional (sink limitation hypothesis). Dampening of CO₂-induced growth stimulation under lower temperatures was similarly observed over the short term in seedlings of several boreal species (Tjoelker *et al.* 1998). Temperatures in July, at peak growth activity, and averaged over the entire current growing season did not strongly influence the CO₂ effect, rather suggesting that low temperatures during the early growing season limited the radial stem growth response to elevated CO₂. The importance of early growing

season temperatures for radial stem growth of *Larix decidua* has been documented previously in a longer-term analysis at Stillberg (1975-2005; Amos 2007) and in dendrochronological studies conducted elsewhere in the Alps (Carrer and Urbinati 2004, Oberhuber *et al.* 2008). Substantially reduced snow volume and earlier spring snowmelt are predicted to occur with climate warming for elevations up to 3000 m in the Swiss Alps, irrespective of winter precipitation conditions (Beniston *et al.* 2003), and our results suggest that this change in climatic conditions could amplify the CO₂-induced growth stimulation of *Larix*. However, earlier snowmelt can expose trees to stochastic early season freezing events, and increased susceptibility to freezing damage observed for *Larix* growing under elevated CO₂ might offset any potential growth advantage over the longer term (Martin *et al.* 2010).

The CO₂ effect observed for *Larix* ring width was also greater in years following a growing season with high temperatures, high solar radiation and low cumulative precipitation. This growth response might have been due to increased photosynthetic CO₂ assimilation with warmer temperatures or to greater CO₂-induced stimulation of photosynthesis at higher temperatures, as theoretically predicted from the Farquhar model (Long 1991). However, it is well documented that rates of photosynthesis for trees growing at treeline are not lower than rates for the same species located at warmer lower elevation sites, suggesting that the photosynthetic rate in high elevation species is relatively insensitive to increases in temperature (Grace *et al.* 2002, Wieser *et al.* 2010). An alternative explanation is that the larger number of days of favourable climatic conditions for carbon uptake simply permitted a greater build-up of carbon reserves under elevated CO₂ that were available for growth in the next year, without a temperature-induced increase in the rate of photosynthesis. Irrespective of the mechanism, these conditions might be more frequent in the future, especially since climate warming in Switzerland has been over twice as large as the mean for the Northern hemisphere and summer temperatures have shown a particularly strong increase (Rebetez and Reinhard 2008). Our results therefore suggest that enhanced above-ground growth of *Larix* with rising CO₂ concentrations will be more pronounced in the future with ongoing climate change.

Conclusions

In this unique nine-year treeline FACE experiment where trees grew at their lower temperature limit, we observed no growth response to elevated CO₂ in *Pinus uncinata*. This result supports the hypothesis that carbon supply does not limit above-ground growth of this species at the high elevation treeline. In contrast, CO₂-induced growth stimulation in *Larix decidua* was sustained through at least the seventh treatment year, indicating that this species benefited from extra carbon despite low growth temperatures. The CO₂ effect on ring width was enhanced under climatic conditions in the current and preceding year known from the literature to positively influence radial stem growth in general. The different responsiveness of these two co-occurring species suggests that under future CO₂ concentrations, especially in combination with warmer and sunnier

growth conditions, *Larix* will have a competitive advantage over less responsive species such as *Pinus*. Consequently, shifts in abundance of these treeline species might occur over the long term, with implications for the associated dwarf shrub heath and the structure of the treeline ecotone.

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Chapter 3.

Growth and community responses of alpine dwarf shrubs to CO₂ enrichment and soil warming

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Abstract

(1) Rising CO₂ concentrations and the associated global warming are expected to have strong impacts on high latitude and high elevation ecosystems, yet long-term multi-factor experiments in these environments are rare.

(2) We investigated how growth and leaf traits of dominant dwarf shrub species (*Vaccinium myrtillus*, *Vaccinium gaultherioides* and *Empetrum hermaphroditum*) and community composition in the understorey of larch and pine trees responded to long-term *in situ* CO₂ enrichment and soil warming at treeline in the Swiss Alps.

(3) *Vaccinium myrtillus* growth was stimulated by elevated CO₂, with no decline over time in the annual shoot growth response, and to an even greater extent by soil warming. *Vaccinium gaultherioides* growth showed a slight positive effect of elevated CO₂, though only in experimental plots with pine, and no response to warming. *Empetrum hermaphroditum* growth was not influenced by either treatment. Vascular plant species richness decreased in elevated CO₂ plots with larch but not with pine, while the number of moss and lichen species decreased under soil warming.

(4) Species-specific dwarf shrub growth responses to environmental changes indicate a competitive advantage of a responsive species, e.g. *V. myrtillus*, over unresponsive species, which can lead to shifts in species composition at the alpine treeline.

Introduction

The current atmospheric CO₂ concentration is higher than it has ever been during the last 25 million years (Pearson and Palmer 2000), and models using coupled carbon-climate cycle simulations predict CO₂ levels to reach between 730 and 1,020 μmol mol⁻¹ by the year 2100 (Meehl *et al.* 2007). As one consequence of rising CO₂ levels, the global mean surface air temperature has increased in recent years and is predicted to rise by 1.8-4.0 K by the year 2100 (Meehl *et al.* 2007). Elevated CO₂ concentrations might enhance carbon uptake by plants through a direct stimulation of photosynthesis, and responses might be particularly strong at high elevation where atmospheric pressure, and therefore CO₂ partial pressure, is lower (Körner 2003). Similarly, the ongoing global warming is already documented to have particularly strong ecological impacts on high latitude and high elevation regions where plants grow close to their low temperature limit (Theurillat and Guisan 2001, Walther 2003, Dorrepaal *et al.* 2009). For these reasons, research on environmental change in alpine and arctic ecosystems has increased substantially in recent years, both with experiments manipulating atmospheric and climate variables (see review by Dormann and Woodin 2002) and with observations of natural vegetation change (e.g. Epstein *et al.* 2004, Wilson and Nilsson 2009).

Elevated CO₂ concentrations have been experimentally applied to high latitude and high elevation vegetation in only a few studies. Field experiments in a late successional alpine grassland in the Swiss Central Alps (Körner *et al.* 1997), in a forest heath ecosystem in subarctic Sweden (Gwynn-Jones *et al.* 1997) and in the wet tussock arctic tundra in

Alaska (Tissue and Oechel 1987) indicate that responses of plant growth and biomass to rising CO₂ concentrations are generally small or non-existent but that co-occurring species often vary in their response (Dormann and Woodin 2002). Longer-term CO₂ enrichment studies of various plant types in diverse environments have revealed that other variables, such as climatic conditions and nutrient availability, can influence the CO₂ effect (Körner 2006, McCarthy *et al.* 2010). In some studies, for example, N limitation has been found to constrain the long-term CO₂ growth response, through increased N immobilization in plant biomass and long-lived soil organic matter and through changes in microbial activity (progressive nitrogen limitation; Luo *et al.* 2004).

In contrast to the small number of CO₂ manipulation experiments, many relatively long-term warming experiments have been completed in (sub)arctic (Chapin and Shaver 1985, Parsons *et al.* 1994, Shevtsova *et al.* 1997, Aerts *et al.* 2009) and alpine (Harte and Shaw 1995, Kudo and Suzuki 2003, Kudernatsch *et al.* 2008) ecosystems. Increased plant growth and biomass production with warming were observed in several experiments, although responses were often small or transient and varied across species and study sites (Rustad *et al.* 2001, Walker *et al.* 2006). Many high latitude and high elevation environments are characterized by low soil nutrient availability due to low temperature constraints on decomposition and mineralization (Nadelhoffer *et al.* 1992, Körner 2003), and higher soil temperatures tend to accelerate these processes in systems that are not water limited (Rustad *et al.* 2001, Cornelissen *et al.* 2007, Kammer *et al.* 2009). Therefore, plant growth responses to warming might be caused by enhanced nutrient availability in addition to a direct effect of increased rates of photosynthesis at higher temperatures. Similar to experimental CO₂ enrichment, warming experiments lasting several years are needed for understanding responses at the plant community and ecosystem scale, especially since changes in resource availability, shifts in competitive interactions, and plant-soil feedback processes can alter responses over time (Wookey *et al.* 2009).

Despite clear predictions that rising CO₂ levels will be accompanied by increased temperatures and other changes in climate, relatively few multi-factor experiments have been conducted within intact systems (Beier 2004). A modelling study including several ecosystem types predicted that warming will reinforce positive effects of elevated CO₂ on net primary productivity (Luo *et al.* 2008). However, existing field studies of natural vegetation combining elevated CO₂ and warming have been conducted almost entirely in grassland systems (but see Andresen *et al.* 2010) and have yielded conflicting results regarding how the combined changes influence plant productivity and nitrogen cycling (Shaw *et al.* 2002, Hovenden *et al.* 2008, Engel *et al.* 2009, Dijkstra *et al.* 2010). While experiments combining CO₂ enrichment and nutrient addition have been conducted in alpine environments (Körner *et al.* 1997; N. Inauen *et al.*, unpublished data), to our knowledge no previous *in situ* studies of high elevation or high latitude systems have simultaneously manipulated CO₂ concentration and temperature.

Dwarf shrubs are a major component of arctic and alpine plant communities, and shifts in growth and reproductive output, abundance and/or distribution of these species under environmental change are likely to have important ecological consequences. In this study, we observed how nine years of free air CO₂ enrichment (FACE) and three years of soil warming affected three dominant ericaceous dwarf shrub species, deciduous *Vaccinium myrtillus* (bilberry) and *Vaccinium gaultherioides* (northern bilberry) and evergreen *Empetrum hermaphroditum* (crowberry), growing in the understory of treeline trees in the Swiss Alps. We hypothesized that 1) these co-occurring dwarf shrubs would vary in their above-ground growth response to long-term CO₂ enrichment, and any initial stimulation would decline over time due to increasing nutrient limitation; 2) soil warming would lead to enhanced dwarf shrub growth due to direct stimulation and/or increased soil nutrient availability; 3) soil warming would alleviate non-carbon constraints on the growth response to elevated CO₂, yielding the largest response when the two experimental treatments were applied together; and 4) species-specific responses of understory vegetation to the experimental treatments would lead to shifts in species composition and richness in the experimental plots.

Materials and Methods

Site and experimental design

The study site is located at Stillberg, Davos in the Central Alps, Switzerland (9° 52' E, 46° 46' N). The CO₂ enrichment and soil warming experiment covers an area of 2500 m² and is situated on a NE-exposed 25 to 30° slope at 2180 m a.s.l., corresponding to or slightly above the natural climatic treeline (Hättenschwiler *et al.* 2002). The site is located within a 5 ha long-term afforestation research area where tree seedlings were planted into the intact dwarf shrub community in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). During the experimental period from 2001-2009, the mean annual precipitation was 1175 mm and the mean annual air temperature was 2.5°C, with February (mean -5.2°C) as the coldest month and July (mean 10.5°C) as the warmest month (WSL climate station at 2090 m a.s.l.).

The experiment consists of 40 hexagonal 1.1 m² plots, 20 with a *Pinus mugo* ssp. *uncinata* (DC.) Domin (mountain pine) individual in the centre and 20 with a *Larix decidua* Mill. (European larch) individual in the centre. These trees are now 39 (pine) and 37 (larch) years old but are ≤ 3.5 m tall and have a stem basal diameter of < 10 cm. The trees are sparsely distributed and do not form a closed canopy; thus, each plot contains a single tree surrounded by a dense cover of understory vegetation consisting of the dominant dwarf shrub species *Vaccinium myrtillus* L., *Vaccinium gaultherioides* Bigelow (group *V. uliginosum* agg.) and *Empetrum nigrum* ssp. *hermaphroditum* (Hagerup) Böcher, plus several herbaceous (e.g. *Avenella flexuosa*, *Gentiana punctata*, *Homogyne alpina*, *Leontodon helveticus*, *Melampyrum pratense*) and non-vascular species. For the

dwarf shrub species targeted in this study, regeneration within the existing heath zone at treeline occurs mainly by vegetative spread (Körner 2003).

The long-term CO₂ enrichment experiment started in early June 2001, at which point the 40 plots were assigned to 10 groups of four neighbouring plots (two larch and two pine trees per group) in order to facilitate the logistics of CO₂ distribution and regulation. Half of these groups were randomly assigned to an elevated CO₂ treatment ($575 \pm 52 \mu\text{mol mol}^{-1}$ mean concentration ± 1 standard deviation from 2001-2009) while the remaining groups served as controls and received no additional CO₂ (c. $380 \mu\text{mol mol}^{-1}$). During daytime hours only, the CO₂ supply system released pure CO₂ gas through laser-punched drip irrigation tubes hung vertically around a hexagonal frame surrounding each plot. Interruptions in CO₂ release due to adverse weather conditions (photon-flux density $< 1000 \text{ mmol m}^{-2} \text{ s}^{-1}$, wind speed $> 75 \text{ km h}^{-1}$, air temperature $< 5^\circ\text{C}$, snow or sleet) and technical failure meant that plants received supplementary CO₂ for 73-87% of the seasonal daytime-only treatment periods. The setup and performance of the CO₂ enrichment facility have been described in detail previously (Hättenschwiler *et al.* 2002, Handa *et al.* 2006, Chapter 2 of this thesis).

In spring 2007, one plot of each tree species identity was randomly selected from each of the 10 CO₂ treatment groups and assigned a soil warming treatment, yielding a balanced split-split plot design with a replication of five individual plots for each combination of CO₂ level, warming treatment and tree species. Soil warming was applied during the 2007-2009 growing seasons using heating cables laid on the ground surface underneath the dwarf shrub layer (Hagedorn *et al.* 2010). Heating was turned on immediately after snowmelt and turned off just before the site was covered in snow for the winter, thereby avoiding an interaction between soil temperature and snow cover duration. Warming increased the growing season mean soil temperatures at 5 cm depth by $3.9 \pm 0.3 \text{ K}$ in 2007, $4.4 \pm 0.5 \text{ K}$ in 2008 and $3.1 \pm 0.4 \text{ K}$ in 2009 (mean ± 1 standard error, $n = 10$). Air temperature was increased up to 20 cm above ground ($0.9 \pm 0.1 \text{ K}$) but no temperature difference was detected at 50 cm height (Hagedorn *et al.* 2010). Warming also had a slight drying effect that was most pronounced in late summer of each year. Averaged over the growing season, volumetric soil water content (0-10 cm depth) was reduced by approximately 15% and air humidity (10 cm height) was reduced by approximately 10%, irrespective of CO₂ treatment and tree species identity (Hagedorn *et al.* 2010). See Fig. A1 in the Appendix for details about how the warming treatment influenced soil temperature and moisture.

Plant growth and plot species composition

During autumn of 2002-2009, after seasonal shoot elongation of the dwarf shrubs was complete, we measured the length of the new shoot increment (distance from bud scales to shoot tip) on the longest ramet branch of five to seven randomly selected ramets in each plot. These specific shoots were selected because they experience less self-shading than shoots closer to the ground and are therefore less likely to have reduced

shoot growth due to competition for light among shoots within an individual ramet. Further, a study of dwarf shrub biomass allocation conducted outside our experimental plots indicated that this measurement is highly correlated with gross annual above-ground biomass production on a given ramet for our three study species (S. Wipf *et al.*, unpublished data). Shoots produced in 2002 were only measured in half of the plots, with a replication of five for each combination of CO₂ treatment and tree species. In all years, ramets located within 10 cm of the plot border were not measured in order to avoid potential edge effects.

We additionally measured the overall understorey vegetation height (mean of three random point measurements) and the maximum ramet height of *V. myrtillus*, *V. gaultherioides* and *E. hermaphroditum* in each plot during late summer of 2008 and 2009. These measurements represent the cumulative treatment effect on vegetation height over all study years up to the sample date and are positively correlated with total above-ground biomass per unit area (understorey height) and per ramet (maximum height of individual species) at our research site (S. Wipf *et al.*, unpublished data). During the peak of the growing season in 2005 and 2009, we documented all vascular and non-vascular (moss and lichen) species present in each plot for calculating species richness.

Leaf traits

During early August 2006-2009, approximately 50 leaves of *V. myrtillus* and of *V. gaultherioides* were harvested from each plot to measure leaf traits. *E. hermaphroditum* leaves were not sampled because individual leaf area and mass could not be measured accurately for the small, folded leaves. Leaves were collected from the whole canopy, with samples evenly distributed across the distinct ramets present throughout a given plot but again avoiding ramets near the plot edges. Leaves were scanned within 12 hours of harvesting and mean leaf area was calculated using the image processing software ImageJ version 1.43k (measurements not available from 2008; Rasband 1997-2008). Any missing leaf parts were filled to correct for losses caused by herbivory (<4% of potential leaf area). Leaves were then dried at 60°C for at least 24 hours and weighed to obtain the average dry leaf mass and specific leaf area (SLA) per plot for each species. Dried leaves were subsequently ground, as one bulk sample per plot for each species, and N concentration was measured using a C-N analyzer (EA-1110, Carlo Erba, Milan, Italy). N concentration was expressed both on a leaf dry mass basis (mg N per g dry mass) and on a leaf area basis (g N per m² leaf area). Uncorrected leaf area was used for calculating both SLA and leaf N per unit area.

Statistical analysis

Treatment effects on annual shoot growth (2002-2009), total ramet height (2008-2009) and leaf traits (2006-2009) were tested for each dwarf shrub species separately with Type I analysis of (co)variance, using repeated measures linear mixed effects models fit

with the restricted maximum likelihood estimation method (REML). To test the long term CO₂ effect, we used a model including CO₂ level (ambient, elevated), plot tree species identity (larch, pine) and their interaction as between-subject fixed factors, and treatment year (categorical variable) and all two- and three-way interactions with year as within-subject fixed factors. For the three years when both CO₂ enrichment and soil warming were applied (2007-2009), likelihood ratio tests indicated that none of the interactions between the two treatments contributed significantly to model fits for any of the parameters measured (see Appendix Table A1). Therefore, we pooled warmed and unwarmed plots for tests of the long-term CO₂ effect (n = 10).

We used a similar statistical approach to test the effect of soil warming during 2007-2009, with temperature treatment (unwarmed, warmed) replacing CO₂ treatment in the models (n = 10). Measurements averaged over 2005 and 2006, the two years leading up to the warming experiment, were included as a covariable in the shoot growth models and measurements from 2006 were included as a covariable in the various leaf trait models. We tested covariable x year and covariable x temperature treatment interactions, but these terms did not contribute significantly to the fit of any model and were therefore excluded. To interpret significant treatment x year interactions in some repeated measures models involving leaf traits, we additionally completed tests for individual years (2006-2009). We did not test the effects of soil warming on total ramet height because no pre-warming measurements were available. We tested treatment effects on species richness measured in 2009, using values from 2005 as a covariable even though some CO₂ effects might have occurred before that point.

For shoot growth models, we accounted for violation of independence of residuals from different treatment years by applying a residual auto-correlation structure (auto-regressive model of order 1 (corAR1); Pinheiro *et al.* 2008). Sub-models of each individual treatment year indicated that the random effects associated with split-plot- and whole-plot-specific intercepts could be omitted, leaving repeated measures models that only included random effects for each individual plot (Zuur *et al.* 2009). Models assuming a normal error distribution were used for all of the above parameters except for non-vascular plant species richness, where a Poisson distribution provided a better fit (Venables and Ripley 2002). For all models, assumptions of normality and homoscedasticity of the residuals were verified visually using diagnostic plots and, when necessary, response variables were log transformed to improve homoscedasticity. For all statistical tests, effects were considered significant at $P < 0.05$. Due to relatively low replication and therefore statistical power, we designated P values ≥ 0.05 but < 0.10 as marginally significant. All analyses were performed using R version 2.11.1, and mixed models were fitted using the nlme package (Pinheiro *et al.* 2008, R Development Core Team 2008-2010).

Shading can influence the growth and survival of understorey plants. We took hemispherical photographs in each plot during the seasonal peak of leaf area in 2008 to quantify the percentage of sky obstructed by the tree trunk and canopy, trees in the area

surrounding the plot, and topographic elements (see Appendix Fig. A2 for details). The amount of shade experienced by understorey plants was generally slightly greater for plots with larch (median 81%) than with pine (median 70%). Additionally, stimulation of larch growth under elevated CO₂ meant that tree canopy cover tended to be greatest for elevated CO₂ plots containing a larch tree (see Chapter 2 of this thesis). For analyses in this paper, the potential influence of shading on understorey plant growth and leaf traits was considered by including this measurement, hereafter referred to as “canopy shading”, as a covariable in statistical models where it significantly influenced the response variable. Interactions between canopy shading and the treatments were also tested but were not included in the final models because they were not significant and did not qualitatively change the other results. For tests of soil warming effects, pre-treatment measurements of the response variable were stronger than canopy shading as a covariable and accounted for overall variability in growing conditions.

Results

Annual shoot growth and total ramet height

Repeated measures tests of the CO₂ effect on annual shoot growth during eight years of treatment yielded a significant stimulation of 12% in *V. myrtillus* ($F_{1,36} = 7.5$, $P = 0.01$), averaged across tree species identities, temperature treatments and years (Fig. 1a). This stimulation was relatively consistent throughout the eight years ($P > 0.9$ for CO₂ x year interaction), with no indication of a decline in the signal over time. Plot tree species identity also significantly influenced *V. myrtillus* shoot growth, with longer annual shoot increments in plots with larch than with pine ($F_{1,36} = 4.6$, $P = 0.04$). For *V. gaultherioides*, the interaction between CO₂ treatment and tree species identity ($F_{1,36} = 5.4$, $P = 0.03$) was significant, with a negative response to elevated CO₂ in plots with larch and a positive response in plots with pine (Fig. 1b). *E. hermaphroditum* did not show a significant response to CO₂ enrichment or tree species identity ($P > 0.65$; Fig. 1c). Annual shoot growth of all three dwarf shrub species varied significantly among individual treatment years ($P < 0.01$) but did not show a clear trend over time. When included as a covariable in models of the individual years 2007-2009, canopy shading as measured in 2008 did not significantly influence shoot growth of any species (data not shown). Further, a harvested sub-sample of annual shoot increments from three age classes demonstrated that, for all three species, longer shoot increments had greater biomass and the length to mass ratio was not significantly influenced by the degree of canopy shading (see Appendix Fig. A3 for details).

Soil warming had a positive effect on *V. myrtillus* shoot growth ($F_{1,35} = 11.8$, $P < 0.01$; Fig. 2a). Relative to the two years preceding warming, mean annual shoot increment length was 31% greater in warmed plots than in unwarmed plots (averaged across tree species identities, CO₂ concentrations and years). Soil warming did not significantly affect shoot growth of *V. gaultherioides* or *E. hermaphroditum*, although the latter

species showed a non-significant trend of stimulation in 2009 (Fig. 2b and c). Shoot increment length measured in 2005 and 2006 influenced growth of each species during the three seasons of soil warming ($P < 0.03$), and the strength of this relationship did not vary significantly with year or temperature treatment. No interactions between temperature treatment, tree species and year were significant for any of the dwarf shrub species (see Appendix Table A2 for ANOVA results).

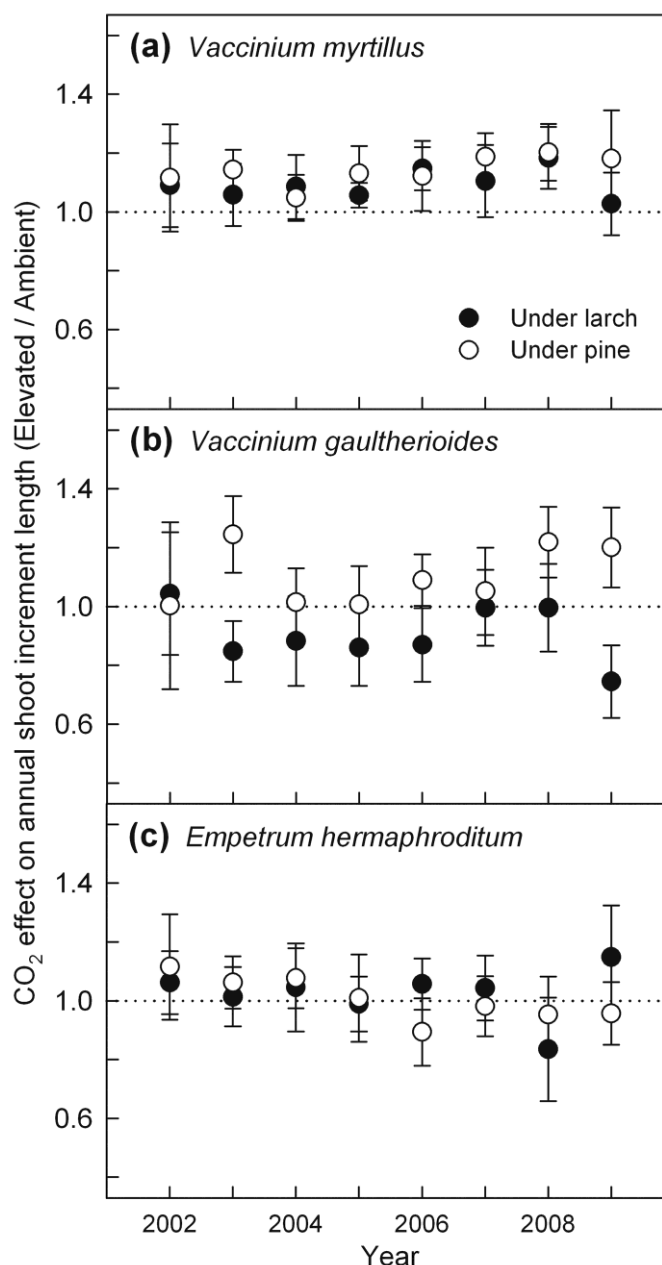


Figure 1. CO₂ effect on the length of annual shoot increments during eight years of enrichment. The CO₂ effect was calculated as the ratio of the mean shoot length of all elevated CO₂ plots to the mean shoot length of all ambient CO₂ plots ($n = 5$ for 2002 and $n = 8-10$ for all other years). Error bars represent ± 1 standard error of the ratio, calculated using rules of error propagation (Kirkup 1994). Plots are separated by tree species identity (open circles = under pine, filled circles = under larch), and means are pooled across warmed and unwarmed plots for 2007-2009.

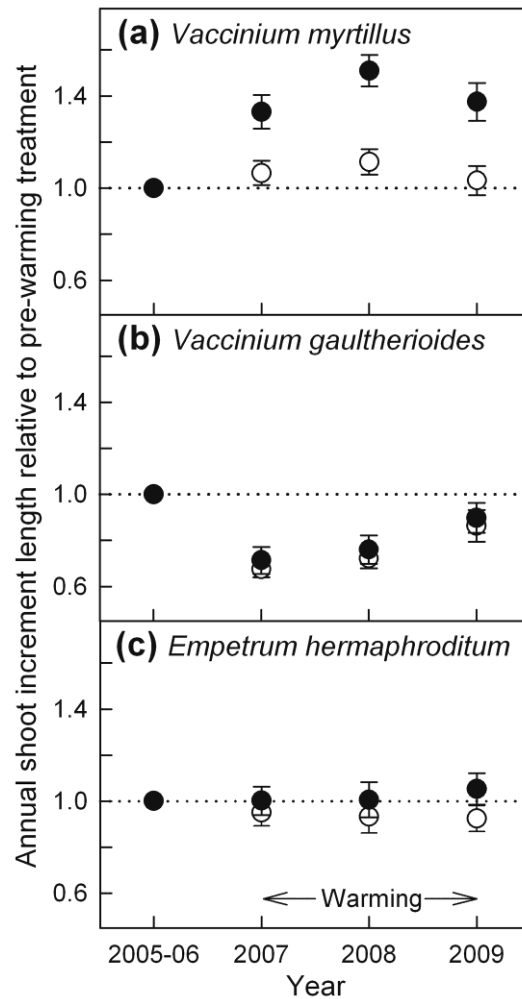


Figure 2. Soil warming effect on the length of annual shoot increments in 2007-2009 (open circles = unwarmed, filled circles = warmed). For each individual experimental plot, average shoot length in a given year was standardized to shoot length averaged over 2005 and 2006, the two years preceding warming treatment. Mean standardized values \pm 1 standard error are shown for each warming treatment group, pooled across plot tree species identities and CO₂ treatment groups ($n = 20$ for *Vaccinium myrtillus* and *Vaccinium gaultherioides*; $n = 15-19$ for *Empetrum hermaphroditum*).

Average understorey vegetation height (measured in 2008 and 2009) was highly correlated with the maximum ramet height of *V. myrtillus*, generally the most abundant species in the plots. Both parameters were significantly enhanced in plots exposed to several years of CO₂ enrichment (understorey height: $F_{1,36} = 9.8$, $P < 0.01$; *V. myrtillus* ramet height: $F_{1,36} = 7.1$, $P = 0.01$; Fig. 3a and b), irrespective of the amount of canopy shading and of tree species identity. *V. gaultherioides* ramet height was not influenced by canopy shading but showed a marginally significant CO₂ x tree species interaction, with a negative CO₂ effect in plots with larch but a positive effect in plots with pine ($F_{1,36} = 4.1$, $P = 0.05$). Additionally, ramet height was lower overall in plots with larch than with pine ($F_{1,36} = 4.3$, $P = 0.05$; Fig. 3c). Maximum ramet height of *E. hermaphroditum* showed a marginally significant decrease with increasing canopy shading ($F_{1,31} = 3.1$,

$P = 0.09$), which partially explained a trend of taller ramets in plots with pine than with larch, but showed no response to elevated CO_2 (Fig. 3d). Overall, these results are consistent with patterns observed for shoot increment length during the eight years of CO_2 enrichment, and they demonstrate that sustained responses in shoot growth yielded cumulative effects at the plot level (see Appendix Table A2 for all ANOVA results except for understorey height).

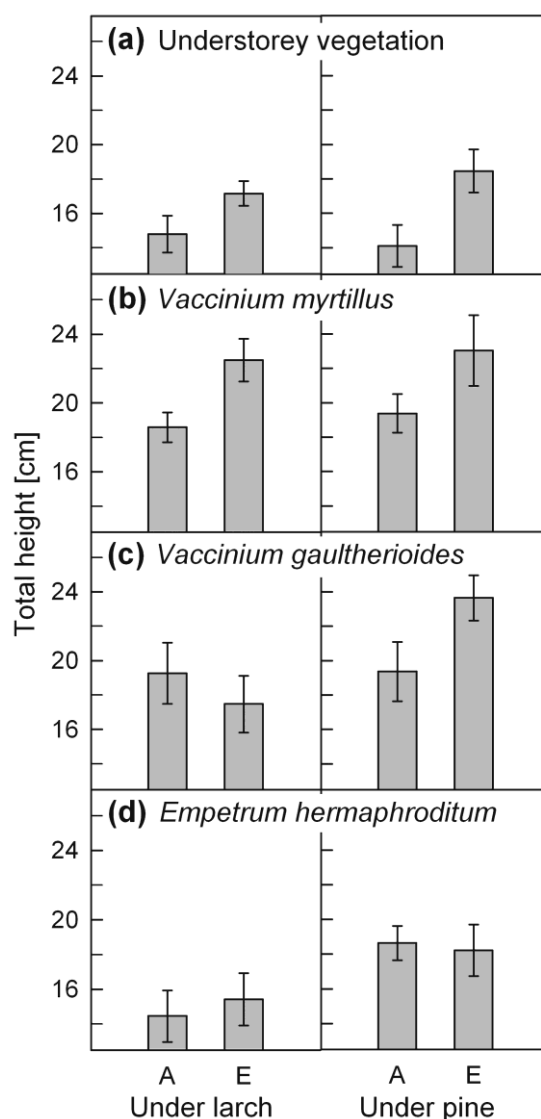


Figure 3. CO_2 effect on the total height of understorey vegetation overall and on the total ramet height of individual dwarf shrub species. Measurements for each plot from 2008 and 2009 were averaged for calculating values shown here. Left and right panels show averages for plots shared with larch and pine, respectively, and values were pooled across warmed and unwarmed plots. (a) Mean plot understorey height for each of the two CO_2 treatment groups, ± 1 standard error (A = ambient CO_2 and E = elevated CO_2 ; $n = 10$). (b-d) Maximum ramet height of each dwarf shrub species, averaged over each of the two CO_2 treatment groups, ± 1 standard error ($n = 10$ for *Vaccinium myrtillus* and *Vaccinium gaultherioides*; $n = 8-10$ for *Empetrum hermaphroditum*).

Leaf morphology and leaf nitrogen concentration

V. myrtillus consistently had a higher SLA than *V. gaultherioides* and SLA of both species increased with increasing canopy shading, meaning that SLA was generally greater in plots with larch than with pine (Table 1, mean values for individual years are shown in Appendix Table A3). SLA of both species was unaffected by CO₂ enrichment ($P > 0.70$). *V. myrtillus* leaf area but not mass increased with increasing canopy shading ($P < 0.01$). Accounting for this shading effect on leaf morphology, *V. myrtillus* dry mass and area per individual leaf showed a marginally significant positive response to elevated CO₂ ($P < 0.08$; Table 1). Thus, CO₂ enrichment led to increased *V. myrtillus* leaf size (mass and area) without significantly altering the area to mass ratio. In contrast, neither leaf mass nor area of *V. gaultherioides* responded significantly to the amount of shading or to CO₂ enrichment (Table 1b). Under soil warming, *V. myrtillus* SLA responded in 2007 only, with a decrease relative to pre-treatment in plots shared with larch but the opposite response in plots with pine (Table 2a and Appendix Table A4a). For *V. gaultherioides*, there was a significant temperature x year interaction for mass and area per individual leaf, with both traits slightly reduced under soil warming in 2007 only (Table 2b and A4b). Soil warming had no additional significant effects on the leaf morphology of either species (Table 2).

Leaf N concentration of the two *Vaccinium* species tended to increase with increasing canopy shading (dry mass basis only for *V. myrtillus*; Table 1, mean values for individual years are shown in Appendix Table A3). Accounting for this shading covariable, CO₂ enrichment negatively affected leaf N concentration per unit dry mass and per unit area in the two *Vaccinium* species (Table 1). For both species, the response depended on tree species identity to some extent: *V. myrtillus* showed a stronger negative CO₂ effect on N concentration in plots with pine, particularly in 2006 and 2007, whereas the negative CO₂ effect generally tended to be stronger in plots with larch for *V. gaultherioides* (Tables 1 and A3). Soil warming had a positive influence on leaf N per unit dry mass and per unit area in *V. myrtillus*, but only during the first year of treatment (Tables 2a and A4a). *V. gaultherioides* similarly had higher N per dry leaf mass in warmed plots, whereas N per unit leaf area increased in warmed plots with pine but not with larch (Tables 2b and A4b).

Table 1. Results from analysis of (co)variance repeated measures test of the effect of CO₂ enrichment and tree species on *Vaccinium myrtillus* and *Vaccinium gaultherioides* leaf traits from 2006-2009.

	SLA (cm ² g ⁻¹) ^a			Mass per leaf (mg)			Area per leaf (cm ²) ^a			N per leaf mass (mg g ⁻¹)			N per leaf area (g m ⁻²) ^a		
	DF	F	P	DF	F	P	DF	F	P	DF	F	P	DF	F	P
(a) <i>Vaccinium myrtillus</i>															
Canopy shading ^b	1, 35	13.09	<0.01	-	-	-	1, 35	18.12	<0.01	1, 35	22.32	<0.01	-	-	-
CO ₂ level	1, 35	0.11	0.74	1, 36	3.90	0.06	1, 35	3.41	0.07	1, 35	11.06	<0.01	1, 36	6.29	0.02
Tree species	1, 35	3.07	0.09	1, 36	5.15	0.03	1, 35	10.00	<0.01	1, 35	7.04	0.01	1, 36	0.25	0.62
Year	2, 70	5.36	<0.01	3, 108	15.64	<0.01	2, 71	15.57	<0.01	3, 107	3.25	0.02	2, 70	10.95	<0.01
CO ₂ x Tree species	1, 35	0.28	0.60	1, 36	0.19	0.67	1, 35	0.03	0.86	1, 35	1.28	0.27	1, 36	0.31	0.58
CO ₂ x Year	2, 70	0.03	0.97	3, 108	0.28	0.84	2, 71	0.82	0.44	3, 107	0.91	0.44	2, 70	1.22	0.30
Tree Species x Year	2, 70	2.99	0.06	3, 108	2.09	0.11	2, 71	8.22	<0.01	3, 107	5.80	<0.01	2, 70	0.38	0.69
CO ₂ x Tree x Year	2, 70	0.67	0.52	3, 108	0.09	0.97	2, 71	1.20	0.31	3, 107	2.79	0.04	2, 70	0.01	0.99
(b) <i>Vaccinium gaultherioides</i>															
Canopy shading	1, 35	24.63	<0.01	-	-	-	-	-	-	1, 35	4.64	0.04	1, 35	15.27	<0.01
CO ₂ level	1, 35	0.15	0.70	1, 36	0.30	0.59	1, 36	0.01	0.92	1, 35	5.04	0.03	1, 35	2.62	0.11
Tree species	1, 35	2.46	0.13	1, 36	1.64	0.21	1, 36	0.22	0.64	1, 35	1.51	0.23	1, 35	0.07	0.79
Year	2, 69	13.13	<0.01	3, 105	5.88	<0.01	2, 69	24.73	<0.01	3, 104	26.12	<0.01	2, 67	14.65	<0.01
CO ₂ x Tree species	1, 35	1.08	0.31	1, 36	2.63	0.11	1, 36	1.60	0.21	1, 35	1.30	0.26	1, 35	3.44	0.07
CO ₂ x Year	2, 69	1.05	0.36	3, 105	1.75	0.16	2, 69	1.54	0.22	3, 104	1.48	0.22	2, 67	0.75	0.48
Tree Species x Year	2, 69	1.86	0.16	3, 105	0.39	0.76	2, 69	1.69	0.19	3, 104	1.06	0.37	2, 67	0.13	0.88
CO ₂ x Tree x Year	2, 69	1.32	0.27	3, 105	0.78	0.51	2, 69	1.41	0.25	3, 104	0.32	0.81	2, 67	0.12	0.89

^a data not available from 2008

^b included as a covariable when statistically significant at P < 0.05

Table 2. Results from analysis of covariance repeated measures test of the effect of soil warming on *Vaccinium myrtillus* and *Vaccinium gaultherioides* leaf traits during 2007-2009.

	SLA (cm ² g ⁻¹) ^a			Mass per leaf (mg)			Area per leaf (cm ²) ^a			N per leaf mass (mg g ⁻¹)			N per leaf area (g m ⁻²) ^a		
	DF	F	P	DF	F	P	DF	F	P	DF	F	P	DF	F	P
(a) <i>Vaccinium myrtillus</i>															
2006 covariable ^b															
Temperature	1, 35	19.11	<0.01	1, 35	36.39	<0.01	1, 35	34.25	<0.01	1, 34	13.62	<0.01	1, 34	17.35	<0.01
Tree species	1, 35	0.14	0.71	1, 35	<0.01	>0.99	1, 35	0.20	0.66	1, 34	0.09	0.77	1, 34	0.04	0.85
Year	1, 35	0.72	0.40	1, 35	0.02	0.89	1, 35	<0.01	0.99	1, 34	0.82	0.37	1, 34	0.09	0.77
Temp x Tree species	1, 35	6.74	0.01	2, 72	17.16	<0.01	1, 35	5.90	0.02	2, 70	3.92	0.02	1, 34	9.55	<0.01
Temp x Year	1, 35	0.43	0.52	1, 35	0.02	0.89	1, 35	0.27	0.61	1, 34	0.45	0.51	1, 34	0.01	0.95
Tree Species x Year	1, 35	3.67	0.06	2, 72	1.08	0.35	1, 35	2.20	0.15	2, 70	2.78	0.07	1, 34	8.09	<0.01
Temp x Tree x Year	1, 35	4.70	0.04	2, 72	1.12	0.33	1, 35	0.08	0.78	2, 70	2.16	0.12	1, 34	0.17	0.68
(b) <i>Vaccinium gaultherioides</i>															
2006 covariable															
Temperature	1, 34	70.22	<0.01	1, 34	58.09	<0.01	1, 34	47.11	<0.01	1, 33	9.89	<0.01	1, 32	7.24	0.01
Tree species	1, 34	2.11	0.16	1, 34	0.14	0.71	1, 34	0.06	0.81	1, 33	3.00	0.09	1, 32	0.17	0.68
Year	1, 34	4.61	0.04	1, 34	1.96	0.17	1, 34	0.17	0.68	1, 33	4.73	0.04	1, 32	0.64	0.43
Temp x Tree species	1, 33	14.15	<0.01	2, 68	3.52	0.04	1, 33	39.43	<0.01	2, 66	44.29	<0.01	1, 31	18.27	<0.01
Temp x Year	1, 34	0.08	0.78	1, 34	0.41	0.52	1, 34	0.70	0.41	1, 33	0.43	0.52	1, 32	3.17	0.08
Tree Species x Year	1, 33	0.22	0.65	2, 68	3.96	0.02	1, 33	5.44	0.03	2, 66	0.61	0.54	1, 31	0.56	0.46
Temp x Tree x Year	1, 33	2.54	0.12	2, 68	0.21	0.81	1, 33	5.62	0.02	2, 66	0.91	0.41	1, 31	0.04	0.85
Temp x Tree x Year	1, 33	0.46	0.50	2, 68	0.69	0.51	1, 33	0.07	0.79	2, 66	0.52	0.60	1, 31	0.71	0.41

^a data not available from 2008^b pre-warming measurement included as a covariable

Understorey vegetation composition

Changes from 2005-2009 in the total number of vascular and non-vascular (moss and lichen) species per plot were negatively influenced by both experimental treatments. In contrast, changes in species richness were not significantly influenced by canopy shading, although more vascular species losses tended to occur in plots with taller *V. myrtillus* cover, irrespective of tree species identity (data not shown). There was a mean loss of approximately two species over the four years in elevated CO₂ plots with larch but no change in ambient CO₂ plots with larch. Plots with pine showed an opposite but weaker pattern, with more pronounced species losses in ambient CO₂ plots (significant CO₂ x tree species interaction; $F_{1,31} = 6.3$, $P = 0.02$). Soil warming had a marginally significant negative effect on total species richness ($F_{1,31} = 3.5$, $P = 0.07$). Separate analyses for vascular and non-vascular species indicated that changes in vascular species were influenced by elevated CO₂, again interacting with tree species identity ($F_{1,31} = 8.0$, $P < 0.01$), but not by warming (Fig. 4). Non-vascular plant species richness was negatively influenced by soil warming, particularly in plots exposed to elevated CO₂ (warming: $P = 0.02$; CO₂ x warming: $P = 0.09$; Fig. 5). The moss species *Polytrichum juniperinum* showed an especially strong response to soil warming: it disappeared from over half of the warmed plots where it had occurred in 2005 (8 of 14), whereas its presence in unwarmed plots increased. ANOVA results and lists of changes in species occurrence are presented in the Appendix in Table A5 and A6, respectively.

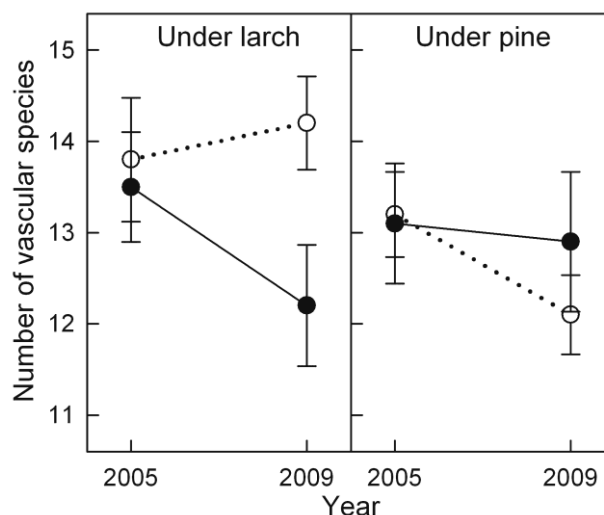


Figure 4. Changes in understorey vascular plant species richness from 2005-2009. The mean number of species per plot is shown for each CO₂ treatment and tree species combination, ± 1 standard error (pooled across temperature treatments; $n = 10$). Open circles = ambient CO₂ and filled circles = elevated CO₂.

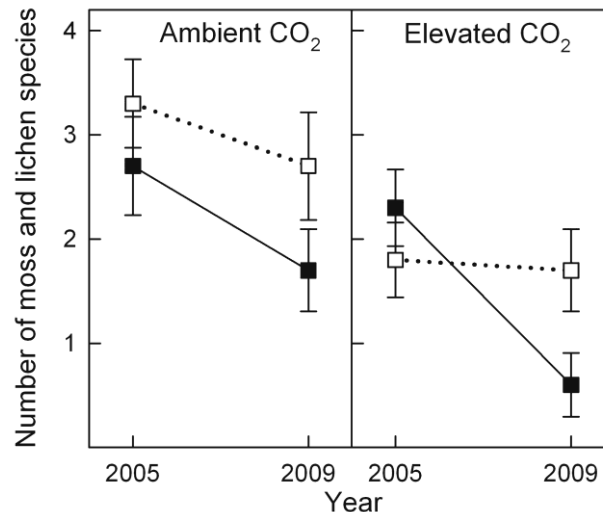


Figure 5. Changes in understorey non-vascular (moss and lichen) plant species richness from 2005-2009. The mean number of species per plot is shown for each CO₂ and temperature treatment combination, ± 1 standard error (pooled across tree species identities; $n = 10$). Open squares = unwarmed and filled squares = warmed.

Discussion

Our study provides a unique dataset of how vegetation growing in a temperate high elevation environment responds to atmospheric and climate changes predicted to occur within the next 50 years. Growth responses to eight years of CO₂ enrichment and three years of soil warming were different for three dominant ericaceous dwarf shrub species growing at treeline in the Swiss Alps. *V. myrtillus* was stimulated by both experimental treatments and generally showed a stronger response than *V. gaultherioides* or *E. hermaphroditum*. Additionally, both elevated CO₂ and soil warming negatively impacted the number of vascular and non-vascular species in the experimental plots.

Species-specific growth responses to simulated atmospheric and climate change

The CO₂ effect on each dwarf shrub species was relatively consistent over several years of CO₂ enrichment, providing a clear indication that these species differ in their responsiveness, irrespective of annual variations in climatic conditions. For *V. myrtillus* in particular, there was no decline over time in the shoot growth response, suggesting that growth stimulation was not constrained by a progressive nutrient limitation or by photosynthetic down-regulation. The relatively strong response of *V. myrtillus* to CO₂ enrichment compared to the other two dwarf shrub species might be related to different leaf traits. For example, higher SLA in *V. myrtillus* compared to *V. gaultherioides* (20-30% in all years measured) and *E. hermaphroditum* (>50%; T. Zumbunn *et al.*, unpublished data), means there is a larger amount of leaf area displayed per unit mass invested and a larger amount of N per unit mass (Poorter *et al.* 2009) in *V. myrtillus* than in the other two species. Efficient light capture and high photosynthetic capacity

associated with these traits could have led to larger assimilation gains under elevated CO₂ (Roumet and Roy 1996) in *V. myrtillus* compared to the other two species.

Contrasting growth responses of the two tree species in the experimental plots to long-term CO₂ enrichment, yielding enhanced larch canopy size but no significant effect on pine (Chapter 2 of this thesis), might indirectly have contributed to some dwarf shrub responses. Canopy shading positively influenced individual leaf area of the relatively shade-tolerant species *V. myrtillus* (Table 1), indicating that production of larger leaves was at least partially a response to increased shading rather than a direct response to CO₂ enrichment. In contrast, *V. myrtillus* shoot growth and ramet height were enhanced under elevated CO₂ but neither parameter was significantly influenced by the amount of shade. These results suggest that increased *V. myrtillus* shoot increment length (and mass, see Appendix Fig. A3) was due to direct stimulation by elevated CO₂. For *V. gaultherioides*, CO₂ effects on shoot growth and ramet height were clearly influenced by tree species identity but, similar to *V. myrtillus*, there was no significant relationship between canopy shading and growth responses. It seems that the differences in plots with larch vs. pine other than light conditions, such as litter production and quality and below-ground competition, played a role in the growth response of this species.

Consistent with our findings, species-specific responses were observed in a three-year mesocosm CO₂ enrichment study at the Abisko research station in northern Sweden that included the same three dwarf shrub species as in our experiment (Gwynn-Jones *et al.* 1997). Similar to our results, *V. myrtillus* was the only species in this subarctic experiment to show a positive growth response to CO₂ enrichment. However, whereas we observed a tree species-specific CO₂ response in *V. gaultherioides* (group *V. uliginosum* agg.) and no effect in *E. hermaphroditum*, at the Abisko site *V. uliginosum* showed no CO₂ response and there was a significant negative effect for *E. hermaphroditum* in one treatment year (Gwynn-Jones *et al.* 1997). Enhanced growth was also observed for *V. myrtillus* exposed to elevated CO₂ for a single season in a greenhouse study in low elevation heathlands of the Netherlands (Arp *et al.* 1998). The consistent growth responses to CO₂ enrichment observed for *V. myrtillus* across multiple studies under various growth conditions indicates an inherent CO₂ responsiveness of this species and suggests that *V. myrtillus* growth and abundance might increase in a future CO₂-enriched atmosphere. In contrast, most species in an alpine grassland in the Swiss Central Alps did not respond to CO₂ enrichment over four years (Körner *et al.* 1997).

Deciduous *V. myrtillus* was the only species to show a significant positive shoot growth response to the warming treatment, with an average stimulation (31%) over twice the size of the mean CO₂ effect (12%). *V. gaultherioides*, also deciduous, showed no response to warming, suggesting that factors other than leaf type were more influential on the responses of individual species. In the Alps, *V. myrtillus* has a lower elevational distribution compared to *V. gaultherioides* and *E. hermaphroditum*, both of which extend to >3000 m a.s.l. (Landolt *et al.* 2010). *V. myrtillus* might therefore be better adapted and more responsive to warmer temperatures. As in our study, *V. myrtillus* had a more

pronounced shoot growth response to soil warming than *E. hermaphroditum* (stimulation only with additional air warming) or *V. gaultherioides* (no growth response) in a five-year study in Abisko, Sweden (Hartley *et al.* 1999). Positive shoot growth responses were similarly observed for dwarf shrubs after the 2nd and 3rd seasons of warming by open top chambers (OTCs) in the Swedish subarctic heath (Parsons *et al.* 1994), although there all three species in common with our experiment responded positively. As in our study, warming by OTCs at a temperate alpine site in northern Japan had no effect on vegetative growth of *V. uliginosum* (Kudo and Suzuki 2003). However, *Empetrum nigrum* var. *japonicum* shoot elongation was strongly stimulated by warming in their study.

Different responses to warming for the same species might be due to genetic differences between regions, especially for *E. nigrum* and *V. uliginosum* which are highly heterogeneous species complexes (Bell and Tallis 1973, Jacquemart 1996). Lower atmospheric pressure (and therefore lower CO₂ partial pressure), contrasting day-night solar radiation and temperatures during summer, and generally higher precipitation in temperate alpine environments compared to arctic regions might also have contributed to the different findings (Körner 2003). Finally, different heating techniques might have played a role in the divergent results: passive warming by OTCs generally results in smaller increases in air and soil temperature than soil warming by heating cables on the ground surface (Rustad *et al.* 2001). Further, warming by OTCs is confounded to some extent with shelter effects, i.e. increased humidity and reduced wind speed and night-time radiative cooling, whereas the heating cables in our study slightly reduced air humidity near the ground surface and did not affect the latter two parameters (Hagedorn *et al.* 2010).

Soil warming had a large short-term positive effect on leaf N concentration of both *Vaccinium* species, supporting the hypothesis that soil warming would initially accelerate N cycling and lead to an enhanced N supply due to faster organic matter decomposition (Melillo *et al.* 2002). Increasing mineral N contents in the soil and $\delta^{15}\text{N}$ values in leaves throughout the three years of warming further suggest an improved N availability in the warmed soils (F. Hagedorn, unpublished data). In a study in open birch forest in northern Sweden, Richardson *et al.* (2002) found that eight years of warming by OTCs had a large positive effect on leaf and shoot N concentration of *V. myrtillus* (73%) but a negative effect on that of *V. gaultherioides* (19%). In another experiment in northern Sweden, Hartley *et al.* (1999) found no effect of soil warming on foliar N concentrations in *V. myrtillus* or *V. gaultherioides*. In our study, warming effects on *V. myrtillus* leaf N concentration were only apparent in the first year of warming. One possible explanation for this response is that enhanced shoot growth in 2008 and 2009 diluted the soil warming-induced increase in total N uptake, yielding an overall higher N content in leaf biomass but no effect on concentrations (Weih and Karlsson 2001). This proposed mechanism assumes that longer, heavier shoots under warming corresponded to greater above-ground biomass production, i.e. that enhanced shoot length under warming was not offset by reduced tillering. Alternatively, it is possible that somewhat drier soil associated with higher temperatures made soil nutrients less mobile and therefore less

available for plants (Knapp *et al.* 2008). Overall, our experimental design did not permit us to distinguish clearly between direct temperature-driven effects and indirect nutrient-driven warming effects on dwarf shrub growth.

We expected increased nutrient availability with soil warming to relieve nutrient constraints on the CO₂ response, leading to a positive CO₂ x warming interactive effect on dwarf shrub growth. Although we observed lower leaf N concentrations in elevated CO₂ plots and a short-term increase in leaf N concentrations in warmed plots, there was no interaction between the experimental treatments. This result could mean that the CO₂ response was not constrained by N limitation and that lower leaf N concentrations observed under elevated CO₂ was an effect of partial photosynthetic down-regulation, altered N allocation without significant losses in photosynthetic capacity, or simply a dilution effect due to enhanced accumulation of starch (Asshoff and Hättenschwiler 2005). Similarly, an alpine grassland community showed no effect of CO₂ enrichment on biomass production, even when combined with 40 kg ha⁻¹ a⁻¹ of NPK fertilization, indicating that nutrient limitation did not cause the system to remain unresponsive to CO₂ in terms of biomass production (Körner *et al.* 1997). Likewise, a FACE x N addition experiment with glacier forefield vegetation yielded strong stimulation by fertilization over three years but no CO₂ effect or interaction between treatments (N. Inauen *et al.*, unpublished data).

Enhanced growth of *V. myrtillus* under simulated atmospheric and climate change suggests that this species might have a competitive advantage over the co-occurring dwarf shrub species in the future. However, at our research site both elevated CO₂ and soil warming increased the sensitivity of *V. myrtillus* to damage from early growing season freezing events, whereas neither *V. gaultherioides* nor *E. hermaphroditum* was affected (Martin *et al.* 2010). Further, *V. myrtillus* was found to be more susceptible than the other two species to negative effects of early snow ablation (Wipf *et al.* 2009). Therefore, stochastic climate events that consistently impact *V. myrtillus* more severely than the other dwarf shrub species could counteract increases in its dominance.

Decline in species richness with elevated CO₂ concentrations and soil warming

The experimental treatments led to changes in vegetation composition at the plot scale during the final four years of the long-term treatment, with a decline in the number of vascular and non-vascular species in the experimental plots. Increased shading in elevated CO₂ plots with larch might have contributed somewhat to the disappearance of more light-demanding understorey species, but the reduction in vascular plant species richness could not be explained statistically by greater canopy shading. However, the trend of greater species loss in plots with taller *V. myrtillus* ramets suggests that increased shading within the understorey canopy and/or increased below-ground competition played a role in the decline. The opposite pattern in plots with pine was surprising because the height of understorey vegetation and of *V. myrtillus* was also enhanced in elevated CO₂ plots shared with this tree species. Three years of

experimental warming had no detectable effect on vascular plant composition, consistent with results from a five-year soil warming treatment in Abisko, Sweden (Hartley *et al.* 1999). In contrast, nine years of warming by greenhouses near Toolik Lake, Alaska led to a decline in species richness (Chapin *et al.* 1995). Given that the response of *V. myrtillus* shoot growth was more pronounced with warming than with CO₂ enrichment, sustained shoot growth enhancement of this abundant species could lead to changes in vegetation composition and species richness over the longer term. The warming treatment in our study did result in a loss of moss and lichen species. Increased vascular plant productivity with warming has been associated with reduced abundance of mosses and lichens in several (sub)arctic studies (see meta-analysis by Walker *et al.* 2006). This relationship has previously been attributed to increased shading by vascular plants (Chapin *et al.* 1995, Cornelissen *et al.* 2001), although soil drying associated with our warming treatment might additionally have played a role in the loss of these species.

Conclusions

In situ experimental manipulation of atmospheric CO₂ concentration and soil temperature yielded different responses for three dwarf shrub species growing at treeline in the Alps. We found no significant interactive effects between soil warming and CO₂ enrichment for any of the observed parameters, suggesting that, regarding these two specific global change factors, future growth of these dwarf shrub species can be predicted from independent responses to each shift. Dwarf shrub growth responses were associated with reductions in the number of vascular and non-vascular plant species under CO₂ enrichment and soil warming, indicating that shifts in understory species composition are likely to occur with ongoing atmospheric and climate change. Increasing dominance by deciduous *Vaccinium myrtillus*, with short-lived leaves producing comparatively high-quality, rapidly decomposing litter, could have important consequences for ecosystem carbon balance and nutrient cycling in the treeline ecotone.

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Appendix

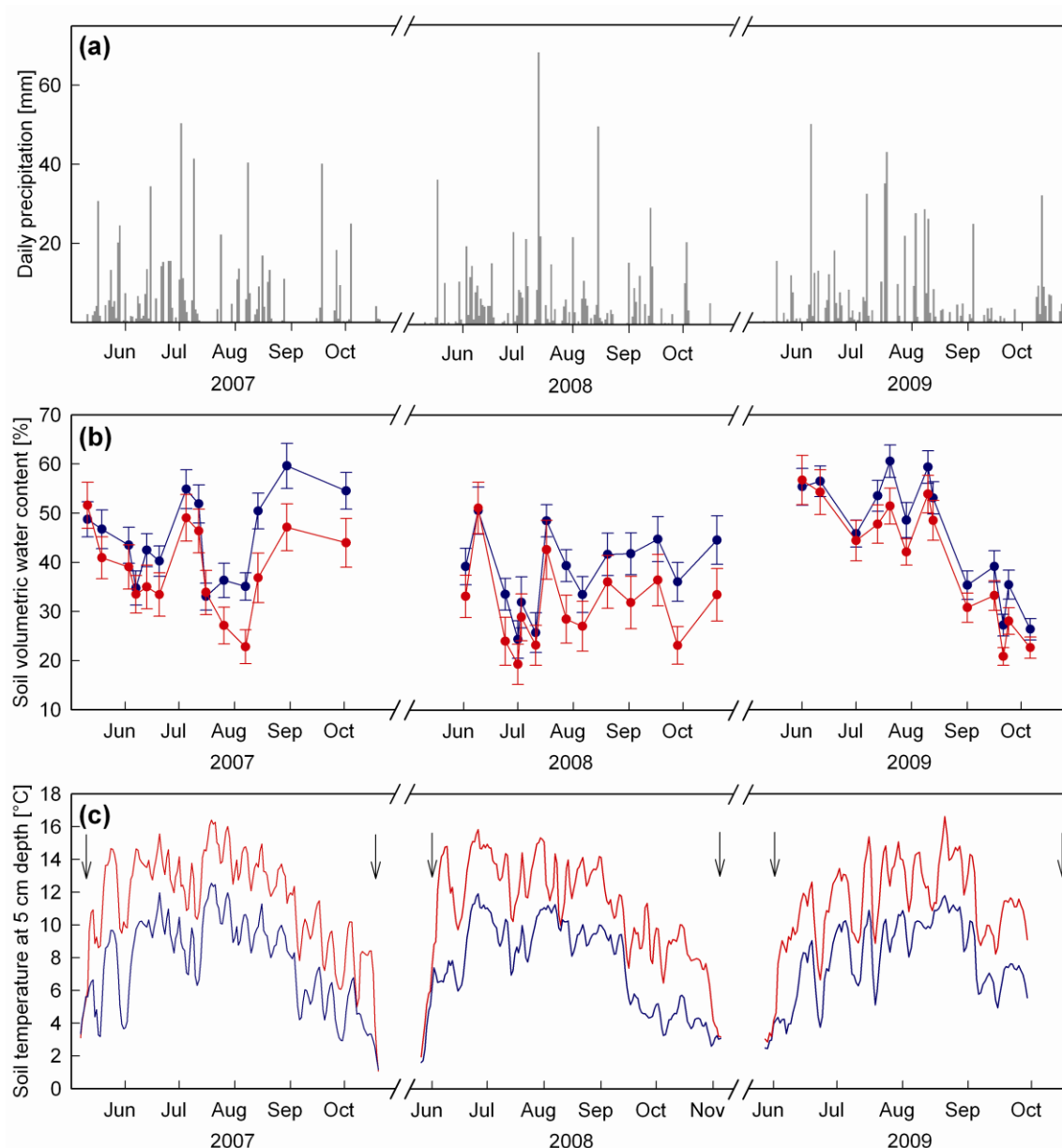


Figure A1. Time series of soil warming effects on soil conditions during three growing seasons of treatment. (a) Daily precipitation data from a climate station located approximately 100 m below the research area. (b) Mean soil volumetric water content at 0-10 cm depth for all unwarmed (blue) and all warmed (red) plots, ± 1 standard error ($n = 20$). (c) Soil temperature at 5 cm depth during each snow-free season, averaged across unwarmed (blue) and warmed (red) plots ($n = 10$). Variation among plots within each treatment group is not shown. Arrows indicate the start and end of each warming period. Data from the end of the 2009 season were not available due to equipment malfunction. Neither CO_2 treatment (ambient, elevated) nor tree species identity (larch, pine) significantly influenced the warming effect on soil temperature or moisture, and values were accordingly pooled across these variables for both (b) and (c).

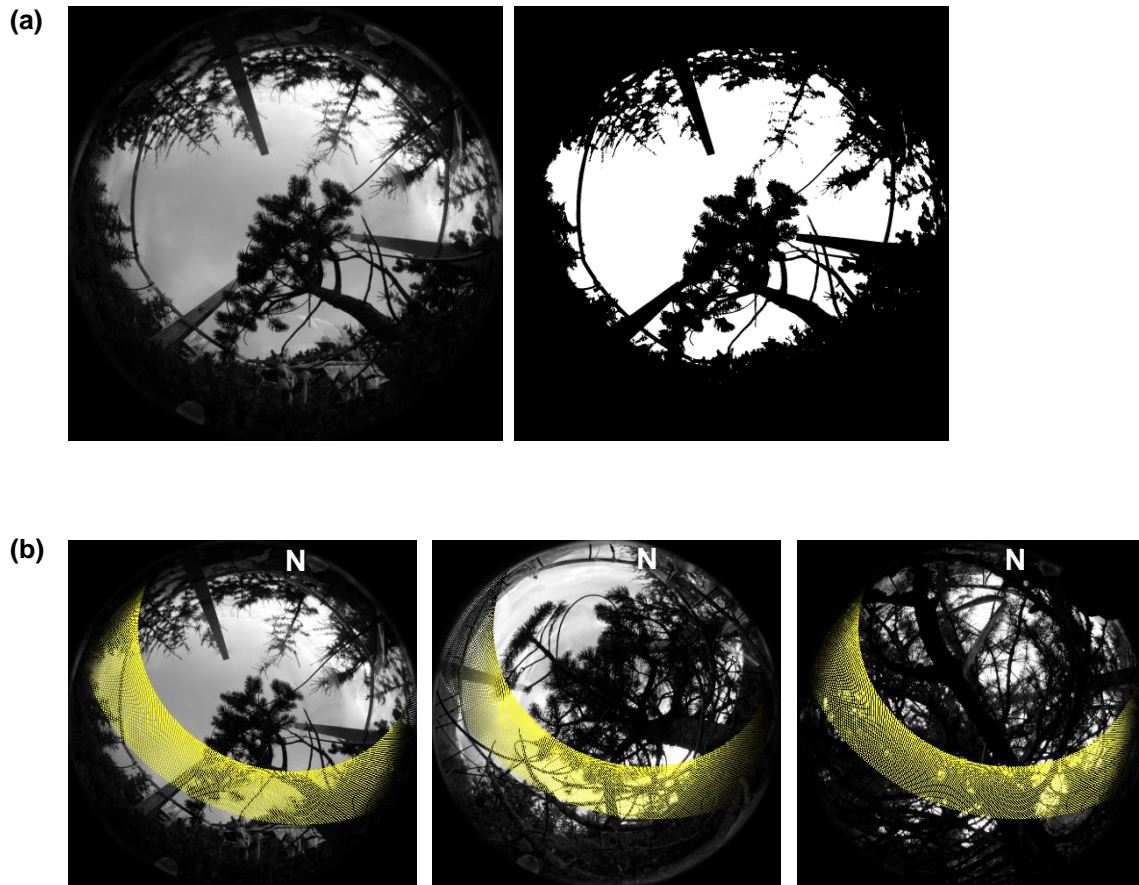


Figure A2. Hemispherical photographs used for estimating the amount of shade experienced by understorey plants. A Nikon Coolpix camera fitted with a fisheye lens was positioned 15-20 cm down slope of the tree trunk in each plot, with the lens oriented horizontally rather than perpendicular to the slope. Using Gap Light Analyzer version 2.0 (Frazer et al. 1999), a darkness threshold isolating open sky was determined manually (a) and the percentage of the photo that was not open sky was calculated (i.e. tree canopy cover, trees in the surrounding area, topographic elements, and experimental infrastructure). Additionally, the sun's path across each photo was plotted for individual dates and for the approximate growing season (1 June to 30 September) to verify visually that this measurement was representative of the relative amount of shade in various plots. Input for these plots included the aspect, latitude, longitude, and elevation of the site. Three examples for the entire growing season are shown in (b).

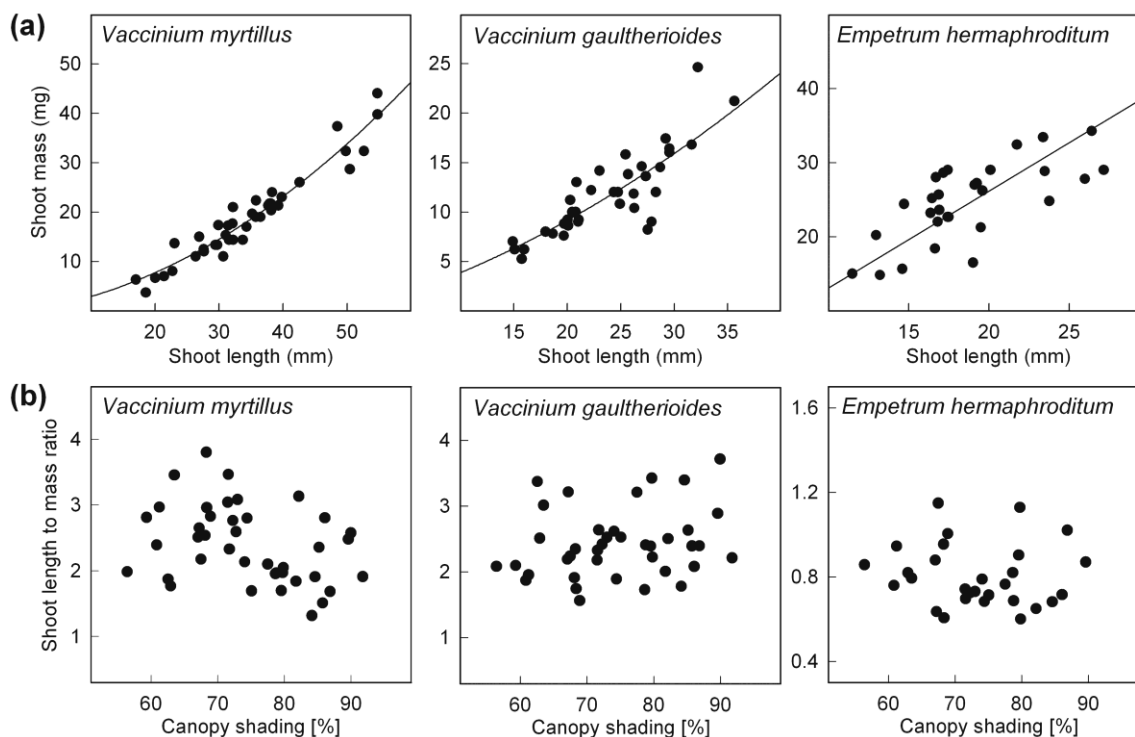


Figure A3. Shoot allometric relationships for the three target dwarf shrub species. (a) Relationship between length and mass of current-year shoot increments (sampled in 2010). These plots demonstrate that, particularly for *Vaccinium myrtillus*, shoot length is highly correlated with shoot mass. (b) The ratio of shoot length to mass as a function of percent canopy shading, as estimated from hemispherical photographs taken in 2008 (see Fig. A2). Heavy shade might be expected to cause production of long, thin shoots (etiolation), which would mean less mass for a given length, but here the length to mass ratio is not strongly influenced by the amount of canopy shading and *Vaccinium myrtillus* even shows a slight trend in the opposite direction.

Table A1. Results of likelihood ratio tests used to justify separate analyses of CO₂ enrichment and soil warming effects on dwarf shrub shoot growth and leaf traits. For each parameter, a repeated-measures model (2007-2009, years when elevated CO₂ and warming were applied in combination) including CO₂ and all CO₂ x temperature interactions was compared with a nested model without these effects. The compared models were each fit using the maximum likelihood estimation method (ML). No significant differences were found between models and AIC values were always lower (indicating a better model fit) for models without these interactive terms.

Measurement	Species	L kelihood Ratio	P
Annual shoot growth	<i>Vaccinium myrtillus</i>	6.88	0.55
	<i>Vaccinium gaultherioides</i>	11.92	0.16
	<i>Empetrum hermaphroditum</i>	7.77	0.46
Specific leaf area	<i>Vaccinium myrtillus</i>	1.27	0.99
	<i>Vaccinium gaultherioides</i>	6.75	0.56
Mass per leaf	<i>Vaccinium myrtillus</i>	2.69	0.99
	<i>Vaccinium gaultherioides</i>	13.74	0.32
Area per leaf	<i>Vaccinium myrtillus</i>	4.12	0.85
	<i>Vaccinium gaultherioides</i>	8.66	0.37
N per unit leaf mass	<i>Vaccinium myrtillus</i>	6.78	0.87
	<i>Vaccinium gaultherioides</i>	16.39	0.17
N per unit leaf area	<i>Vaccinium myrtillus</i>	1.75	0.99
	<i>Vaccinium gaultherioides</i>	7.18	0.52

Table A2. Analysis of (co)variance results for annual shoot growth and total ramet height. The response variable was log-transformed for all shoot growth analyses. For tests of the warming effect, covariable x year and covariable x temperature treatment interactive effects did not contribute significantly to the model fit for any species and were therefore excluded from the analyses. Likewise, year effects were excluded from analyses for total ramet height because they did not contribute significantly to models fits.

	Vaccinium myrtillus			Vaccinium gauthieroides			Empetrum hermaphroditum		
	DF *	F	P	DF	F	P	DF	F	P
(a) Annual shoot growth									
<i>Elevated CO₂ (2002-2009)</i>									
CO ₂ level	1, 36	7.46	0.01	1, 36	0.03	0.86	1, 35	0.20	0.66
Tree species	1, 36	4.63	0.04	1, 36	4.14	0.05	1, 35	0.05	0.82
Year	7, 1686	38.22	<0.01	7, 1483	17.47	<0.01	7, 1306	28.92	<0.01
CO ₂ level x Tree species	1, 36	0.35	0.56	1, 36	5.40	0.03	1, 35	<0.01	0.95
CO ₂ level x Year	7, 1686	0.30	0.95	7, 1483	0.65	0.72	7, 1306	0.59	0.77
Tree species x Year	7, 1686	0.88	0.53	7, 1483	2.29	0.03	7, 1306	1.49	0.17
CO ₂ x Tree x Year	7, 1686	0.13	>0.99	7, 1483	1.05	0.40	7, 1306	0.92	0.49
<i>Warming (2007-2009)</i>									
2005-2006 covariable									
Temperature	1, 35	5.30	0.03	1, 34	7.82	<0.01	1, 31	6.09	0.02
Tree species	1, 35	0.77	0.39	1, 34	0.31	>0.99	1, 31	0.20	0.66
Year	2, 697	4.49	0.01	2, 492	16.34	<0.01	2, 363	0.75	0.48
Temperature x Tree species	1, 35	0.64	0.43	1, 34	0.59	0.45	1, 31	0.82	0.37
Temperature x Year	2, 697	0.59	0.56	2, 492	0.05	0.96	2, 363	0.95	0.39
Tree species x Year	2, 697	1.53	0.22	2, 492	1.81	0.17	2, 363	0.43	0.65
Temp x Tree x Year	2, 697	0.92	0.40	2, 492	0.82	0.44	2, 363	0.93	0.40
(b) Total ramet height (2008 & 2009)									
Canopy shading									
CO ₂ level	1, 36	7.11	0.01	1, 36	0.50	0.48	1, 31	3.07	0.09
Tree species	1, 36	0.29	0.60	1, 36	4.31	0.05	1, 31	2.70	0.11
CO ₂ level x Tree species	1, 36	<0.01	0.97	1, 36	4.10	0.05	1, 31	1.05	0.31

* The estimation of denominator degrees of freedom in linear mixed effects models is still a highly debated topic (see discussion on the R-help mailing list: <https://stat.ethz.ch/pipermail/r-help/2006-May/094765.html>). P values presented here are based on values estimated in the nlme package in R (Pinheiro et al., 2008) and might be somewhat anti-conservative.

Table A3. Mean values \pm 1 standard error and analysis of (co)variance results for the effect of CO₂ enrichment and tree species identity on *Vaccinium myrtillus* and *Vaccinium gautherioides* leaf traits in individual years. No multiple comparison correction was applied.

	Year	Ambient CO ₂	Elevated CO ₂	Canopy shading		CO ₂ level		Tree species		CO ₂ x Tree species	
				DF = 1, 34-35	P	DF = 1, 34-36	F	DF = 1, 34-36	F	DF = 1, 34-36	F
(a) <i>Vaccinium myrtillus</i>	2006	8.4 \pm 0.3	9.4 \pm 0.5	-	-	3.50	0.07	7.63	<0.01	0.03	0.86
	2007	7.6 \pm 0.2	8.2 \pm 0.4	-	-	2.05	0.16	0.44	0.51	0.02	0.88
	2008	7.4 \pm 0.3	8.0 \pm 0.3	-	-	2.32	0.14	4.61	0.04	0.35	0.56
	2009	8.5 \pm 0.3	9.3 \pm 0.4	-	-	2.16	0.15	2.38	0.13	0.22	0.64
	2006	1.54 \pm 0.08	1.80 \pm 0.10	16.55	<0.01	2.29	0.14	14.40	<0.01	0.67	0.42
	2007	1.36 \pm 0.04	1.51 \pm 0.07	13.57	<0.01	0.89	0.35	1.82	0.19	0.05	0.82
	2009	1.43 \pm 0.06	1.61 \pm 0.05	6.39	0.02	3.18	0.08	3.41	0.07	0.30	0.59
	2006	180 \pm 5	192 \pm 8	5.13	0.03	0.08	0.78	4.55	0.04	1.04	0.32
	2007	178 \pm 3	181 \pm 4	24.07	<0.01	0.01	0.92	10.05	<0.01	0.03	0.87
	2009	169 \pm 5	176 \pm 5	4.93	0.03	0.16	0.70	0.06	0.81	<0.01	0.98
	2006	19.1 \pm 0.4	18.1 \pm 0.7	15.24	<0.01	11.89	<0.01	13.59	<0.01	6.74	0.01
	2007	19.1 \pm 0.3	18.4 \pm 0.4	10.94	0.02	9.71	<0.01	9.92	<0.01	0.17	0.68
	2008	19.6 \pm 0.4	19.4 \pm 0.4	5.88	0.02	1.40	0.25	0.35	0.56	0.18	0.67
2009	19.1 \pm 0.4	18.8 \pm 0.4	9.56	<0.01	2.51	0.12	<0.01	0.99	0.19	0.67	
2006	1.07 \pm 0.03	0.95 \pm 0.02	-	-	11.31	<0.01	1.20	0.28	0.31	0.58	
2007	1.08 \pm 0.02	1.01 \pm 0.02	-	-	7.62	<0.01	0.05	0.82	0.48	0.49	
2009	1.15 \pm 0.04	1.09 \pm 0.04	-	-	1.06	0.31	0.07	0.79	0.08	0.78	
2006	10.5 \pm 0.6	10.3 \pm 0.7	-	-	0.07	0.79	0.32	0.57	3.35	0.08	
2007	10.3 \pm 0.6	9.4 \pm 0.5	-	-	2.20	0.15	2.20	0.15	1.28	0.27	
2008	9.3 \pm 0.7	8.9 \pm 0.6	-	-	0.30	0.59	1.57	0.22	2.40	0.13	
2009	9.4 \pm 0.6	10.0 \pm 0.6	-	-	0.43	0.52	0.82	0.37	0.81	0.37	
2006	1.55 \pm 0.08	1.56 \pm 0.07	-	-	0.03	0.87	0.82	0.37	2.57	0.12	
2007	1.52 \pm 0.08	1.44 \pm 0.05	-	-	0.82	0.37	0.53	0.47	0.27	0.60	
2009	1.27 \pm 0.06	1.32 \pm 0.07	-	-	0.31	0.58	0.10	0.75	1.00	0.33	
2006	147 \pm 4	156 \pm 6	14.94	<0.01	0.08	0.77	1.69	0.20	2.52	0.12	
2007	147 \pm 3	154 \pm 5	21.16	<0.01	<0.01	0.97	10.04	0.01	1.89	0.18	
2009	137 \pm 5	135 \pm 5	11.85	<0.01	1.37	0.25	<0.01	0.95	0.03	0.86	
2006	22.6 \pm 0.6	21.2 \pm 0.6	2.80	0.10	6.77	0.02	<0.01	0.98	2.16	0.15	
2007	22.8 \pm 0.6	21.6 \pm 0.6	2.37	0.13	4.22	0.05	3.08	0.09	0.45	0.51	
2008	24.8 \pm 0.5	24.8 \pm 0.5	6.45	0.02	0.69	0.41	2.46	0.13	0.38	0.54	
2009	23.0 \pm 0.5	22.1 \pm 0.6	1.27	0.27	2.37	0.13	0.63	0.43	0.66	0.42	
2006	1.55 \pm 0.04	1.38 \pm 0.05	5.19	0.03	4.40	0.04	0.27	0.61	3.11	0.09	
2007	1.55 \pm 0.03	1.42 \pm 0.04	7.36	0.01	4.58	0.04	1.42	0.24	3.43	0.07	
2009	1.72 \pm 0.07	1.67 \pm 0.06	6.78	0.01	0.02	0.89	0.61	0.44	0.30	0.59	
(b) <i>Vaccinium gautherioides</i>	2006	8.4 \pm 0.3	9.4 \pm 0.5	-	-	3.50	0.07	7.63	<0.01	0.03	0.86
	2007	7.6 \pm 0.2	8.2 \pm 0.4	-	-	2.05	0.16	0.44	0.51	0.02	0.88
	2008	7.4 \pm 0.3	8.0 \pm 0.3	-	-	2.32	0.14	4.61	0.04	0.35	0.56
	2009	8.5 \pm 0.3	9.3 \pm 0.4	-	-	2.16	0.15	2.38	0.13	0.22	0.64
	2006	1.54 \pm 0.08	1.80 \pm 0.10	16.55	<0.01	2.29	0.14	14.40	<0.01	0.67	0.42
	2007	1.36 \pm 0.04	1.51 \pm 0.07	13.57	<0.01	0.89	0.35	1.82	0.19	0.05	0.82
	2009	1.43 \pm 0.06	1.61 \pm 0.05	6.39	0.02	3.18	0.08	3.41	0.07	0.30	0.59
	2006	180 \pm 5	192 \pm 8	5.13	0.03	0.08	0.78	4.55	0.04	1.04	0.32
	2007	178 \pm 3	181 \pm 4	24.07	<0.01	0.01	0.92	10.05	<0.01	0.03	0.87
	2009	169 \pm 5	176 \pm 5	4.93	0.03	0.16	0.70	0.06	0.81	<0.01	0.98
	2006	19.1 \pm 0.4	18.1 \pm 0.7	15.24	<0.01	11.89	<0.01	13.59	<0.01	6.74	0.01
	2007	19.1 \pm 0.3	18.4 \pm 0.4	10.94	0.02	9.71	<0.01	9.92	<0.01	0.17	0.68
	2008	19.6 \pm 0.4	19.4 \pm 0.4	5.88	0.02	1.40	0.25	0.35	0.56	0.18	0.67
2009	19.1 \pm 0.4	18.8 \pm 0.4	9.56	<0.01	2.51	0.12	<0.01	0.99	0.19	0.67	
2006	1.07 \pm 0.03	0.95 \pm 0.02	-	-	11.31	<0.01	1.20	0.28	0.31	0.58	
2007	1.08 \pm 0.02	1.01 \pm 0.02	-	-	7.62	<0.01	0.05	0.82	0.48	0.49	
2009	1.15 \pm 0.04	1.09 \pm 0.04	-	-	1.06	0.31	0.07	0.79	0.08	0.78	
2006	10.5 \pm 0.6	10.3 \pm 0.7	-	-	0.07	0.79	0.32	0.57	3.35	0.08	
2007	10.3 \pm 0.6	9.4 \pm 0.5	-	-	2.20	0.15	2.20	0.15	1.28	0.27	
2008	9.3 \pm 0.7	8.9 \pm 0.6	-	-	0.30	0.59	1.57	0.22	2.40	0.13	
2009	9.4 \pm 0.6	10.0 \pm 0.6	-	-	0.43	0.52	0.82	0.37	0.81	0.37	
2006	1.55 \pm 0.08	1.56 \pm 0.07	-	-	0.03	0.87	0.82	0.37	2.57	0.12	
2007	1.52 \pm 0.08	1.44 \pm 0.05	-	-	0.82	0.37	0.53	0.47	0.27	0.60	
2009	1.27 \pm 0.06	1.32 \pm 0.07	-	-	0.31	0.58	0.10	0.75	1.00	0.33	
2006	147 \pm 4	156 \pm 6	14.94	<0.01	0.08	0.77	1.69	0.20	2.52	0.12	
2007	147 \pm 3	154 \pm 5	21.16	<0.01	<0.01	0.97	10.04	0.01	1.89	0.18	
2009	137 \pm 5	135 \pm 5	11.85	<0.01	1.37	0.25	<0.01	0.95	0.03	0.86	
2006	22.6 \pm 0.6	21.2 \pm 0.6	2.80	0.10	6.77	0.02	<0.01	0.98	2.16	0.15	
2007	22.8 \pm 0.6	21.6 \pm 0.6	2.37	0.13	4.22	0.05	3.08	0.09	0.45	0.51	
2008	24.8 \pm 0.5	24.8 \pm 0.5	6.45	0.02	0.69	0.41	2.46	0.13	0.38	0.54	
2009	23.0 \pm 0.5	22.1 \pm 0.6	1.27	0.27	2.37	0.13	0.63	0.43	0.66	0.42	
2006	1.55 \pm 0.04	1.38 \pm 0.05	5.19	0.03	4.40	0.04	0.27	0.61	3.11	0.09	
2007	1.55 \pm 0.03	1.42 \pm 0.04	7.36	0.01	4.58	0.04	1.42	0.24	3.43	0.07	
2009	1.72 \pm 0.07	1.67 \pm 0.06	6.78	0.01	0.02	0.89	0.61	0.44	0.30	0.59	

Table A4. Mean values (relative to pre-treatment year 2006) \pm 1 standard error and analysis of covariance results for the effect of soil warming and tree species identity on *Vaccinium myrtillus* and *Vaccinium gauthieroides* leaf traits in individual years. No multiple comparison correction was applied.

	Year	Unwarmed relative to 2006	Warmed relative to 2006	2006 covariable DF = 1, 32-35		Temperature DF = 1, 32-35		Tree species DF = 1, 32-35		Temp x Tree species DF = 1, 32-35	
				F	P	F	P	F	P	F	P
(a) <i>Vaccinium myrtillus</i>											
Mass per leaf	2007	0.90 \pm 0.03	0.91 \pm 0.04	19.68	<0.01	0.44	0.51	1.03	0.32	0.38	0.54
	2008	0.85 \pm 0.04	0.92 \pm 0.04	15.02	<0.01	0.78	0.38	0.60	0.44	0.01	0.91
	2009	1.00 \pm 0.04	1.03 \pm 0.04	25.88	<0.01	0.07	0.80	0.02	0.88	0.09	0.76
Area per leaf	2007	0.89 \pm 0.03	0.90 \pm 0.04	37.40	<0.01	1.52	0.23	0.03	0.86	0.74	0.40
	2009	0.90 \pm 0.04	0.98 \pm 0.05	16.97	<0.01	0.08	0.78	0.02	0.89	0.08	0.78
SLA	2007	1.00 \pm 0.03	0.98 \pm 0.02	57.76	<0.01	2.88	0.10	13.09	<0.01	6.64	0.02
	2009	0.92 \pm 0.03	0.97 \pm 0.03	5.19	0.03	1.09	0.30	0.09	0.77	0.01	0.90
N per leaf mass	2007	0.98 \pm 0.02	1.05 \pm 0.02	43.56	<0.01	6.06	0.02	2.38	0.13	0.89	0.35
	2008	1.04 \pm 0.03	1.08 \pm 0.04	3.20	0.08	0.05	0.82	0.55	0.46	0.08	0.78
	2009	1.03 \pm 0.03	1.04 \pm 0.04	4.43	0.04	1.05	0.31	0.07	0.79	0.32	0.57
N per leaf area	2007	1.00 \pm 0.02	1.08 \pm 0.02	25.53	<0.01	10.43	<0.01	1.19	0.28	1.34	0.26
	2009	1.13 \pm 0.04	1.09 \pm 0.04	10.26	<0.01	1.66	0.21	0.01	0.91	0.05	0.83
(b) <i>Vaccinium gauthieroides</i>											
Mass per leaf	2007	1.03 \pm 0.04	0.92 \pm 0.03	63.74	<0.01	4.43	0.04	1.65	0.21	0.55	0.46
	2008	0.89 \pm 0.05	0.91 \pm 0.05	30.54	<0.01	0.31	0.58	0.81	0.37	<0.01	0.99
	2009	0.93 \pm 0.04	0.94 \pm 0.05	46.71	<0.01	0.05	0.82	1.80	0.19	0.89	0.35
Area per leaf	2007	1.01 \pm 0.03	0.94 \pm 0.03	50.83	<0.01	2.09	0.16	0.40	0.53	0.48	0.49
	2009	0.81 \pm 0.04	0.87 \pm 0.04	25.48	<0.01	0.81	0.37	1.98	0.17	0.44	0.51
SLA	2007	0.98 \pm 0.02	1.02 \pm 0.02	94.11	<0.01	1.50	0.23	13.39	<0.01	0.15	0.70
	2009	0.89 \pm 0.02	0.95 \pm 0.04	17.08	<0.01	0.93	0.34	0.109	0.74	0.30	0.59
N per leaf mass	2007	0.98 \pm 0.03	1.06 \pm 0.03	11.77	<0.01	4.13	0.05	5.11	0.03	0.73	0.40
	2008	1.10 \pm 0.03	1.18 \pm 0.03	5.83	0.02	2.76	0.11	4.76	0.04	0.01	0.91
	2009	1.01 \pm 0.03	1.06 \pm 0.03	5.69	0.02	0.57	0.46	1.22	0.28	0.59	0.45
N per leaf area	2007	1.00 \pm 0.02	1.04 \pm 0.04	21.32	<0.01	1.61	0.21	0.89	0.35	1.69	0.20
	2009	1.18 \pm 0.05	1.17 \pm 0.07	0.89	0.35	<0.01	0.96	0.12	0.73	2.23	0.15

Table A5. Analysis of covariance and analysis of deviance results for species richness in the experimental plots.

	Total species richness			Vascular plant species richness			Moss and lichen species richness			
	df	F	P	df	F	P	df	Dev	Resid Dev	P
2005 covariable	1, 31	27.31	<0.01	1, 31	38.83	<0.01	1, 38	18.24	40.4	<0.01
CO ₂ level	1, 31	1.51	0.23	1, 31	0.73	0.40	1, 37	1.08	39.30	0.30
Temperature	1, 31	3.51	0.07	1, 31	0.02	0.89	1, 36	5.79	33.51	0.02
Tree species	1, 31	0.06	0.81	1, 31	0.36	0.55	1, 35	0.80	32.71	0.37
CO ₂ level x Temperature	1, 31	1.36	0.25	1, 31	0.40	0.53	1, 34	2.92	29.79	0.09
CO ₂ level x Tree species	1, 31	6.33	0.02	1, 31	8.00	0.01	1, 33	0.32	29.46	0.57
Temperature x Tree species	1, 31	0.20	0.66	1, 31	0.25	0.62	1, 32	0.15	29.32	0.70
CO ₂ x Temp x Tree	1, 31	0.62	0.44	1, 31	0.58	0.45	1, 31	0.01	29.31	0.94

Table A6. Vascular, lichen and moss species most frequently lost from the experimental plots from 2005 to 2009. For each species, the number of plots of each CO₂ and soil temperature treatment combination occupied in 2005 (pre-warming) and in 2009 and the difference between years are given. The change in the total number of plots occupied is also shown. Ten plots were assigned to each of the four treatment combinations, yielding 40 plots total.

Species	Plant growth form	Ambient CO ₂ , unwarmed		change	Ambient CO ₂ , warmed		change	Elevated CO ₂ , unwarmed		change	Elevated CO ₂ , warmed		change	Total change
		2005	2009		2005	2009		2005	2009		2005	2009		
<i>Loiseleuria procumbens</i>	evergreen dwarf shrub	5	4	-1	7	5	-2	5	4	-1	8	4	-4	-8
<i>Hieracium alpinum</i>	forb	5	4	-1	8	7	-1	6	3	-3	4	2	-2	-7
<i>Solidago virgaurea</i>	forb	6	6	0	6	4	-2	8	6	-2	5	4	-1	-5
<i>Melampyrum pratense</i>	forb	6	9	+3	9	5	-4	8	7	-1	9	7	-2	-4
<i>Cetraria islandica</i>	lichen	8	5	-3	6	6	0	6	5	-1	7	3	-4	-8
<i>Dicranum scoparium</i>	moss	7	7	0	6	3	-3	3	3	0	5	1	-4	-7
<i>Polytrichum juniperinum</i>	moss	7	7	0	7	4	-3	2	5	+3	7	2	-5	-5

Chapter 4.

Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO₂

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Abstract

The frequency of freezing events during the early growing season and the vulnerability to freezing of plants in European high-altitude environments could increase under future atmospheric and climate change. We tested early growing season freezing sensitivity in ten species, from four plant functional types (PFTs) spanning three plant growth forms (PGFs), from a long-term *in situ* CO₂ enrichment (566 vs. 370 μmol mol⁻¹) and two-year soil warming (+4 K) experiment at treeline in the Swiss Alps (Stillberg, Davos). By additionally tracking plant phenology, we distinguished indirect phenology-driven CO₂ and warming effects from direct physiology-related effects on freezing sensitivity. The freezing damage threshold (lethal temperature 50) under ambient conditions of the ten treeline species spanned from $-6.7 \pm 0.3^{\circ}\text{C}$ (*Larix decidua*) to $-9.9 \pm 0.6^{\circ}\text{C}$ (*Vaccinium gaultherioides*). PFT, but not PGF, explained a significant amount of this interspecific variation. Long-term exposure to elevated CO₂ led to greater freezing sensitivity in multiple species but did not influence phenology, implying that physiological changes caused by CO₂ enrichment were responsible for the effect. The elevated CO₂ effect on freezing resistance was significant in leaves of *Larix*, *Vaccinium myrtillus* and *Gentiana punctata* and marginally significant in leaves of *Homogyne alpina* and *Avenella flexuosa*. No significant CO₂ effect was found in new shoots of *Empetrum hermaphroditum* or in leaves of *Pinus uncinata*, *Leontodon helveticus*, *Melampyrum pratense* and *V. gaultherioides*. Soil warming led to advanced leaf expansion and reduced freezing resistance in *V. myrtillus* only, whereas *Avenella* showed greater freezing resistance when exposed to warming. No effect of soil warming was found in any of the other species. Effects of elevated CO₂ and soil warming on freezing sensitivity were not consistent within PFTs or PGFs, suggesting that any future shifts in plant community composition due to increased damage from freezing events will likely occur at the individual species level.

Introduction

Plants growing near treeline in the Alps experience seven to eight months of winter and have adapted to survive seasonal low temperatures and persistent snow cover. During autumn, plants change physiologically and undergo a hardening process to reach maximum freezing resistance in mid-winter. As a consequence, very low temperatures during the winter have not been found harmful for fully hardened plants. Additionally, with the exception of windswept areas, low-stature plants and sometimes even trees growing near treeline are thermally insulated by snow cover during winter, which protects them against low temperatures (Sakai and Larcher 1987, Körner 2003). However, freezing conditions can occur year-round in high elevation environments and are particularly common in temperate regions during the early part of the alpine growing season and in early autumn. Physiologically active (dehardened) plants, especially newly developed tissue, are comparatively vulnerable to freezing temperatures and often suffer damage from episodic freezing events during the early growing season. Damage caused by

freezing can have lasting impacts on plant performance, as tissue damage means both a loss of stored carbon and nutrients and of the capacity for photosynthetic carbon gain.

The particular species assemblages occurring in alpine and treeline locations indicate that the present plant species are able to persist over the long term, despite occasional freezing events during the growing season. What is unclear, however, is how plants will cope with altered frequencies and temporal distributions of freezing temperatures in the future, given expected changes in climate and in plant phenology and physiology resulting from the progressive increase in atmospheric CO₂ concentration. Overall warmer conditions and an increase in high temperature extremes during summer relative to mean climatic conditions are expected (Schär *et al.* 2004, IPCC 2007). However, there is also a realistic possibility of an increased frequency of freezing events during periods when plants are active in mountain environments, simply because a warmer climate might advance phenology in many taxa without a concurrent decline in the risk of freezing temperatures (Inouye 2000, 2008). Photoperiodic sensitivity of many alpine plant species safeguards against premature dehardening during warm spells in early spring, but temperature often has a stronger influence on vegetative and reproductive phenology later in the spring when days are longer (Keller and Körner 2003). Further, some opportunistic high-elevation species develop shortly after release from dormancy (snow cover), regardless of photoperiod (Keller and Körner 2003). Declining snow depth and duration in the Alps due to climate warming, already evident during the last century, will likely cause earlier dehardening in these species (Keller *et al.* 2000, Beniston *et al.* 2003, Wipf *et al.* 2009). If late-spring freezing events remain constant or even increase, earlier plant development due to warmer temperatures and/or earlier snow melt will increase the probability of freezing damage to sensitive new plant tissue. On the other hand, mature leaves and shoots are typically less vulnerable to freezing conditions than newly-formed tissue (Taschler *et al.* 2004, Sierra-Almeida *et al.* 2009), and plant tissue that is more developmentally advanced as a result of warmer growing conditions might actually be less susceptible to freezing damage as the growing season progresses.

More frequent and more serious freezing damage has been observed in alpine *Delphinium barbeyi* in the American Rocky Mountains during springs with early snowmelt (Inouye *et al.* 2002) and in dwarf shrubs experiencing experimentally advanced snowmelt in the Swiss Alps (Wipf *et al.* 2009). Similarly, several studies have found that warmer temperatures impact growing season freezing sensitivity through changes in springtime development. Accelerated tissue dehardening, and therefore reduced freezing resistance during spring, has been observed after experimental warming throughout winter (without snow cover manipulation) in subarctic *Vaccinium myrtillus* (Taulavuori *et al.* 1997a, 1997b), in boreal *Pinus sylvestris* saplings (Repo *et al.* 1996), and in *Betula pubescens* seedlings (Taulavuori *et al.* 2004). However, no effect of experimental warming during the growing season was found for subarctic *Empetrum hermaphroditum* (Ögren 2001). To our knowledge, no studies have previously investigated the possibility of warming-induced shifts in the dehardening process of alpine treeline plants.

Atmospheric CO₂ enrichment has also been found to reduce freezing resistance but, unlike warming, this phenomenon has only rarely been observed in conjunction with advanced phenology (but see Repo *et al.* 1996). Reduced growing season freezing resistance in response to elevated CO₂ has been documented for forbs and grasses grown in a native temperate grassland (Obrist *et al.* 2001) and for field-grown dwarf shrubs from a subarctic heath community (Beerling *et al.* 2001). The same result was found for saplings of chamber-grown *Pseudotsuga menziesii* (Guak *et al.* 1998) and *Ginkgo biloba* (Terry *et al.* 2000), and for *Eucalyptus pauciflora* seedlings in an OTC field experiment (Lutze *et al.* 1998, Barker *et al.* 2005, Loveys *et al.* 2006). Although mechanisms for increased sensitivity of CO₂-enriched plants have not been determined, several authors have speculated that previously-documented physiological and chemical changes in response to elevated CO₂ are involved. No shift in freezing resistance under elevated CO₂ has been found for *Picea abies* saplings (Wiemken *et al.* 1996), for subarctic *Vaccinium vitis-idaea* (Taulavuori *et al.* 2001), or for *Picea mariana* seedlings (Bigras and Bertrand 2006). Enhanced freezing resistance has even been observed for dormant winter buds of *Betula alleghaniensis* seedlings (Wayne *et al.* 1998) and for *Yucca* seedlings from the Mojave Desert (Loik *et al.* 2000).

The contrasting freezing resistance responses to elevated CO₂ reported in previous studies may be related to species-specific effects, to differences in growing conditions or in duration of CO₂ exposure, or to different ontogeny, which is particularly important for trees (Sakai and Larcher 1987). It is also possible that results were influenced by methodological differences including detached or attached plant tissue, rate and duration of freezing, and damage assessment technique (Taschler and Neuner 2004, Bannister 2007). Finally, shifts in freezing resistance under elevated CO₂ have consistently been reported as less than 2 K (e.g. Lutze *et al.* 1998, Beerling *et al.* 2001, Obrist *et al.* 2001), and studies reporting no CO₂ effect often used freezing temperature increments too large to detect such subtle differences (e.g. Wiemken *et al.* 1996, Taulavuori *et al.* 2001, Bigras and Bertrand 2006).

Plant growth form (PGF) and plant functional type (PFT) classification systems can be valuable tools for predicting plant responses to climate change in cases where differences among groups remain consistent under changing environmental conditions. For example, Chapin *et al.* (1996) found that, for a range of arctic species, those from the same PFT showed similar long-term responses to changes in nutrient availability and soil moisture. Also, similar phenological and growth responses to experimental warming have been reported within PFTs (Arft *et al.* 1999, Dunne *et al.* 2003). However, shifts in the freezing resistance of co-occurring species under atmospheric and climate change have not yet been compared at the PGF or PFT level. As plant height (Squeo *et al.* 1991) as well as deciduousness and woodiness (Taschler and Neuner 2004) have been found to influence freezing resistance, both PGF and PFT classifications are potentially informative for predicting changes.

In the present study, we investigated growing season freezing sensitivity of a range of different plant species from a long-term *in situ* CO₂ enrichment and two-year soil warming experiment at treeline in the Swiss Alps. In this experiment, we previously observed increased *in situ* leaf tissue damage under elevated CO₂ in *Vaccinium myrtillus* ($F_{1,8} = 5.54$, $P = 0.047$) but not in *V. gaultherioides* ($F_{1,8} = 0.01$, $P = 0.93$) after a natural freezing event in June 2005 (I.T. Handa *et al.*, unpublished data). We also found a trend of increased damage under elevated CO₂ in *Larix decidua* after freezing conditions in late May 2007 ($F_{1,8} = 3.80$, $P = 0.087$; M. Martin, unpublished data). These observations prompted us to examine this effect further through an experimental freezing study including ten prominent species from four PFTs spanning three PGFs. We aimed to determine how atmospheric CO₂ enrichment and soil warming affect the freezing sensitivity of different alpine treeline species, PFTs and PGFs. By additionally tracking phenology of the selected species, we determined if treatment effects on freezing resistance were associated with changes in phenology. Our primary hypotheses were that (1) CO₂ enrichment would negatively affect early growing season freezing resistance but that this effect is intrinsic to CO₂ and not associated with a phenological shift induced by the treatment and (2) soil warming would advance phenology, leading to more mature leaf tissue in the early growing season and, therefore, reduced freezing sensitivity of fully expanded leaves. We also predicted that (3) plants of different PFT and PGF groups would have distinct freezing damage thresholds under current conditions, and plants within the same group would respond similarly to the experimental treatments.

Materials and methods

Site description

The study site is located at Stillberg, Davos in the Central Alps, Switzerland (9° 52' E, 46° 46' N), where a free air CO₂ enrichment (FACE) experiment was set up in 2001 (Hättenschwiler *et al.* 2002). The FACE experiment is situated at or slightly above the natural climatic treeline (2180 m a.s.l.) on a NE-exposed 25 to 30° slope. 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 (WSL). For the FACE experiment, 40 hexagonal 1.1 m² plots were established, 20 with a European larch (*Larix decidua*) in the centre and 20 with a mountain pine (*Pinus mugo* ssp. *uncinata*) in the centre. Although these trees planted at treeline are ca. 35 years old, they are not taller than 3 m and have a stem basal diameter of less than 10 cm. The trees are widely spaced and do not form a closed canopy, thus allowing for a dense heath-like understorey layer in each experimental plot surrounding the tree base (see Hättenschwiler *et al.* 2002 for additional information about the site and experimental setup). CO₂ enrichment (multiple-year mean $566 \pm 75 \mu\text{mol mol}^{-1}$ vs. ca. $370 \pm 3 \mu\text{mol mol}^{-1}$ ambient) has been supplied throughout each growing season since 2001; the technical setup and performance of the CO₂ enrichment facility has been described in detail previously (Hättenschwiler *et al.* 2002, Handa *et al.* 2005). In the summer of 2007, a soil warming treatment (growing season

mean +4 K) was introduced to the FACE experiment by laying heating cables on the ground underneath the dwarf shrub layer (details in Hagedorn *et al.* 2010).

The resulting experimental design was a completely randomized split-split-plot model: 10 whole-plots, each consisting of four individual trees and their associated understorey layer, were randomly assigned to a CO₂ treatment, two split-plots within each whole-plot were randomly assigned a soil warming treatment, and finally one of the two tree species (larch or pine) was present at the split-split-plot level (hereafter simply referred to as 'plot'). For the freezing experiment, therefore, each tree species had up to five replicates of the four individual combinations of CO₂ and warming treatments. Three of the pines died prior to 2008, which resulted in a replication of four in three of the treatment combinations. The maximum replication of five was also applicable to measurements made on understorey plants in the freezing experiment because we distinguished between larch or pine presence in the plots, resulting in a total of eight treatment combinations. Each understorey species was present in at least 30 of the 40 experimental plots to allow for sufficient replication of each treatment combination.

Air temperature data (height at 2 m above ground) was available from a climate station located on Stillberg at 2090 m a.s.l., approximately 100 m below the FACE site. Additionally, HOBO Pro v2 dataloggers (Onset Computer Corporation, Bourne, MA, USA, part U23-003) were installed in the canopy of eight trees within the experiment (4 pine, 4 larch; height at 1 to 2 m above ground) to obtain a closer estimate of daily air temperature minima throughout the growing season.

Plant species and field sampling

We sampled a total of 10 plant species from the experimental plots, spanning four plant functional types (classification after Chapin *et al.* 1996): a) deciduous woody: tree *Larix decidua* Mill. (European larch) and dwarf shrubs *Vaccinium myrtillus* L. (bilberry) and *Vaccinium gaultherioides* Bigelow (group *V. uliginosum* agg.; northern bilberry); b) evergreen woody: tree *Pinus mugo* ssp. *uncinata* (DC.) Domin (mountain pine) and dwarf shrub *Empetrum nigrum* ssp. *hermaphroditum* (Hagerup) Böcher (crowberry); c) forb: *Gentiana punctata* L., *Homogyne alpina* (L.) Cass., *Leontodon helveticus* Mérat, and *Melampyrum pratense* L.; and d) graminoid: *Avenella flexuosa* (L.) Drejer. The selected species also represented three plant growth forms: tree, shrub, and herb. Names of the listed species are according to Lauber and Wagner (2007).

We completed six sampling events during the early growing season of 2008 (days 169 to 209), with up to three plant species tested on a single date (Fig. 1). Each species was sampled only once, as limited plant material available for destructive sampling and space constraints in the freezing units prevented multiple tests. The one exception was *Larix*, for which both short shoots (leaf fascicles on last-year-wood; day of year 169) and long shoots (expanding new-wood growth; day 188) were used. Newly-formed leaf and shoot tissue was sampled upon full leaf expansion. As a result, each species was tested at a

similar point relative to leaf phenology, but calendar date and number of days since snowmelt varied.

Springtime phenology was quantified for each species present in a given plot by estimating the percentage of plant cover that had experienced a) leaf bud break and b) at least 50% of full leaf expansion (Hartley *et al.* 1999). We inspected individual ramets from all regions of each plot and averaged out any small-scale variation due to microsite differences for a percentage estimate at the plot level for a given species. Observations were made weekly from snowmelt to early July (days of year 154, 161, 168 and 175).

Freezing experiments

Freezing resistance was determined using detached tissue collected between 0700 and 0900 in the morning. For *Vaccinium myrtillus*, *V. gaultherioides*, and *Melampyrum*, one or two (depending on availability) entire individual leaves per experimental plot were used for each freezing treatment. For forbs with large leaves (*Leontodon*, *Gentiana* and *Homogyne*), two 5 mm leaf discs were used instead (Gurvich *et al.* 2002, Bannister 2007). Whole leaf fascicles were used for *Pinus* and short shoots of *Larix* (first sampling), and the newly-grown shoot increment (with leaves attached) was used for *Empetrum* and long shoots of *Larix* (second sampling). For *Larix* and *Pinus*, material was sampled from the top half of the tree on the downslope (NE) side. Grass blades (*Avenella*) were cut into approximately 1 cm sections to standardize the size of sample material. All samples were rinsed with deionized water to remove surface impurities, blotted dry, and subsequently wrapped in aluminium foil to (a) prevent desiccation during freezing and (b) ensure a homogenous thermal environment during freezing cycles (Obrist *et al.* 2001). Samples were kept in darkness at 5°C until freezing, which occurred within 12 h of collection.

Freezing conditions were implemented overnight and different freezing temperature treatments were applied concurrently, rather than sequentially as is common practice, to most closely simulate naturally-occurring summer freezing events. One sample per species per experimental plot was inserted into each of six polystyrene-insulated cylindrical aluminium freezing chambers (radius 4 cm, depth 7 cm), as in Obrist *et al.* (2001). Each of the six freezing chambers was used for a different final target temperature in order to create a temperature gradient spanning the species-specific critical range. Samples from each tested species were arranged on small racks within the freezing chambers; this arrangement was consistent for all freezers with respect to experimental plots to account for any temperature gradient effect within the chambers. Temperature inside each chamber was regulated separately by a computer-controlled Peltier cooling element and constantly monitored by thermocouples attached to extra leaf samples. After initial reduction to 0°C, temperature within each freezer was linearly decreased 1 K every 20 minutes (3 K h⁻¹) and then held at a target value. The precise duration at each specific target value was shorter with each increment to a colder target temperature (20 min shorter per 1 K), in accordance with typical natural freezing events

at the site. This slow cooling process also helped reduce the possibility of either excessive supercooling or failure to nucleate (Sakai and Larcher 1987), artefacts that have been observed for small, excised samples (Neuner *et al.* 1997). The span of target temperatures for the different freezers varied for each freezing cycle, based on preliminary tests for each species, ranging from -3 to -14°C. After being held at the target temperature for about 5 hours, freezing chambers were switched off to gradually return samples to room temperature, not exceeding 6 K h⁻¹. Samples were then kept in darkness at 5°C. In addition to the six custom-made freezing chambers, we included a control (5°C) and a maximum damage (-30°C) treatment using a standard household commercial unit, providing a total of eight treatment units.

Damage assessment

Leaf tissue damage was determined both by the electrolyte leakage (EL) method (Bernier-Cardou and Bigras 2001) and by visual scoring (e.g. Obrist *et al.* 2001, Taschler and Neuner 2004). After freezing, samples were unwrapped and transferred to polypropylene vials with 10 ml of deionized water, shaken, and stored in darkness at 8°C for 24 h to allow ions to leach across damaged cell membranes. Conifer needle samples were cut into 5 mm segments beforehand, as this step has been shown to improve freezing damage detection with the EL method (Burr *et al.* 2001). Electrical conductivity of the solution was measured once, and sealed vials were then heated at 100°C using an oven for 1 h to completely disrupt all cell membranes. Another 24 h lapsed (conditions as above) followed by a second electrical conductivity measurement, thus allowing us to express electrolyte leakage after freezing as a percent of maximum electrolyte leakage [Relative Electrolyte Leakage (REL)]. REL values are not influenced by the amount of material sampled, by routine mechanical damage caused by the sample preparation method, e.g. leaf disc punching, or by the inherent membrane permeability of individual species.

Freezing resistance of plant tissue was expressed as Lethal Temperature 50 (LT50), the estimated temperature at which half of the samples were killed (Sakai and Larcher 1987). There is generally only a narrow range of temperatures between no damage (low REL) and complete damage (high REL), which allows a relatively precise calculation of this value. LT50 was determined by fitting a sigmoid curve (Boltzmann equation, Origin v.8 software, OriginLab Corporation, Northampton, MA, USA) through the eight individual REL data points for each species from an individual plot and calculating the inflection point (Burr *et al.* 1990, Taschler *et al.* 2004). In cases where the sigmoid curve did not have a clear inflection point, the corresponding LT50 value was omitted from statistical analysis (10% of the total number of LT50 value calculations). Damage induced by freezing was also assessed visually and quantified as the proportion of plant tissue with altered colouration and/or loss of turgor (Obrist *et al.* 2001). Though not feasible for all species, this basic yet reliable technique (Ritschie 1991) helped to verify that high REL values actually corresponded to freezing damaged tissue. Pooled across the species for which visible assessment was possible (*V. myrtillus*, *V. gaultherioides* and *Homogyne*),

REL measurements and visual damage score showed a strong correlation ($R^2 = 0.845$). This comparison rendered the electrolyte leakage method a reliable proxy for freezing damage; hence, only REL results were used for further data analysis.

Statistical analysis

LT50 values were analyzed according to our completely randomized split-split-plot design. Type I analysis of variance was performed by fitting a linear mixed model, using the restricted maximum likelihood method (REML) to account for cases where species were not present in all plots. The model for all tested plants together included plant species, CO₂ and temperature treatments, and all two- and three-way interactions as fixed effects. As tree type (larch or pine presence in a plot) was not applicable to the two tree species in the model, this factor could not be included as a fixed effect. For the complete model, we explored different ways of including LT50 values of *Larix* short and long shoots: only short shoots, only long shoots, both shoot types, and mean values from the two types. Different approaches yielded nearly identical results, and mean values were ultimately used in the complete model. Based on the results from this overall analysis, we also tested for treatment effects on LT50 for each species individually. For *Larix* and *Pinus*, “tree” was omitted from the data structure (split-plot model). Short and long shoots of *Larix* were tested separately at this more detailed level of analysis to investigate potential differences in the response of short and long shoots.

The effect of plant growth form (PGF: tree, shrub or herb) and its interaction with the experimental treatments were tested with a statistical model analogous to that for all plant species together. The effect of plant functional type (PFT) was tested in the same way as PGF. *Avenella flexuosa* was excluded from the PFT analysis because it was the only grass species, leaving a total of three PFTs: deciduous woody (three species), evergreen woody (two species) and forb (four species). We used a Tukey multiple comparisons test to compare the LT50 values of individual PFTs (Hothorn *et al.* 2008). Although post-hoc tests can give inaccurate results when applied to structurally complex statistical models (Quinn and Keough 2002), the lack of significant interactions between main effects in the overall model justified such an analysis in this case.

For statistical analysis of phenology, we considered only *Larix*, *Pinus*, *Vaccinium myrtillus*, *V. gaultherioides*, and *Empetrum* because limited presence and unclear phenological stages prevented accurate analysis of the forb and grass species. Statistical tests were completed for each species and observation date separately, using the same fixed and random effects as in the model for LT50 values of individual species described above. The percentages were arcsine(sqrt) transformed in the model to satisfy the requirement of linearity. Percent budburst and/or percent expanded leaves were only tested statistically on dates where variation was observed among the plots for a given species (not all 0 or 100%). Leaf budburst (all four observation dates) and leaf expansion (last three dates) were tested for *V. myrtillus*, whereas only budburst was tested for *V. gaultherioides* (all four dates). Expansion of short shoot leaves on all four dates was

analyzed for *Larix*. Budburst on the last observation date (175) was the only data used for both *Empetrum* and *Pinus*.

For all statistical analyses, assumptions of linearity and constant variance were checked visually using diagnostic plots. Results with P values ≤ 0.05 were considered significant. Replication was limited to five in this study due to the pre-existing design of the long-term FACE experiment, and consequently results with lower statistical significance might have had ecological significance. To acknowledge this possibility, P values > 0.05 but ≤ 0.10 were considered marginally significant. All analyses were performed using R version 2.7.2 (R Development Core Team 2008-2010).

Results

Snowmelt date and air temperature

Snow was completely melted from each of the experimental plots between days 145 and 154 of 2008. Daily minimum air temperature in the canopy of FACE trees ranged from 0 to 10°C during the sampling period between day 169 and day 209 (Fig. 1).

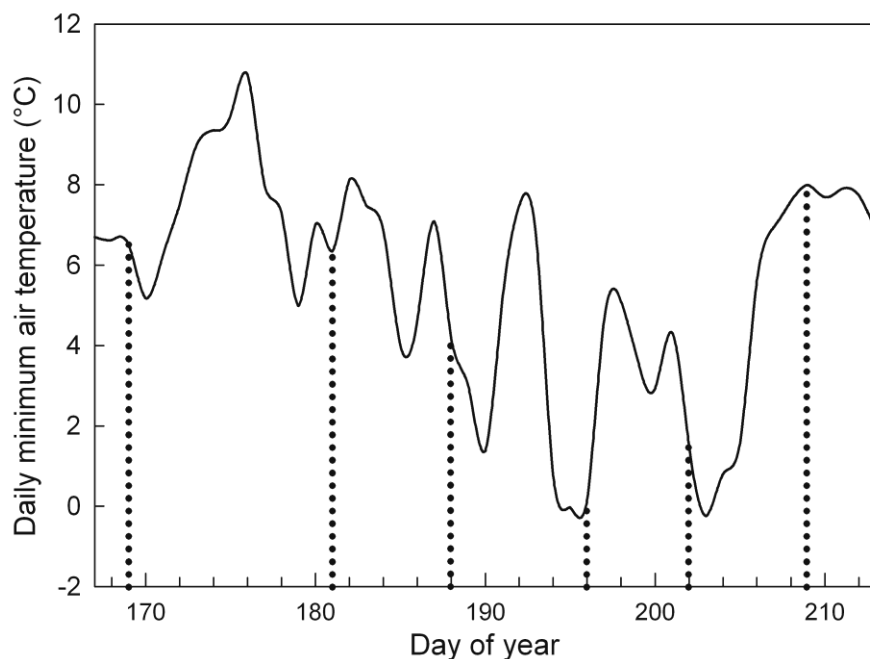


Figure 1. Period of plant sampling during the summer of 2008. The six freezing trials (dotted vertical lines) included the species: day 169 = *Larix decidua* (short shoots); 181 = *Gentiana punctata*; 188 = *Larix decidua* (long shoots); 196 = *Homogyne alpina*, *Leontodon helveticus*, and *Melampyrum pratense*; 202 = *Empetrum hermaphroditum*, *Vaccinium gaultherioides*, and *Vaccinium myrtillus*; 209 = *Avenella flexuosa* and *Pinus uncinata*.

Plant leaf phenology

Overall, we observed only few significant treatment effects on plant phenology, and none of the interactions between the main factors CO₂, warming, and tree species identity in the plot (if applicable) were significant. Of the five species for which we could analyze leaf phenology, only *Empetrum* showed an effect of CO₂ enrichment (marginally significantly lower percent budburst in elevated CO₂ plots on day 175, $F_{1,8} = 3.61$, $P = 0.094$). *Vaccinium myrtillus* was the only species that showed some effect of soil warming on phenology (Fig. 2). Leaves expanded earlier in warmed plots, with a higher percent of leaves >50% expanded on days 161 ($F_{1,8} = 4.36$, $P = 0.070$, marginally significant) and 168 ($F_{1,8} = 5.48$, $P = 0.047$, significant) but not on days 154 or 175 (Fig. 2). There was no significant warming effect on leaf budburst. Neither of the experimental treatments significantly affected leaf phenology of *Larix* or *Pinus*.

Leaf phenology of the two deciduous dwarf shrub species was influenced by the identity of the tree species in the plot, irrespective of the other treatments. *V. gaultherioides* growing under pine showed earlier budburst than when growing under larch, with significant differences on days 161 ($F_{1,16} = 8.00$, $P = 0.012$) and 168 ($F_{1,16} = 12.12$, $P = 0.003$), and a non-significant trend in the same direction on the two other observation dates (day 154, $F_{1,16} = 2.84$, $P = 0.111$ and day 175, $F_{1,16} = 2.28$, $P = 0.151$; Fig. 3). The opposite pattern was observed in *V. myrtillus*, for which plants growing under pine showed marginally significantly lower percent budburst on day 161 only ($F_{1,16} = 3.95$, $P = 0.064$) and marginally significantly lower percent expanded leaves on day 168 only ($F_{1,16} = 3.95$, $P = 0.064$; Fig. 2).

Freezing sensitivity of co-occurring plant species, functional types and growth forms

The freezing damage threshold (LT50) of the ten treeline species under ambient CO₂ and temperature conditions spanned from $-6.7 \pm 0.3^\circ\text{C}$ (*Larix*) to $-9.9 \pm 0.6^\circ\text{C}$ (*V. gaultherioides*), and plant species varied significantly in their LT50 in our complete model ($F_{9,241} = 27.69$, $P < 0.0001$; Fig. 4). PFT explained a significant amount of the observed interspecific variation ($F_{2,230} = 7.18$, $P = 0.001$): the deciduous woody group was significantly more resistant than the forbs ($z = 2.52$, $P = 0.031$), while the evergreen woody group had intermediate resistance and did not differ significantly from the other two groups. PGF did not significantly influence freezing resistance ($F_{2,269} = 0.48$, $P = 0.621$). Within the tree growth form, deciduous *Larix* was the most sensitive to freezing conditions out of all tested species, whereas new foliage of evergreen *Pinus* was one of the hardiest. The two deciduous dwarf shrub species varied widely in their freezing resistance, with *V. gaultherioides* showing the most resistant leaves of all species tested. Both deciduous dwarf shrubs tolerated colder temperatures than the evergreen species *Empetrum*, the opposite pattern as that observed in the trees. Among the herbaceous species, *Homogyne*, *Leontodon* and *Melampyrum* had LT50 values within 0.3 K of each other, but *Gentiana* and the alpine grass species *Avenella* showed greater resistance to freezing.

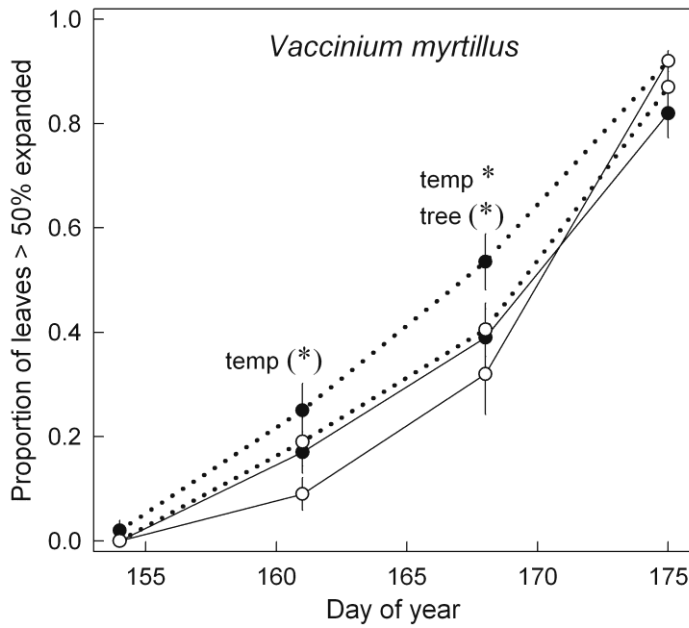


Figure 2. Effects of soil warming (solid line = control temperature, dotted line = warmed) and identity of the tree species (larch or pine; filled circle = growing under larch, open circle = growing under pine) in the experimental plot on leaf phenology of *Vaccinium myrtillus*. The proportion of leaves at least 50% expanded are shown. Mean values across all plots per treatment combination \pm 1 standard error are presented. Treatment effects are marked as marginally significant: $P \leq 0.10$, (*); and significant: $P \leq 0.05$, *. For each of the four treatment combinations shown, $n = 10$ plots (averaged across CO_2 treatments).

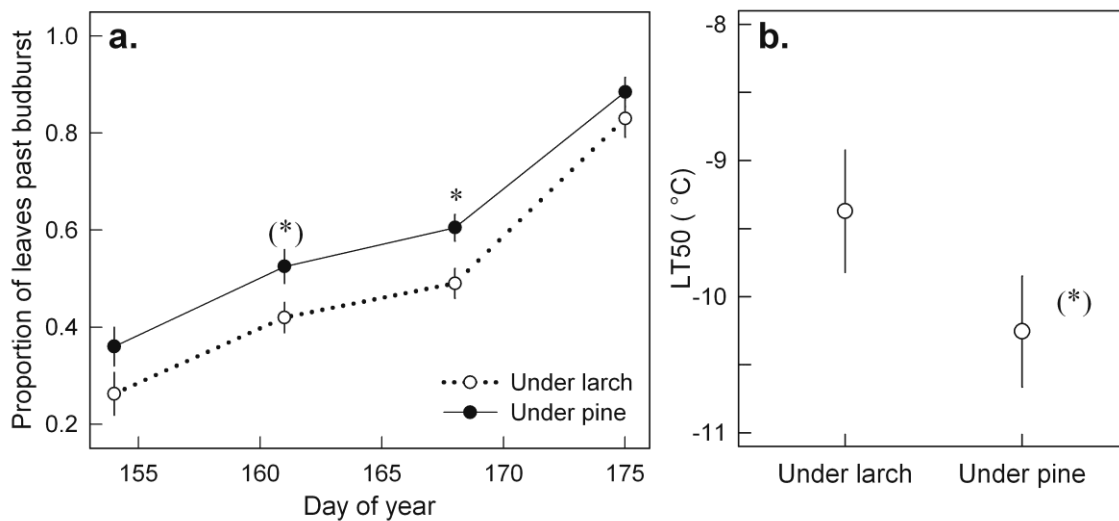


Figure 3. Phenology (a) and freezing resistance (b) of *Vaccinium gaultherioides* as affected by the tree species (larch or pine) under which it was growing. (a) time series of the proportion of leaves past budburst under the two different tree species ($n=20$ plots, mean across CO_2 and warming treatments); (b) effect of tree species on freezing resistance ($n=20$ plots, mean across CO_2 and warming treatments). Mean values across all plots per treatment combination \pm 1 standard error are presented. Treatment effects are marked as marginally significant: $P \leq 0.10$, (*); and significant: $P \leq 0.05$, *.

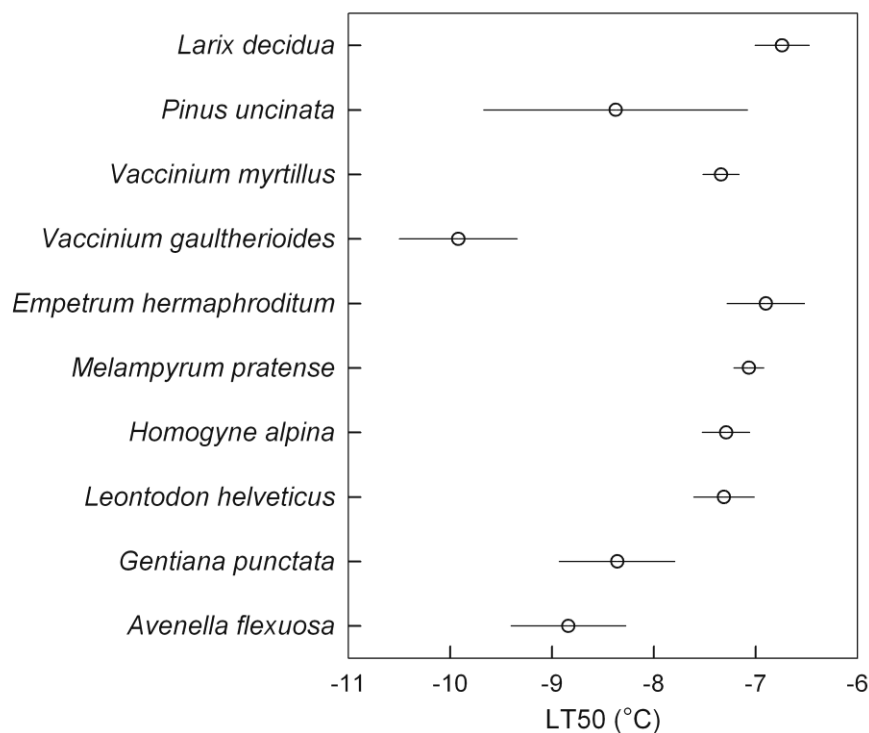


Figure 4. Freezing sensitivity of individual treeline plant species. Only plants that experienced ambient CO₂ and no soil warming treatments are shown. Mean LT50 values across all plots per treatment combination ± 1 standard error are presented. Sample size in each treatment group was determined by species presence in the plots and missing samples. n = 10 for *Larix decidua*, mean across short and long shoots; n = 4 for *Pinus uncinata*; n = 10 for *Vaccinium myrtillus*, *Vaccinium gaultherioides*, *Homogyne alpina*, and *Leontodon helveticus*; n = 9 for *Melampyrum pratense*; n = 5 for *Empetrum hermaphroditum* and *Gentiana punctata*. For all understory species, mean across larch and pine tree identity are shown.

Effects of elevated CO₂ and soil warming on freezing sensitivity

Plants at the Stillberg treeline site experienced increased susceptibility to early growing season freezing events when grown under elevated atmospheric CO₂ (LT50 across all species: $F_{1,8} = 16.38$, $P = 0.004$). When plant species were investigated individually, the negative CO₂ effect on freezing resistance was significant for three, and marginally significant for two, of the 10 studied species. LT50 was reached at significantly higher temperatures in *Larix* short shoots ($+1.0 \pm 0.3$ K; $F_{1,8} = 8.93$, $P = 0.017$), in *V. myrtillus* ($+0.5 \pm 0.2$ K; $F_{1,8} = 9.91$, $P = 0.014$), and in *Gentiana* ($+1.2 \pm 0.4$ K; $F_{1,7} = 9.21$, $P = 0.019$) growing under elevated CO₂ compared to ambient CO₂ (Fig. 5). The negative effect of CO₂ on freezing resistance was marginally significant in *Homogyne* ($+0.7 \pm 0.3$ K; $F_{1,8} = 3.92$, $P = 0.083$) and *Avenella* ($+1.2 \pm 0.6$ K; $F_{1,8} = 3.91$, $P = 0.083$; Fig. 5). At the given resolution of our experiment, no CO₂-induced effect on freezing resistance was observed in *Larix* long shoots, *Pinus*, *Empetrum*, *V. gaultherioides*, *Leontodon* and *Melampyrum* (Fig. 5).

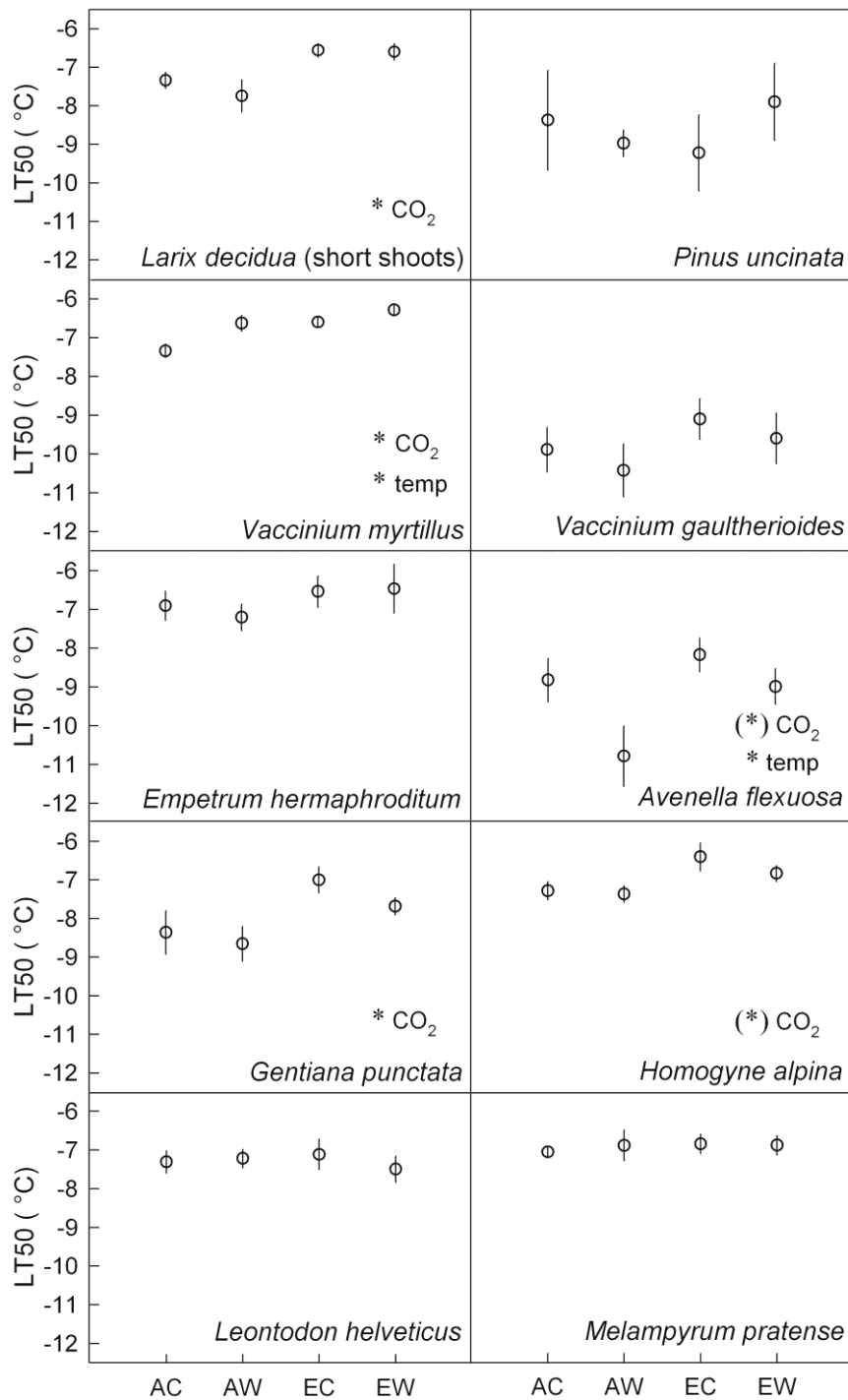


Figure 5. CO₂ (ambient = A, elevated = E) and soil warming (control = C, warmed = W) treatment effects on the freezing resistance (LT50) of treeline plant species at Stillberg. Mean LT50 values across all plots per treatment combination ± 1 standard error are presented. Treatment effects are marked as marginally significant: P ≤ 0.10, (*); and significant: P ≤ 0.05, *. Sample size in each treatment group was determined by species presence in the plots and missing samples. For trees, n = 3 to 5; for understorey plants, n = 5 to 10 and means across larch and tree identity are shown.

Soil warming did not have a consistent effect on freezing sensitivity across the studied species (LT50 across all species: $F_{1,8} = 2.14$, $P = 0.182$). However, species differed in how their freezing sensitivity was affected by warming, and we found a marginally significant species by warming interaction in the analysis of all species together ($F_{9,241} = 1.69$, $P = 0.092$). Soil warming significantly increased freezing sensitivity in *V. myrtillus* ($+0.5 \pm 0.2$ K; $F_{1,8} = 9.25$, $P = 0.016$), but the opposite was found for *Avenella* (-1.4 ± 0.6 K; $F_{1,8} = 5.37$, $P = 0.049$; Fig. 5). None of the remaining eight species showed a significant warming effect on freezing sensitivity (Fig. 5).

The identity of the tree species in the experimental plot (larch or pine) had only a marginally significant effect on freezing sensitivity in two of the understorey plants. Under pine, freezing damage was detected at a warmer temperature for *Empetrum* ($+1.1 \pm 0.5$ K; $F_{1,2} = 9.57$, $P = 0.091$) but at a lower temperature for *V. gaultherioides* (-0.9 ± 0.6 K; $F_{1,12} = 3.34$, $P = 0.092$; Fig. 3). None of the two- or three-way interactions involving elevated CO₂, soil warming and tree species identity were significant. Responses to the experimental treatments were not consistent within the three PFTs or within the three PGFs (Fig. 5).

Discussion

Distinct freezing damage threshold for co-occurring plant species

Our freezing study was novel for including several species, from three plant growth forms and four functional types, growing in a natural treeline setting. During the early growing season, all plant species sampled had a mean freezing damage threshold (LT50) between -6.7 and -9.9°C when grown under ambient conditions. The narrow span of this range, despite high diversity of growth form and life strategy among species, can be explained by the common local temperature regime to which the sampled plants are adapted and acclimated (Gurvich *et al.* 2002, Körner 2003). Similar peak growing season LT50 values from -5.5 to -9.5°C have been reported for alpine forbs and grasses at the Furka Pass (2470 m a.s.l.) in the Swiss Alps (Körner 2003). While the definition of freezing damage, e.g. initial vs. complete damage, varies among studies, a similar range of critical temperatures has been observed for non-dormant trees, dwarf shrubs, and herbaceous plants growing in other high-elevation (Sakai and Larcher 1987, Taschler and Neuner 2004) and high-latitude (Beerling *et al.* 2001) locations.

We predicted that plants of different functional types and growth forms growing at the treeline would have distinct critical freezing temperatures under current conditions. Across all treatments, classification into three PFTs (grass PFT excluded) based on deciduousness and woodiness explained some of the variation among species. The forb group was significantly more sensitive to freezing than the deciduous, but not the evergreen, woody group. No difference was observed between deciduous and evergreen woody species, likely because the effect of deciduousness on dwarf shrubs was in the

opposite direction as that on trees. This surprising result could reflect other characteristics of the individual species available for testing, such as microclimate preference and changes in freezing resistance throughout the early growing season. Given the high variability observed among the 10 species sampled, a clear understanding of the importance of deciduousness or woodiness in the freezing resistance of plants growing near treeline would require sampling many more species than were available in our experimental plots.

Regarding plant growth form, we predicted that trees would be more sensitive to freezing than low stature dwarf shrubs and herbaceous plants because they experience greater conductive heat exchange (Squeo *et al.* 1991). However, PGF did not help explain differences among species in LT50 and there was high variability in freezing resistance within PGFs. It is possible that a survey including many species would reveal patterns regarding PGFs, although a previous study including 33 treeline and alpine species also showed no correlation between plant growth form and freezing resistance (Taschler and Neuner 2004). Within the tree growth form, deciduous *Larix* was the species most sensitive to freezing conditions during the early growing season, whereas new foliage of evergreen *Pinus* tolerated lower temperatures. In contrast, the average growing season LT50 value of *Larix decidua* long shoots, sampled from June through August at an Austrian treeline site, was found to be more freezing resistant than other woody species (Taschler *et al.* 2004).

Among shrubs, the two closely related deciduous *V. myrtillus* and *V. gaultherioides* diverged widely in their freezing resistance. *V. gaultherioides* extends >3000 m in the Alps and, as to be expected, was more freezing-tolerant than *V. myrtillus*, which has a montane centre of distribution and has previously been found to have low freezing tolerance when active (Taschler and Neuner 2004). *V. gaultherioides* occupies microhabitats with a shorter snow duration than *V. myrtillus* (Körner 2003), which likely also contributed to its greater freezing resistance (Bannister *et al.* 2005). Similarly, the evergreen dwarf shrub *Empetrum* is known for its obligatory snow cover requirement in winter (Tybirk *et al.* 2000) and reached LT50 at a higher temperature than the other two dwarf shrubs. Acute freezing desiccation upon snow removal during winter has been reported for *Rhododendron ferrugineum*, another species requiring snow cover for protection against winter freezing conditions (Larcher and Siegwolf 1985). However, new shoots of *Empetrum* were tested approximately three weeks closer to budburst than the deciduous dwarf shrubs, and this difference in timing could have also played a role in its lower freezing resistance. Within the herbaceous PGF, three of the four forb species had similar thresholds of freezing damage and were close to the -7°C threshold commonly observed for herbaceous alpine and arctic plants (Körner 2003, Körner and Alsos 2008). The grass *Avenella* was more freezing resistant, consistent with previous evidence that graminoids tend to resist lower temperatures than broad-leaved species (Gurvich *et al.* 2002, Körner 2003, Taschler and Neuner 2004, Hacker and Neuner 2008).

Comparisons of LT50 values among plant species, functional types and growth forms are unavoidably influenced by sampling date. In our study, we chose to sample each species as soon as possible after full leaf expansion to minimize differences in LT50 among species due to differences in leaf developmental stage and also to standardize across species the stage tested for CO₂ and warming treatment effects. This decision, along with spatial constraints in the freezing chambers, led us to sample individual species on different dates over a 40-day period. Therefore, our comparison of LT50 values among species might reflect variation in the recent temperature history before sampling. However, relatively consistent air temperature, specifically no freezing conditions, for the duration of the study likely reduced this timing effect (Fig. 1). The alternative approach, though not feasible in our study, of sampling all species on the same date would have permitted a more realistic comparison of relative freezing resistance on one specific date but would have been more influenced by differences in developmental stage of the compared species.

For comparisons of LT50 values between species, it is also important to note that the freezing process potentially initiated with different degrees of supercooling for different species. In particular, small, cut samples have a tendency to supercool to lower temperatures before ice nucleation occurs compared to intact leaves and stems (Neuner *et al.* 1997). Constraints on the availability of plant material and space in the freezing chambers necessitated segmentation of *Avenella* leaf blades and the use of leaf discs for *Gentiana*, *Homogyne* and *Leontodon*, and consequently these species might have been more likely to experience delayed ice nucleation. However, our protocol using a slow, natural cooling rate followed by several hours at the target freezing temperature reduced the possibility of excessive supercooling (Sakai and Larcher 1987). We observed a range of LT50 values that corresponded closely to values documented previously for alpine plants (Körner 2003), which suggests that the freezing process was indeed realistic. Therefore, while it is important to recognize the potential effect of excessive supercooling on the observed LT50 values, we are confident that any delayed ice nucleation did not strongly affect our comparison among species. Important for the main study objectives, we also expect that any supercooling effects in a given species were systematic across all experimental treatments. As freezing occurred at higher temperatures under elevated CO₂ than in ambient conditions, the same nucleation delay in both elevated and ambient CO₂ treatments would have tended to reduce the CO₂ signal by freezing at lower temperatures than with undelayed nucleation. Hence, our analysis and conclusions regarding the effect of elevated CO₂ are conservative.

Effects of simulated atmospheric and climate change on plant freezing resistance

In our study, multiple treeline plant species grown in a CO₂-enriched atmosphere showed increased sensitivity to early growing season freezing events. This result supports the well-established pattern of reduced freezing resistance in plants growing under elevated CO₂ (for a review see Woldendorp *et al.* 2008) and provides the first evidence of this effect for treeline plants *in situ*. However, the three dwarf shrub species we studied

showed different responses than their European subarctic counterparts. We found that freezing resistance of *V. myrtillus* decreased in response to elevated CO₂ at our alpine treeline site, whereas no response was detected in low-elevation *in situ* studies of the same species in a subarctic heathland community (Abisko Scientific Research Station; Beerling *et al.* 2001). Conversely, *V. gaultherioides* and *Empetrum hermaphroditum* were not affected by CO₂ enrichment in our study but both experienced a slight (less than 1 K) but significant increase in ice nucleation temperature under elevated CO₂ during the early growing season at the subarctic Abisko site (Beerling *et al.* 2001). These inconsistencies could be due to the potential difference in physiological characteristics between populations in northern Scandinavia and in the Alps. In particular, *Empetrum* species and *V. gaultherioides* are two highly heterogeneous species complexes with, at present, unclear systematic positions (Bell and Tallis 1973, Jacquemart 1996). The freezing resistance response of an individual species or species complex to CO₂ enrichment can apparently vary depending on general environmental conditions or plant genotype, making habitat-specific field studies important for predicting future plant responses. It is also possible that our experimental design, using 1 K steps near the expected critical temperature, was not sensitive enough to capture the differences detected by determining ice nucleation temperatures at a resolution of 0.1 K (Beerling *et al.* 2001).

There were minimal shifts in springtime phenology of the observed species when growing in a CO₂-enriched atmosphere, which follows findings from previous field studies of subarctic heathland dwarf shrubs (Abisko site; Gwynn-Jones *et al.* 1997), mature subarctic *Picea abies* (Slaney *et al.* 2007), and young field-grown maple trees (Norby *et al.* 2003). Leaf elongation in *Larix decidua* trees was advanced after one growing season of CO₂ enrichment (spring 2002) at the Stillberg site, but there were no significant phenological shifts under elevated CO₂ in subsequent years (Handa *et al.* 2005). Overall, the combined result of increased freezing sensitivity of plants growing under elevated CO₂ without a shift in phenology suggests a physiological, rather than phenological, mechanism behind the CO₂ effect. To our knowledge, specific physiological changes caused by CO₂ enrichment that reduce the ability of plant cells to tolerate freezing conditions have yet to be determined. Proposed mechanisms include altered biomembrane lipid composition and reduced availability of calcium-binding sites in cell walls and membranes (Beerling *et al.* 2001) and a reduction in membrane-stabilizing or osmotically-active antifreeze compounds (Obrist *et al.* 2001).

Given the observed earlier start of key phenological events, such as bud break and leaf expansion, of photoperiod-insensitive taxa in response to climate warming (Keller and Körner 2003), we hypothesized that any such soil warming effects at the alpine treeline would lead to a temporal shift of the period when plants are most sensitive to freezing conditions. However, the warming treatment had little, if any, impact on phenology of the studied plant species. *V. myrtillus* was the only species with a warming effect on phenology, showing earlier leaf expansion in response to warming. Surprisingly, we observed an increased freezing sensitivity in *V. myrtillus* experiencing soil warming.

Since warming accelerated phenology, we expected the opposite response of higher resistance in the more mature leaf tissue on a given sampling date (Taschler *et al.* 2004). With our experimental protocol, we cannot interpret the apparent 0.5 K greater freezing sensitivity in more mature leaf tissue of *V. myrtillus*; a higher temporal resolution of freezing resistance dynamics in expanding young foliage would address this question. It seems that the state of freezing resistance is not simply a linear function of leaf aging, but may depend on various physiological processes with different dynamics. As elevated CO₂ also increased freezing sensitivity in *V. myrtillus*, this species, one of the most dominant species at our study site, may be particularly sensitive to freezing events during the early growing season under future atmospheric and climate change. Conversely, *Avenella* exposed to soil warming showed reduced freezing sensitivity, as would be expected with accelerated phenological development. However, phenological observations of this species were not possible.

We acknowledge the limitation of one sampling period per species, for the sake of a larger sampling across many different species, for detecting and understanding warming effects on freezing sensitivity. Also, for some of the less abundant species, plant material was too limited in the CO₂-treated area for several consecutive samplings to achieve better coverage of bud break and leaf expansion dynamics. In interpreting the effects of soil warming, we also note that this treatment was only initiated after all plots were snow-free to avoid the confounding effect of differences in snow cover in our experiment. Earlier snowmelt in a warmer climate, resulting in increased plant exposure and advanced phenology in photoperiod-insensitive taxa, can lead to increased damage from springtime freezing events (see Inouye and Wielgolaski 2003) but could not be tested in the present experiment. On the other hand, there are predictions of enhanced late winter snowfalls under climate change and thus the possibility of greater snow pack at elevations >2000 m a.s.l. (IPCC 2007). Thus, the conditions simulated in our experiment are likely to occur during at least some years in the future, and there is even a realistic possibility of prolonged snow cover duration at very high altitudes.

A totally unexpected but rather interesting finding was related to tree species identity in the experimental plots. Irrespective of CO₂ or warming treatments, *V. gaultherioides* growing under pine had advanced budburst and subsequently had greater freezing resistance than those in plots containing larch. This result suggests that earlier budburst under pine, possibly due to different light conditions or nutrient composition of the tree leaf litter (M. Martin, unpublished data), caused leaves to be more fully mature and therefore hardier when exposed to freezing conditions.

Plant functional type classification as a predictor for freezing resistance in a future climate

Replicate species within deciduous woody (3), evergreen woody (2), and forb (4) PFTs and within tree (2), shrub (3), and herb (5) PGFs permitted us to test the hypothesis that plants within the same classification group would have a similar freezing resistance

response to treatments simulating atmospheric and climate change (Dorrepaal *et al.* 2005). Contrary to our prediction, effects of elevated CO₂ and soil warming on freezing sensitivity were not consistent within the three PGFs or within the three testable PFTs. Plant functional type and growth form groupings were clearly less meaningful than species-specific environmental preferences, such as altitudinal distribution and snow cover demand (Gurvich *et al.* 2002, Körner 2003), for predicting responses to the treatments. Our result suggests that future shifts in the treeline plant community composition due to freezing events are likely to occur at the individual species level.

Conclusions

Our results are the first to demonstrate a CO₂-enrichment-induced decrease in freezing resistance of European treeline plants. Elevated atmospheric CO₂ concentration acted directly on freezing sensitivity and not via altered phenology. Soil warming showed little to no influence on the phenology and freezing resistance of the sampled species. Advanced phenology occurred in only one of the ten species tested, while LT50 was shifted in only two species and in opposite directions. Warmer temperatures could, however, have a greater impact if earlier snowmelt dates affect species with poor photoperiod control of phenology. Plant responses to the experimental treatments were largely species-specific, and grouping into PGFs or PFTs did not contribute to a more general prediction of expected freezing sensitivity under future atmospheric and climate change. In summary, our results suggest that leaf tissue damage caused by episodic early season freezing events will increase in frequency for some species in the coming decades. The resulting shifts in relative freezing resistance among co-occurring species could, in turn, alter competitive interactions among species.

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Chapter 5.

Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline

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Abstract

Climate warming will probably have particularly large impacts on carbon fluxes in high altitude and high latitude ecosystems due to their great stocks of labile soil C and high temperature sensitivity. At the alpine treeline, we experimentally warmed undisturbed soils by 4 K for one growing season with heating cables at the soil surface and measured the response of net C uptake by plants, of soil respiration, and of leaching of dissolved organic carbon (DOC). Soil warming increased soil CO₂ effluxes instantaneously and throughout the whole vegetation period (+45%; +120 g C m⁻² y⁻¹). In contrast, DOC leaching showed a negligible response of a 5% increase (not significant). Annual C uptake of new shoots was not significantly affected by elevated soil temperatures, with a 17, 12, and 14% increase for larch, pine, and dwarf shrubs, respectively, resulting in an overall increase in net C uptake by plants of 20 to 40 g C m⁻² y⁻¹. The Q₁₀ of 3.0 measured for soil respiration did not change compared to a three-year period before the warming treatment started, suggesting little impact of warming-induced lower soil moisture (-15% relative decrease) or increased soil C losses. The fraction of recent plant-derived C in soil respired CO₂ from warmed soils was smaller than that from control soils (25 vs. 40% of total C respired), which implies that the warming-induced increase in soil CO₂ efflux resulted mainly from mineralization of older SOM rather than from stimulated root respiration. In summary, one season of 4 K soil warming, representative of hot years, led to C losses from the studied alpine treeline ecosystem by increasing SOM decomposition more than C gains through plant growth.

Introduction

Feedbacks between terrestrial ecosystems and the ongoing climate changes are one of the key uncertainties in predicting global warming (Davidson and Janssens 2006, Heimann and Reichstein 2008). Although the effects of rising temperatures on single processes have been studied intensively, a comprehensive understanding of whole ecosystem responses to global warming still remains elusive (see meta-analysis by Rustad *et al.* 2001). Arctic and alpine regions are likely to be particularly affected by climate warming because observed temperature increases in these areas are higher than anywhere else (IPCC 2007, Rebetez and Reinhard 2008). Moreover, high latitude/altitude ecosystems might be more sensitive than other types of ecosystems because plant growth is often limited by low temperature (Körner 1998), and soil respiration is more sensitive to warming at lower temperatures (Kirschbaum 1995). In addition, high latitude/altitude ecosystems store the greatest fraction of their carbon stocks in soils (IPCC 2007) and, compared to temperate ecosystems, cold soils were found to comprise more labile soil organic matter (SOM) because decomposition and humification processes are slow (Jenny 1929, Sjögersten *et al.* 2003).

Plant growth responses to increased temperature have been studied *in situ* in both arctic (see review by Dormann and Woodin 2002) and alpine (Kudo and Suzuki 2003)

ecosystems. Results indicate delayed growth stimulation by two or more years after initiation of warming in these ecosystems dominated by slow-growing plants of determinate growth (Shaver *et al.* 1986, Parsons *et al.* 1994). The magnitude of growth responses and thus of net C uptake rates, however, have been found to be highly ecosystem and plant species specific (e.g. Hartley *et al.* 1999). As the alpine treeline has received little attention in experimental warming studies (but see Danby and Hik 2007), growth responses to warming in these ecosystems remain largely unknown.

Since temperature drives soil respiration rates (Davidson and Janssens 2006), we might expect increasing C losses from ecosystems under climate warming. The stimulation of soil respiration by increased temperatures, however, could be counterbalanced by changing carbon inputs from plants (Oberbauer *et al.* 2007), by declining soil moisture (Saleska *et al.* 1999), by an 'acclimatization' through physiologically adapting microbial communities, or by declining resource availability (Luo *et al.* 2001, Melillo *et al.* 2002). Moreover, soil respiration is essentially driven by recent photosynthates (Högberg *et al.* 2001), and thus, the response of soil CO₂ effluxes to rising temperatures also depends on how plants and their C allocation to below-ground sinks respond to warming (Schindlbacher *et al.* 2009).

In contrast to soil respiration, little is known about the temperature dependency of DOC leaching, the second major pathway of C loss from ecosystems (Harrison *et al.* 2008). Although DOC production is at least partly microbial driven, concentrations of DOC in the field are generally only weakly related to temperature since (i) water fluxes are important co-drivers, (ii) inputs of fresh litter vary across seasons, (iii) DOC is a net product of DOC generation and consumption, and (iv) DOC is also released by physico-chemical processes (Michalzik *et al.* 2001). Laboratory and soil transplant studies using 'undisturbed' soil columns suggest that the Q_{10} of DOC production is much lower than that of soil respiration (Christ and David 1996, Harrison *et al.* 2008). However, interpretation of these short-term experiments is limited by the physical disturbance of soils during sampling.

Although C fluxes between ecosystems and the atmosphere have been studied intensively, the reasons for intra-annual variability and the responses to extreme meteorological conditions, such as the hot and dry year 2003, are still not well understood (Ciais *et al.* 2005, Heimann and Reichstein 2008). A promising approach to improve our knowledge of the complex impacts of climate changes and of climate variability is the *in situ* manipulation of climatic conditions. So far, experimental warming in colder climates has mainly occurred at high latitudes (Oechel *et al.* 2000, Oberbauer *et al.* 2007) and in boreal forests (Niinistö *et al.* 2004, Bronson *et al.* 2008). Results indicate ecosystem-dependent responses in C fluxes with initial C losses in dry tundra and boreal forests but dampened effects under anoxic conditions. The only study at high altitude was conducted in a dry alpine meadow in Colorado, where soil heating had stronger indirect than direct effects on soil C cycling by changing plant species composition and inducing moisture limitations on soil respiration (Saleska *et al.* 1999).

Our study aimed at estimating how carbon fluxes in alpine treeline ecosystems with undisturbed soils and thick organic layers respond to *in situ* soil warming. In our experiment, we warmed soils by a constant 4 K throughout the snow-free period with heating cables laid out on the soil surface. The soil warming was conducted within a long-term CO₂ enrichment study, which provided a unique ¹³C label for recent plant-derived C in soils. Our objectives were (1) to estimate the *in situ* temperature dependency of soil respiration and DOC leaching, (2) to determine if soil warming induces a moisture limitation for soil C fluxes and plant growth; and (3) to quantify how the one-year warming treatment, as a proxy for hot years, affects the C balance of alpine treelines.

Materials and Methods

Study site description

The soil warming study was carried out at Stillberg (2180 m a.s.l.) in the Central Alps near Davos, Switzerland, where a long-term research site was established in the late 1950's to study climate-growth relationships (Senn and Schönenberger 2001). Long-term average annual precipitation is 1050 mm, mean maximum snow depth is 1.50 m, mean annual temperature is 1.4°C, and average January and July temperatures are - 5.8 °C and 9.4 °C, respectively. The terrain is rather steep, with slopes of 25 to 30° facing north-east. Parent material is Paragneiss. Soil types are sandy Ranker and Podzols (Lithic Cryumbrepts and Typic Cryorthods). The organic layers are Humimors dominated by 5 to 20 cm thick Oa horizons (Bednorz *et al.* 2000, Hagedorn *et al.* 2008). Soil characteristics are given in Table 1.

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

Horizon	Depth (cm)	Soil density ^a (g cm ⁻³)	Fraction sand ^b (%)	pH (CaCl ₂)	Soil organic C (%)	C/N (mass ratio)
Oi	7 - 6	0.07	ND	ND	46.3	46.2
Oe	6 - 5	0.13	ND	ND	45.1	28.1
Oa	5 - 0	0.16	ND	4.2	40.8	27.2
AE	0 - 20	0.85	60	3.8	4.7	21.3
Bh	20 - 45	0.91	61	4.0	3.9	25.1
Bs	45 - 80	1.10	65	4.2	4.2	29.1

^a Fine earth per volume soil

^b Particle sizes > 63 µm

Experimental set-up

The combined CO₂ enrichment and soil warming experiment was conducted at the upper end of an afforestation experiment established in 1975 slightly above the natural treeline. Three treeline species, *Larix decidua* L., *Pinus cembra* L., and *Pinus mugo* ssp. *uncinata*

Ramond, were planted across an area of 5 ha spanning an altitudinal range of 2080 to 2230 m a.s.l. Thirty-two years later, the trees at the upper end of the plantation are currently approximately 2 m tall and they form a sparse open canopy with dense understorey vegetation composed predominantly of ericaceous dwarf shrubs, such as *Vaccinium myrtillus*, *Vaccinium gaultherioides*, and *Empetrum hermaphroditum*, and of common herbaceous species, such as *Gentiana punctata*, *Homogyne alpina* and *Melampyrum pratense*.

CO₂ enrichment. In 2001, we established experimental CO₂ enrichment within a relatively homogeneous 2500 m² area (Hättenschwiler *et al.* 2002). Forty plots, each with an individual *Larix* or *Pinus* tree in the centre and at least 1 m apart from each other, were organized into 10 groups of four neighbouring plots to facilitate logistics of CO₂ distribution and regulation. Five of these 10 groups were randomly assigned to an elevated CO₂ treatment, while the remaining groups served as controls, resulting in a split-plot design. The 20 elevated CO₂ plots were enriched with CO₂ using a FACE-set-up, in which pure CO₂ is released through 24 vertically hanging laser-punched drip irrigation tubes fixed on steel frames enclosing an area of 1.1 m² (Hättenschwiler *et al.* 2002). Concentrations of CO₂ were recorded every 10 minutes in all cardinal points in one plot per group. These concentrations were used to adjust the CO₂ addition. The multiple-year growing season average of CO₂ concentration was 566 ± 75 ppm_v under elevated CO₂ and 370 ± 3 ppm_v under ambient CO₂ (see Hättenschwiler *et al.* 2002, Handa *et al.* 2006). In 2006, we modified the CO₂ enrichment system by putting the steel frames and the vertical tubes down to a height of 50 cm thereby adding CO₂ to the dwarf shrubs and lower parts of trees. In addition, laser perforated tubes were woven into the tree crowns and adjusted to match 570 ppm_v CO₂ using a portable IRGA. The mean CO₂ concentrations under elevated CO₂ were 580 ± 60 ppm_v within the rings and 555 ± 70 ppm_v in the tree crowns.

Soil warming. The warming experiment was installed in half of the plots (10 at ambient CO₂ and 10 at elevated CO₂) during summer 2006. On the ground surface of each plot, woven under the above-ground shoots of the dwarf shrubs, 26 m of 420 W-heating cables (SPS-S004026, Chromalox-ETIREX, Soissons, France) were laid out in spirals with a 5 cm distance between neighbouring cables. Preliminary tests were conducted during 2005 and 2006 to determine the cable length, density and power required to achieve the target temperature, while at the same time assuring that maximum cable surface temperatures would not exceed 45°C. The 4 K soil warming treatment was achieved by switching the power supply on and off in one minute intervals and was applied continuously (day and night). In 2007, the warming treatment began directly after snowmelt (23th May) and was switched off just before the site was covered in snow for winter (17th October) to avoid an interaction between soil temperature and snowmelt or snow cover.

Sampling and field measurements

Soil and air temperatures were measured at -3, -5 and -10 cm in the soil plus 5, 10, 20, 100 and 200 cm above the ground surface in four to ten plots per warming treatment using temperature sensors (Hobo Pro v2, Onset Computer Corporation and ibuttons, Maxim Integrated Products DS1922L). Air humidity was measured with ibutton DS1923 sensors. *Volumetric soil moisture* was repeatedly measured by Frequency Domain Reflectometry with a Theta sonde ML2x probe (Delta-T, UK) at fixed locations in all 40 plots. Readings were converted using a soil-specific equation derived from a lab calibration with soil from all groups. *Gravimetric soil moisture* was determined in mid July 2007 by taking five soil samples (0 to 5 cm depth) per plot with a soil corer (2 cm) and measuring the decrease in weight after drying soil samples at 105°C.

Soil solution was collected in all plots by installing two ceramic suction cups (SoilMoisture Equipment Corp., Santa Barbara, USA) per plot at 3 to 7 cm depth. All suction cups were located within the Oa horizon that dominated the organic layer. At each sampling event, we collected soil water by evacuating suction cups with a constant 400 hPa for about 16 hours (overnight). In addition to the suction cups, we installed zero-tension lysimeters (8x8 cm plexiglass plates with a PE-net) at 5 cm soil depth. The sampling devices were installed at a fixed depth because space within the 1.1 m² plots was too limited to open pits for identifying diagnostic horizons. All lysimeters were connected to 1-liter glass bottles buried in the ground. After collection, soil water samples were stored in cooling boxes for transport to the institute for analysis.

CO₂-efflux from soils and its $\delta^{13}\text{C}$. 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 (40 plots total) was pressed to a depth of 2 cm into the organic layer in between dwarf shrubs in the middle of the plots. Soil respiration rates were estimated from increases in CO₂ concentrations with time after scrubbing the chamber air to ambient CO₂ levels. The pump rate through the system was kept small with 0.2 ml min⁻¹. For ¹³CO₂ measurements, we closed the chambers with PVC-lids at least four hours after stopping the CO₂ enrichment to avoid contamination with ¹³C-depleted CO₂. Twenty to thirty minutes after closing the collars, we took gas samples by retrieving 15 ml of air with a 20 ml syringe through a septum and by injecting the air into 12 ml pre-evacuated glass vials closed with an airtight rubber septum (volume of 12 ml, Exetainer gas testing vials, Labco Limited, High Wycombe, UK). The glass vials were evacuated with a vacuum of 800 hPa immediately before the sampling.

Plant shoot growth was measured in order to estimate carbon uptake and how the one-year warming treatment affected C balance at the site (see calculations below). The length of all new shoots of both tree species was measured during early autumn of 2006 and 2007. For the dominant dwarf shrubs *Vaccinium myrtillus*, *V. gaultherioides* and

Empetrum hermaphroditum, three measurements of each species per plot were used to estimate mean 2006 and 2007 shoot growth.

Chemical Analysis

All solution samples from the field were passed through 0.45- μm cellulose-acetate filters (Schleicher & Schuell, ME25) within two days of collection and then stored at 4°C until analysis. *Microbial biomass* was determined with the chloroform-fumigation-extraction method (Vance *et al.* 1987) using a soil:solution ratio of 1:5 for the extraction with 0.5 M K_2SO_4 . Concentrations of dissolved organic C in soil extracts and waters were determined with a TOC/TN analyser (TOC-V Shimadzu Corp. Tokyo, Japan).

Stable isotopes. The $\delta^{13}\text{C}$ values of soil CO_2 were measured with a gasbench II linked to a mass spectrometer (Delta Plus XL, Thermo Finnigan, Bremen interfaced with a Delta-S Finnigan MAT, Bremen, Germany) after depressurizing the vials with a needle (Joos *et al.* 2008). 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_v). Results of the C isotope analysis were expressed in δ units (‰). The $\delta^{13}\text{C}$ values were referenced to the Pee Dee Belemnite (PDB) standard.

Calculations and statistics

Net plant C uptake. The warming effect on net C uptake by plants was roughly estimated by multiplying measured growth effects by estimates of plant biomass production. Biomass accumulation by trees was obtained by first estimating growth functions from measured tree height changes every 0, 4, 6, 10, 15, 20, and 30 years after planting seedlings at the Stillberg site (Senn and Schönenberger 2001; P. Bebi and C. Rixen, unpublished data). The fitted functions were then applied to the trees in the soil warming experiment. Finally, species-specific allometric regressions between height and total biomass (above-ground and coarse roots) based on 25 trees excavated at the study site (Bernoulli and Körner 1999) were used to calculate the net increase in tree biomass from 2006 to 2007. Biomass productivity of dominant dwarf shrubs in 2007 was similarly calculated from shoot length measurements, applying allometric relationships established from 21 understorey plots at the Stillberg site (S. Wipf *et al.*, unpublished data). Fine root productivity for all plants combined, not included in the above calculations, was determined from ingrowth core data and ^{13}C measurements of fine roots (Handa *et al.* 2008). For both trees and dwarf shrubs, net C uptake by plants was calculated by multiplying biomass by 0.5.

Temperature dependencies of soil respiration and DOC concentrations were estimated by the Q_{10} function:

$$R = R_{10} * Q_{10}^{((T-10)/10)} \quad [1]$$

In which R is the measured soil respiration or DOC concentration, R_{10} is the simulated soil respiration or DOC concentration at 10°C, Q_{10} is the temperature sensitivity (over a range of 10°C), and T is the soil temperature. R_{10} and Q_{10} were estimated by fitting measured data using the Levenberg-Marquard algorithm (Origin 7.0, OriginLab).

Annual soil CO₂ effluxes were calculated from applying the fitted Q_{10} functions to measured mean daily soil temperatures for each of the plots.

Stable isotopes. The $\delta^{13}\text{C}$ of soil-respired CO₂ was calculated by a mixing model with the sampled CO₂ from the soil chambers being composed of ambient CO₂ and soil-respired CO₂ (Subke *et al.* 2004). The fraction of soil-respired 'new' C (f_{new}) derived from the CO₂ addition (being respired from the rhizosphere or litter) was calculated by relating the difference in ^{13}C of respired CO₂ between ambient and elevated CO₂ to the difference in ^{13}C in plant leaves (mean of tree needles and dwarf shrub leaves).

$$f_{\text{new}} = \frac{(\delta^{13}\text{C}_{\text{flux, pool, elevated}} - \delta^{13}\text{C}_{\text{flux, pool, ambient}})}{(\delta^{13}\text{C}_{\text{plant, elevated}} - \delta^{13}\text{C}_{\text{plant, ambient}})} \quad [2]$$

Statistics. Effects of CO₂ enrichment and warming on all measured parameters were tested by ANOVA using a full factorial split-plot model. Analyses were performed with R (R Development Core Team 2008-2010). Concentrations of DOC, DON, and *in situ* soil respiration were log transformed before the analysis. The warming effect was calculated by dividing warmed plots by control plots in 2007 and relating this ratio to the ratio in the pre-warming year 2006. Standard errors were estimated using laws of error propagation.

Results

Temperature and moisture

The experimental warming at the soil surface resulted in a mean increase in soil temperatures by 4.7 ± 0.8 , 3.7 ± 0.2 and 3.2 ± 0.2 K at 3, 5, and 10 cm depth, respectively (mean \pm 1 standard error; Fig. 1). Air temperatures were increased by 4.3 ± 1.3 , 2.6 ± 0.5 and 0.9 ± 0.1 K at 5, 10 and 20 cm height above ground. No air warming effect was detected at tree canopy heights of 100 and 200 cm (data not shown). Diurnal temperature fluctuations were more pronounced at 5 than at 10 cm soil depth (Fig. 1) but did not differ between warmed and control plots. The relative warming effect was consistent throughout the snow-free period and did not change with weather or soil moisture conditions. Mean air humidity at 10 cm height was 90 ± 1 % in control plots and 81 ± 2 % in warmed plots. As a result of the warming, soil moisture decreased during the growing season (Fig. 2). In the late growing season 2008, volumetric water contents at 0 to 10 cm depths were approximately 20% lower in the warmed than in the control plots. Averaged over the 2007 growing season, the warming effect on volumetric soil water content was 15% ($P < 0.05$). Gravimetric water content in mid July was decreased by 18

$\pm 6\%$ due to the warming. The decline in soil moisture was significant when standardized to pre-season values. Elevated CO_2 did not affect soil moisture, regardless of soil warming treatment.

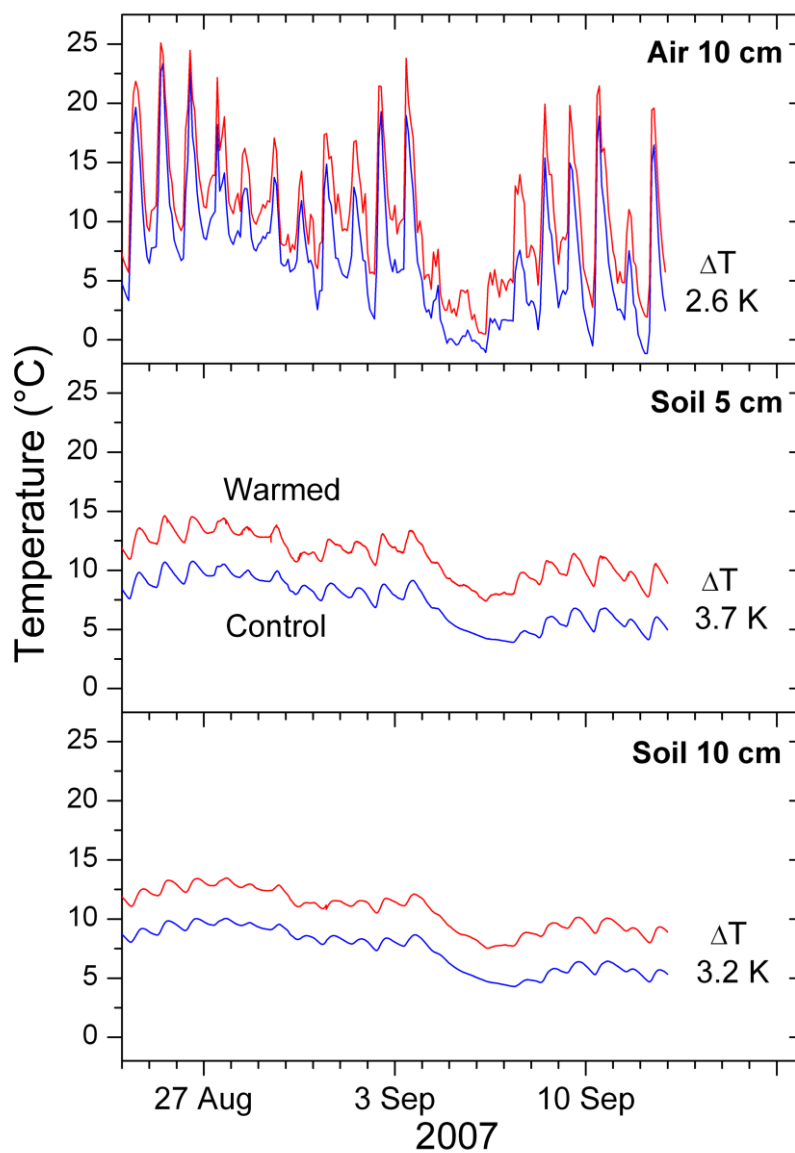


Figure 1. Effects of experimental warming at the soil surface on air and soil temperatures in late summer 2007. Values for control plots (blue line) and warmed plots (red line) are each based on the mean of 8 to 10 plots.

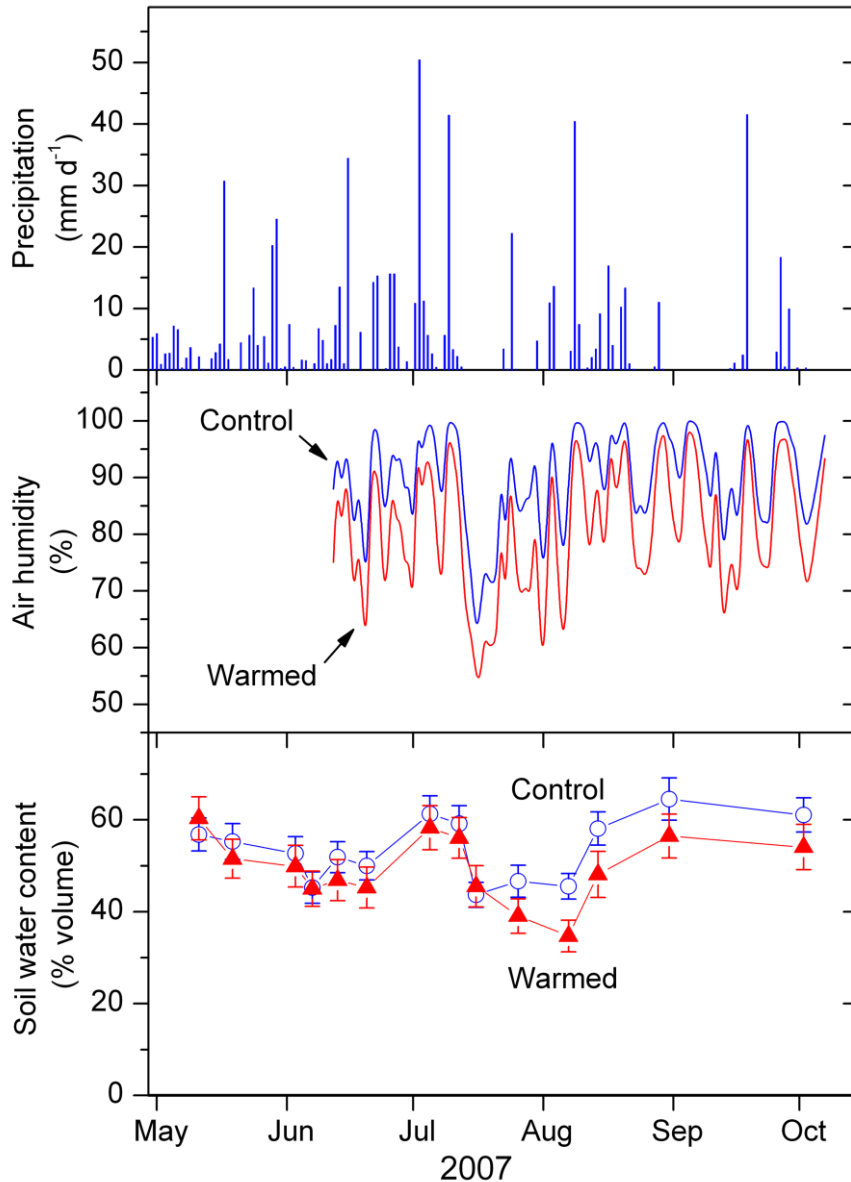


Figure 2. Effects of experimental soil warming on air humidity (10 cm height) and volumetric soil water content (0-10 cm depth) in 2007. Each air humidity value represents the mean of 9 plots, and each soil water content value represents the mean of 20 plots \pm 1 standard error.

Soil respiration and DOC leaching

Ambient temperatures 2004 to 2007. CO_2 effluxes from soils and concentrations of DOC in Oa horizons at 5 cm depth differed in their dependence on soil temperature (Fig. 3). While soil respiration closely followed an Arrhenius-type relationship with temperature, DOC concentration correlated only weakly with temperature. The calculated Q_{10} for overall soil respiration was 3.0 ± 0.4 ($r^2 = 0.66$; $P < 0.001$) whereas that for DOC concentration was 1.2 ± 0.1 ($r^2 = 0.27$; $P < 0.05$). Frequent monitoring of CO_2 effluxes throughout 2007 indicated lower respiration rates at a given temperature in the early than in the late growing season (Fig. 4).

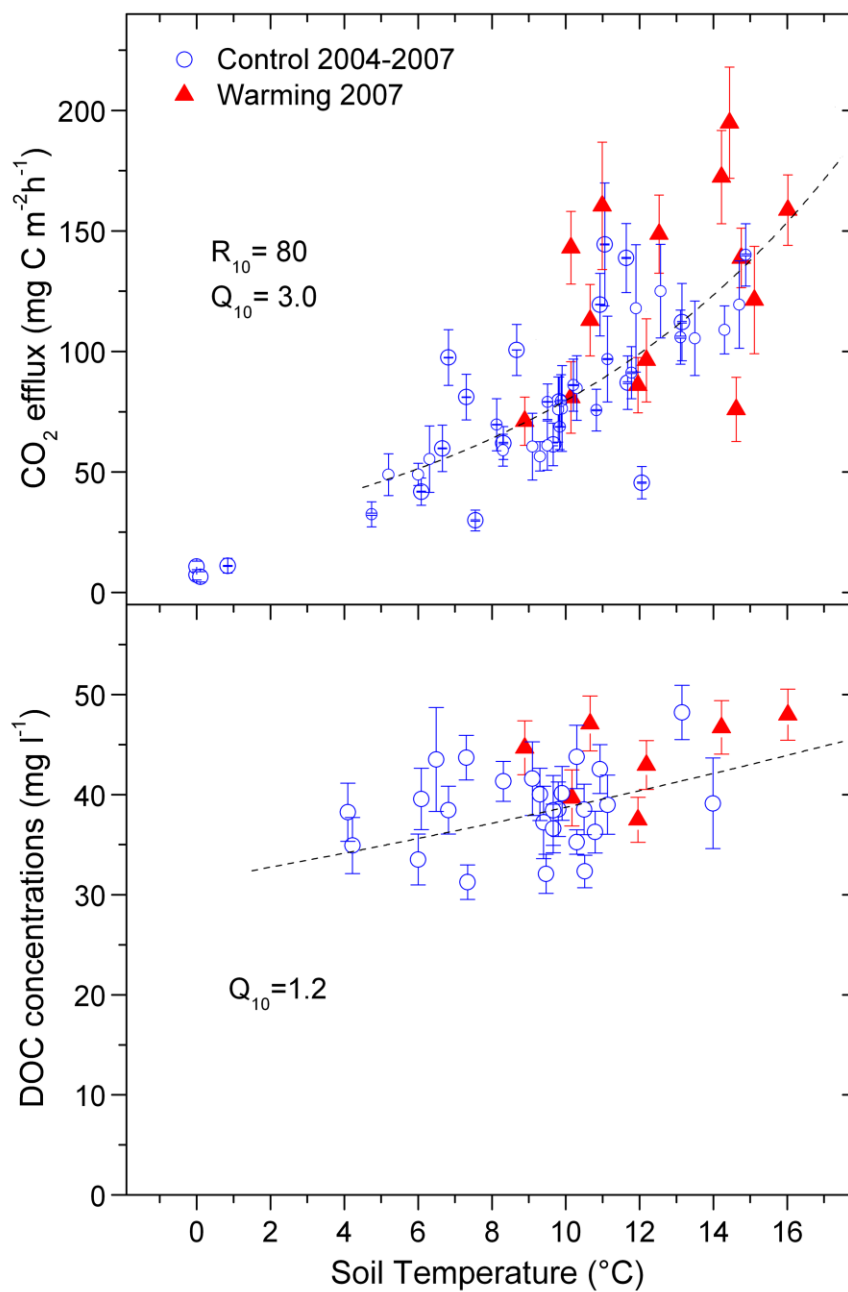


Figure 3. The relationship between soil temperature at 5 cm depth and DOC concentrations and soil respiration rates. Each point represents the mean of 20 plots \pm 1 standard error.

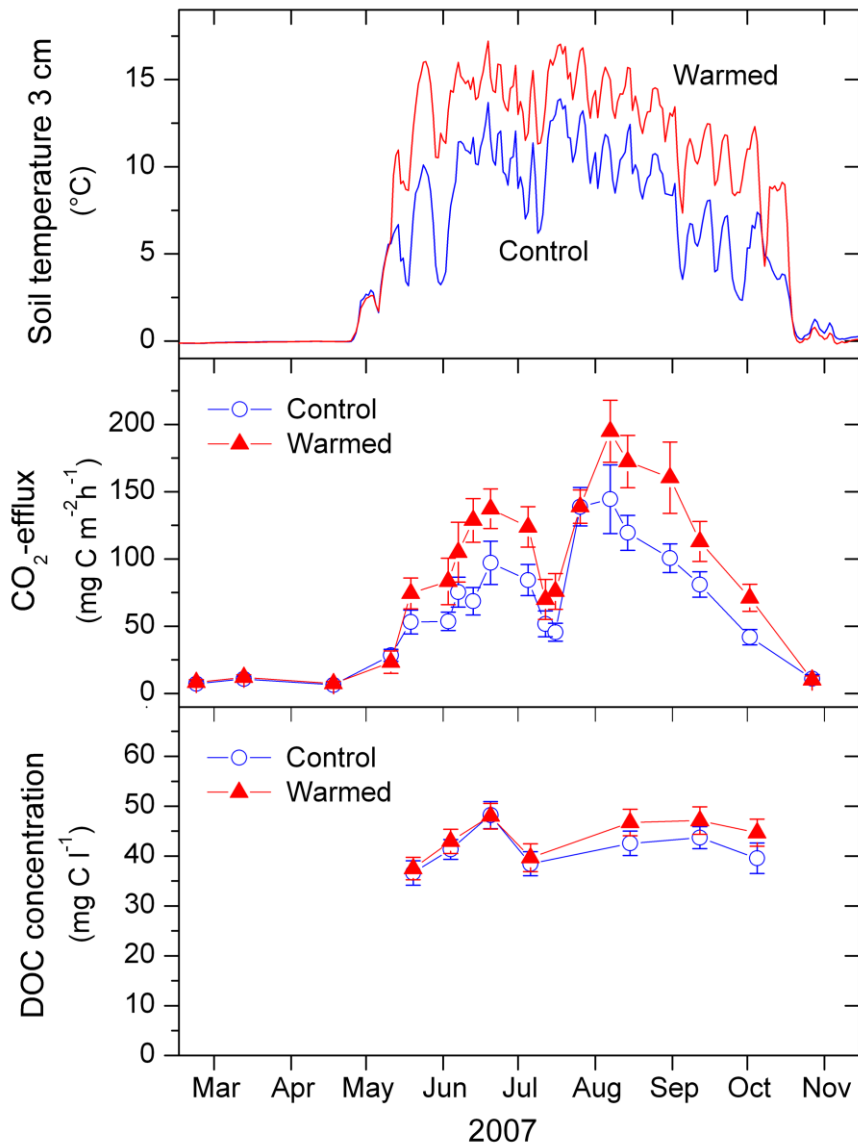


Figure 4. Effects of 4 K soil warming on soil respiration rates and DOC concentrations in the Oa horizon at 5 cm depth. Each point represents the mean of 20 plots (± 1 standard error, bottom two panels).

Responses to soil warming

In the pre-treatment period 2004 to 2006, soil respiration rates differed by not more than 5% between control and warmed plots. During the five-month growing season in 2007, soil warming by 4 K increased soil CO₂ effluxes by 30 to 100% with a mean increase of 45% ($P < 0.05$; Fig. 4). The warming effect on soil respiration did not change throughout the summer. In mid July, however, there was a short dry period during which experimental soil warming had negligible impacts on soil respiration. The temperature

dependency of soil respiration was not affected by soil warming. In the 2007 growing season, overall Q_{10} s were 2.5 ± 1.2 at ambient temperatures and 2.6 ± 0.8 in 4 K warmer soils. Soil warming increased soil respiration rates despite slightly negative effects on microbial biomass (not significant; Fig. 7), indicating that the warming stimulated the activity but not the quantity of soil microbes.

Applying the fitted Q_{10} functions to measured soil temperatures indicated that annual $\text{CO}_2\text{-C}$ effluxes were $300 \pm 30 \text{ g C m}^{-2} \text{ y}^{-1}$ (mean ± 1 standard error) in the pre-treatment year 2006 and $350 \pm 40 \text{ g C m}^{-2} \text{ y}^{-1}$ in the control plots in 2007. The greater CO_2 effluxes in 2007 were related to a one-month earlier snowmelt and, thus, to a substantially longer growing season than in the previous year. Experimental soil warming increased annual soil respiration by an additional $120 \pm 40 \text{ g C m}^{-2} \text{ y}^{-1}$.

Compared to CO_2 effluxes, DOC fluxes were much smaller, with 20 to 35 g DOC m^{-2} being leached from the organic layer (Hagedorn *et al.* 2008). Also, the effects of the 4 K soil warming on DOC concentrations sampled with suction cups at 5 cm depth in the Oa horizons were much smaller than for soil respiration (Fig. 4). Concentrations of DOC were not increased before August, more than two months after the warming started, and even then the effect was not significant. In the late growing season, the maximum increase in DOC concentrations was $10 \pm 10\%$. The results of the zero-tension lysimeter study support these negligible warming effects on DOC concentrations (Fig. 5). In September 2007, after four months of soil warming, DOC concentrations at 5 cm depth were $11 \pm 20\%$ higher in the warmed than in the unwarmed soils. However, the pre-warming difference of the same lysimeters was $7 \pm 17\%$.

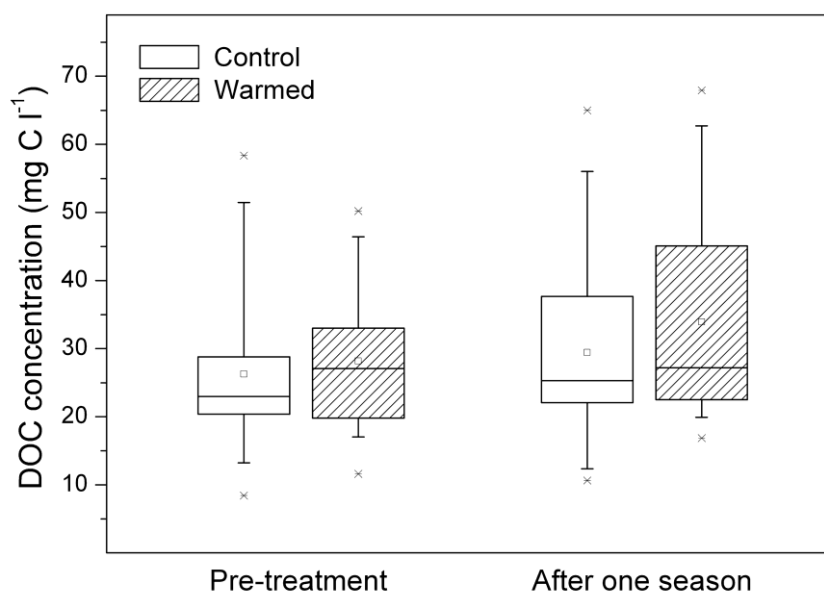


Figure 5. Effects of the 4 K soil warming on DOC concentrations in leachates of zero-tension lysimeters at 5 cm depth. Box plots show the mean (small squares), median (lines), quartiles (box edges), 5th and 95th percentiles (error bars), and maximum/minimum (crosses) for each temperature treatment group (n = 20).

Soil warming effects on new and old SOM

The addition of 200 ppm_v CO₂ depleted in ¹³C (-29‰) allowed the tracing of recent plant-derived C in the plant and soil system. In 2007, the mean decline in ¹³C in leaves of trees and dwarf shrubs was $-6.7 \pm 0.7\text{‰}$. This isotopic signal was clearly reflected in soil-respired CO₂ but was significantly smaller in the warmed than in the unwarmed soils (Fig. 6). Assuming a simple mixing model suggests that the contribution of new plant-derived C, either through rhizosphere respiration or through decomposing new litter, was approximately 40% in the unwarmed soils but only 25% in the warmed soils (averaged across seven sampling campaigns throughout the season). Multiplying these fractions of recent C with measured CO₂ effluxes suggests that the increased soil respiration rates from warmed soils were solely related to an accelerated mineralization of old soil carbon, while respiration rates from new plant-derived C remained constant. We might have underestimated the respiration losses from root-derived C because the collars for measuring CO₂ effluxes were placed in between dwarf shrubs and thus at microsites with smaller root densities and because some roots might be supplied with plant-derived C from outside the plots. However, because CO₂ effluxes were measured in the same way in both treatments, such potential underestimates would not influence the warming effect on respiration of new C. In addition, they would not affect respiration rates from old soil C.

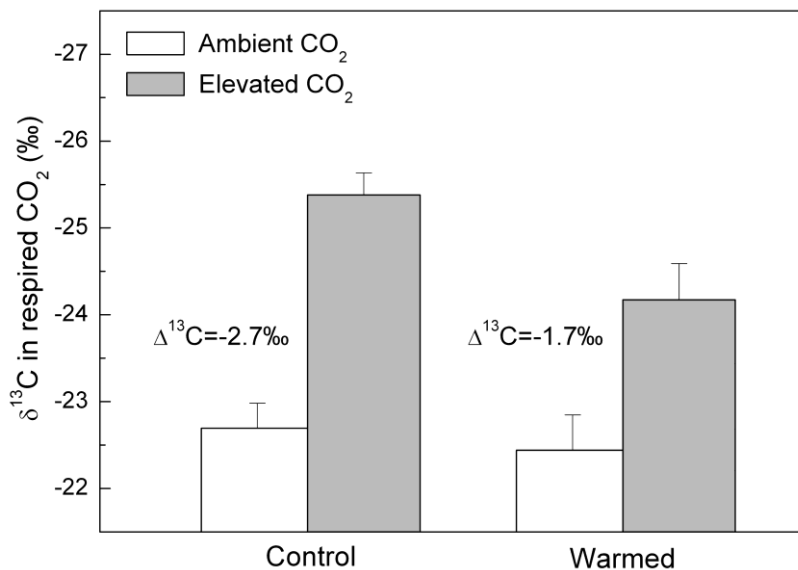


Figure 6. Effects of 4 K soil warming on $\delta^{13}\text{C}$ values of soil-respired CO₂. The CO₂ enrichment decreased air $\delta^{13}\text{C}$ values by 6.7‰. Each bar shows the mean of 10 plots + 1 standard error.

C uptake by plants

Soil warming for one season caused slight increases in shoot growth of trees (larch $+17 \pm 34\%$, pine $12 \pm 32\%$) and dwarf shrubs ($+14 \pm 12\%$), though no increases were statistically significant (Fig. 7). Regarding larch, total shoot growth per tree during 2007,

the first season of warming, was on average only half of growth in the previous year due to damage from an early summer frost event. Our growth data allows a first rough extrapolation of growth data to net annual C uptake by plants: calculated growth rates indicated annual net C uptake rates of 20 to 40 g C m⁻² y⁻¹ for the 32-year old treeline trees. Biomass calculations of dwarf shrubs suggested an annual shoot growth production of 40 to 150 (mean = 90) g C m⁻² y⁻¹ for the experimental plots (S. Wipf *et al.*, unpublished data). Ingrowth core data and recovery of the added ¹³C-depleted CO₂ in roots indicated a fine root production of 75 g C m⁻² y⁻¹ (Handa *et al.* 2008). In total, this yields a total net annual C uptake by the treeline vegetation of 200 to 250 g C m⁻² y⁻¹. When we assume that overall plant C uptake responds similarly to warming as shoot growth, the warming-induced stimulation of net C uptake is between 20 and 40 g C m⁻² y⁻¹. A more comprehensive study of growth responses, including below-ground production and stem diameter increases, over a longer period are needed to validate this assumption.

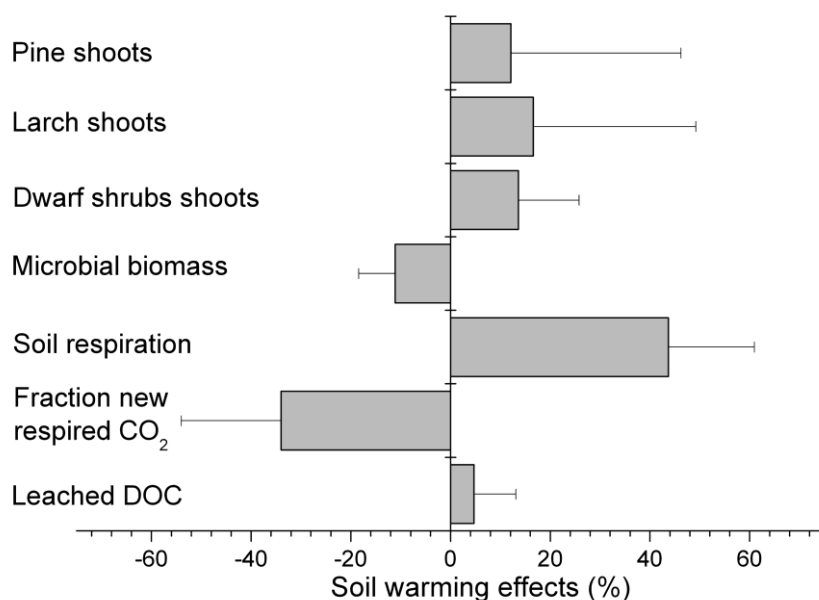


Figure 7. Effects of the one-season soil warming by 4 K on shoot growth of pine, larch, and dwarf shrubs, on the soil microbial biomass pool, on soil respiration rates, on fractions of new C (litter + roots) in soil-respired CO₂, and on mean DOC concentrations. The effect size was calculated as: (mean of all warmed plots / mean of all control plots) in 2007 divided by the same ratio in the pre-warming year 2006 (n = 20). Error bars show 1 standard error of the overall ratio.

Discussion

Contrasting response of C fluxes to warming

The experimental warming created temperature increases that were comparable with those experienced in the warm European summers of 2003 and 2006: in June and August 2003 and July 2006, temperature anomalies reached more than 6 K over major

parts of Central Europe (Luterbacher *et al.* 2004, Rebetez *et al.* 2009). Our treatment resulted in a quite homogenous soil warming down to a soil depth of at least 10 cm where more than 90% of the roots (Handa *et al.* 2008) and most of the labile C in the organic layer are located (Bednorz *et al.* 2000). Warming also induced a decline in soil moisture by 15 to 20% (relative to pre-treatment), which was comparable to the 10 to 20% drier soils in our experiment during the heat wave of 2003 compared to other years (F. Hagedorn, unpublished data).

Our results showed distinctly different effects of experimental soil warming on C fluxes in the plant and soil system. In the soil, CO₂ efflux was closely linked to soil temperature and showed instantaneous responses to experimental warming as well as sustained stimulations over one growing season. In contrast, warming had little effect on DOC leaching with no immediate responses at the beginning of the warming treatment and an overall insignificant 5% increase after one growing season.

The strong temperature dependency of soil respiration at short and longer time scales is consistent with other field and lab studies (e.g. Kirschbaum 1995, Schindlbacher *et al.* 2009). It supports the theoretically expected tight relationship between biochemical processes and temperature below critical temperatures as long as other factors such as moisture are not co-limiting (Davidson and Janssens 2006). In fact, the sustained temperature stimulation of soil respiration observed here across the whole season suggests that soil moisture was not an important co-limiting factor at our study site with well-drained sandy soils and 1100 mm of annually well-distributed rainfall. As a consequence, the 15% decline in soil moisture induced by the warming did not significantly reduce the response of soil respiration except for a two-week dry and hot period in mid July 2007 (Fig. 4). Our field-based Q_{10} of soil respiration of 2.5 to 3 during the growing season agreed well with the Q_{10} of 2.5 estimated in a laboratory incubation of soils of the study site (Reichstein *et al.* 2000). The extrapolation of our soil respiration measurements using the fitted Q_{10} functions suggests that the 4 K-warming increased the annual CO₂ efflux in 2007 by 120 g CO₂-C m⁻² y⁻¹, which is 40 to 50% higher than what was calculated for the control plots. In relative terms, the increase in soil respiration we measured was similar to the initial responses to a 5 K warming in a montane forest in Austria (Schindlbacher *et al.* 2009), but higher than the 24 to 43% increase in boreal forests (Niinistö *et al.* 2004, Bronson *et al.* 2008) and the 20 to 35% increase in the temperate Harvard Forest (Melillo *et al.* 2002). However, since CO₂ efflux rates at our alpine treeline site were smaller than those measured in other forests with more moderate climates and longer growing seasons, the absolute increase in respired C due to soil warming was lower in our study. Niinistö *et al.* (2004) and Melillo *et al.* (2002) showed that the response of soil CO₂ efflux strongly declined after one and five years of treatment, respectively, very likely due to depletion in labile soil carbon. At the alpine treeline, however, we would expect longer lasting warming effects because the 5 to 20 cm thick organic layer stores particularly large amounts of SOM (4 to 20 kg C m⁻²), which is predominantly non-stabilized and readily decomposable (Reichstein *et al.* 2000).

Soil warming induces C losses

While C losses via soil respiration responded strongly to warming, ecosystem C gains by plant growth were only slightly affected in this first treatment year (Fig. 7). It is not surprising that plants showed relatively small growth responses in the first growing season because most plant species present at our site are characterized by determinate growth, and response time to changing growth conditions are likely to lag behind by at least one growing season (Hartley *et al.* 1999, Danby and Hik 2007). Additionally, our treatment did not warm the air above the dwarf shrub layer, which might have contributed to the low responsiveness observed for tree growth in particular. However, responses during the hot year 2003 support our results in that tree growth at our site and in other Swiss alpine and subalpine forests was hardly affected by a 5 to 6 K warmer growing season (Jolly *et al.* 2005, Handa *et al.* 2006) whereas soil respiration was accelerated (F. Hagedorn, unpublished data). We therefore conclude that our findings of net C losses from the warmed plots are indeed representative of stochastically occurring hot years. However, it remains to be shown whether or not the net C balance in the following years will change due to potential long-term plant growth responses to warming or due to declining substrate availability in soils.

The consequences of the stronger response of soil CO₂ effluxes for the ecosystem net C balance depend on which one of the two major components of soil respiration was driving the increase in soil respiration. If the autotrophic component (i.e. plant roots) was stimulated, then the increase in soil respiration would simply mean a faster C cycling rate through the plant and soil system. A greater heterotrophic respiration rate, however, would signify an increased C loss from soil organic matter. The ¹³C tracing provided clear evidence for a stronger response of the heterotrophic component to the soil warming. The difference in δ¹³C of soil-respired CO₂ was reduced in the warmed plots (Fig. 6), indicating that the relative contribution of recent plant-derived C to soil respiration declined by about 40% (Fig. 7). Therefore, our results show that the 4 K soil warming particularly increased the mineralization of more than seven-year-old SOM and hence, the warming turned the treeline ecosystem into a C source rather than simply causing faster C cycling.

Our results allow a rough estimate of the initial net C losses by the soil warming. Relating the small growth response of about 15% to net primary productivity of about 200 to 250 g C m⁻²y⁻¹ including roots (S. Wipf *et al.*, unpublished data), typical for closed alpine vegetation (Körner 2003), suggests that the warming-induced increase in plant C uptake amounted to 30 to 40 g C m⁻² y⁻¹. This is about one-third of the acceleration in soil CO₂ efflux from warmed soils via respiration. A stronger warming-response of roots than shoots could have biased the estimated increase in plant C uptake. However, our data suggests that roots were rather less responsive because respiration rates of new C (largely from roots) were not affected by the warming. This conclusion is supported by decreasing root biomass in heated soils of a boreal forest (Bronson *et al.* 2008). In our study, the difference between increased SOM mineralization and plant C uptake

indicates that soil warming increased net C losses by about 80 to 110 g C m⁻² y⁻¹, which corresponds closely to the net C losses of 100 g C m⁻² y⁻¹ in response to warming of a montane meadow in Colorado by 1.5 K (Saleska *et al.* 1999). However, in contrast to our study, their result was attributed to the indirect warming effect of declining soil moisture in the dry meadow receiving only 150 mm of summer precipitation. Drought conditions induced by their treatment dampened the increase in soil respiration but negatively affected plant C uptake, resulting in a net C loss in warmed plots. In comparison to the Colorado study, mean rainfall during the vegetation period is four times higher at our site and amounted to 550 mm even in the very dry summer of 2003. Soil moisture is not considered a limiting factor for plant growth in treeline systems of this region (Körner 2003), which is also supported by no measurable effects of the 2003 drought on tree growth (Handa *et al.* 2006). Moreover, soil moisture rarely limited soil respiration during our experiment, as indicated by the similar temperature dependency of respiration in warmed and control plots. Most other parts of the Alps receive even more precipitation than our site located in the relatively dry Inner Alps (Federal Office for Water and Geology 2001). We therefore expect increased C losses due to warming-induced acceleration in soil respiration at most alpine treeline sites as the frequency of hot and dry summers will likely increase during the next century (Stott *et al.* 2004).

Negligible response of DOC leaching

Temperature apparently did not control leaching of DOC to the same extent as soil respiration. There was no immediate response of DOC concentration to increased temperature, a relatively weak 10%-increase after two months of experimental warming, and essentially no seasonal variation in response to differences in temperature. Because warming generally decreases drainage, the absolute amount of DOC leached from the organic layer was very likely even less affected. A poor relationship between temperature and DOC leaching on a seasonal basis has been observed previously in field studies in temperate forests, where this finding was attributed to the stronger impact of water fluxes than temperature or to seasonally changing DOC sources (Michalzik *et al.* 2001). In a preliminary warming test in 2006, we excluded water fluxes as a potential co-driver for DOC leaching by regularly irrigating soils and found the same lack of response as in 2007 (F. Hagedorn, unpublished data). The impact of varying 'fresh' DOC inputs is also considered to be negligible in our study, as we directly compared warmed with untreated soils. Despite the fact that we can largely exclude potential confounding factors, soil warming still had only negligible effects on DOC concentrations in the Oa horizons. At first glance, this seems surprising because DOC production is thought to be mediated by microbial activity (Christ and David 1996, Kalbitz *et al.* 2000) and thus, we would expect DOC concentrations to increase with increasing temperatures. One explanation for the apparently minor direct control by temperature could be that microbial-driven DOC generation rates are small compared to the total pool of leachable DOC. Consequently, the DOC pool might build up over relatively long time periods, and any temperature-driven microbial effects would only be observable in the longer term. The increase in DOC leaching after two months of warming supports a delayed DOC

response (Fig. 4), which is consistent with time lags of about two months between DOC leaching and temperature observed in a seasonal monitoring study and in transplant experiments with soil cores (Fröberg *et al.* 2006, Harrison *et al.* 2008). However, our study suggests that other mechanisms must also have contributed because in late summer, after four months of soil warming and after substantial amounts of DOC (10 to 15 g DOC m⁻²) had been leached, the warming effect on DOC leaching was still substantially smaller than the effect on soil respiration. Perhaps the production and consumption of DOC were tightly balanced, resulting in negligible net changes in DOC leaching. Laboratory studies by Marschner and Bredow (2002) with agricultural soils corroborate this mechanism; they show that contents of extractable DOC actually decrease with rising temperatures, due to the utilization of labile DOC by microorganisms.

Conclusions

Our results indicate that net C uptake by plants, soil respiration and DOC leaching in an alpine treeline ecosystem responded very differently to a 4 K temperature increase over one season. While soil respiration rates increased instantaneously with increased temperature, DOC leaching showed a delayed and much smaller response, probably due to a balancing out of DOC production and degradation. Tracing of ¹³C depleted CO₂ added for seven years showed that the accelerated CO₂ effluxes from warmed soils were not driven by increased mineralization of recent plant litter and root respiration but by mineralization from older soil organic matter. These soil C losses clearly exceeded the estimated C accumulation by plants, which showed little response to one growing season of soil warming. We conclude that soil warming, representative of hot but also drier years, leads to C losses from alpine treeline ecosystems.

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Chapter 6.

Summary

This PhD dissertation summarizes responses of two co-occurring tree species, *Larix decidua* and *Pinus mugo* ssp. *uncinata*, and the associated understorey dwarf shrub heath to nine years of CO₂ enrichment and three years of soil warming at the Swiss alpine treeline (Stillberg, Davos). Chapters 2 and 3 focus on the long-term above-ground growth responses of trees and dwarf shrubs, respectively, whereas Chapter 4 describes an experimental freezing study conducted on several plant species during a single growing season. Chapter 5 is an investigation of how the first season of soil warming influenced soil processes and the carbon balance of this treeline ecosystem.

Tree growth

Nine years of free air CO₂ enrichment led to an overall stimulation of above-ground growth in *Larix* but no significant growth response in *Pinus*. Accounting for pre-treatment vigour of individual trees, tree ring increments throughout the experimental period were larger in *Larix* growing under elevated CO₂ but not in *Pinus*. The magnitude of the CO₂ response in *Larix* ring width varied over time, with a significant stimulation occurring in treatment years three to seven (marginal in year six). These tree ring width measurements confirmed the species-specific responsiveness to elevated CO₂ observed in an independent set of tree cores during the first four treatment years (Handa *et al.* 2006) and indicated that this pattern continued in the following five years. Our longer-term results for mean lateral shoot length were also consistent with earlier results (Handa *et al.* 2005, 2006), with a consistent but non-significant trend of longer shoots in *Larix* exposed to elevated CO₂ but not in *Pinus*. At the whole tree level, species-specific responses were apparent in leaf canopy cover, total new shoot production and stem basal area, indicating a cumulative effect of increased tree size in *Larix* after nine years of exposure to elevated CO₂ but no effect in *Pinus*. Due to the expanding tree canopy, the *Larix* ring width response in years three to seven could have caused the cumulative CO₂ effect on tree size even if no further stimulation occurred (Körner 2006), so it remains unclear if responsiveness was sustained over the longer term.

The tree ring record during years of CO₂ enrichment provided us with the opportunity to investigate how interannual variability in climatic conditions influenced the responsiveness to elevated CO₂. We found that the CO₂ effect on *Larix* ring width was enhanced under climatic conditions in the current and preceding year known from the literature to positively influence radial stem growth in general. Specifically, ring width was stimulated more by elevated CO₂ in years with relatively high spring temperatures and an early snowmelt date, suggesting that temperatures were less limiting in these years and greater benefit was gained from extra carbon assimilated under elevated CO₂. The

magnitude of CO₂ stimulation was also greater after relatively high temperatures and high solar radiation in the preceding growing season, perhaps reflecting gains due to larger carbon reserves. These results indicate that stimulation of *Larix* growth might be especially pronounced in a future warmer climate.

Findings from this chapter apply to juvenile individuals of these treeline species, which experience a steady-state consumption and recycling of resources below ground but have minimal constraints on canopy expansion with respect to light or space. Therefore, it remains uncertain how rising CO₂ concentrations will affect seed germination, seedling establishment, or growth of older trees under closed-canopy conditions. Further, our understanding of tree below-ground responses to CO₂ enrichment is limited: ingrowth root core samples indicated that the first four years of CO₂ enrichment did not influence the standing root crop or the rate of new fine root production of either tree species (Handa *et al.* 2008), but observations of longer-term tree growth focused almost exclusively on above-ground responses. Nonetheless, above-ground growth benefits of *Larix* but not *Pinus* in the observed life stage could have long-lasting consequences for the growth and survival of these two competing treeline species and for the structure of the treeline ecotone.

Dwarf shrub growth and understorey community composition

Growth responses to CO₂ enrichment and soil warming were different for three dominant ericaceous dwarf shrub species growing in the understorey of larch and pine at the high elevation treeline. *Vaccinium myrtillus* responded positively to both experimental treatments and was generally more responsive than *Vaccinium gaultherioides* or *Empetrum hermaphroditum*. *V. myrtillus* responded to CO₂ enrichment with increased annual shoot increment length (+12%, pooled across temperature treatments, tree species and years) and slightly larger leaf size, measured in multiple treatment years, which contributed to enhanced total ramet height in the final three years of the experiment. In contrast, *V. gaultherioides* showed a slightly positive CO₂ effect on shoot growth and total ramet height in plots with pine but a slightly negative effect in plots with larch. The response was different again for *E. hermaphroditum*, for which none of the measured parameters were influenced by elevated CO₂, regardless of plot tree species identity. The CO₂ effect on each dwarf shrub species was relatively consistent over several years of enrichment, providing a clear indication that these species differ in their responsiveness irrespective of annual variations in climatic conditions. For *V. myrtillus* in particular, there was no decline over time in the shoot growth response, suggesting that long-term growth stimulation was not constrained by a progressive nutrient limitation or by photosynthetic down-regulation.

V. myrtillus was the only species to show a significant positive shoot growth response to the warming treatment. Relative to the two years preceding warming, the mean length of annual shoot increments of this species was 31% greater in warmed plots than in unwarmed plots (pooled across CO₂ concentrations, tree species and years). Soil

warming had a large short-term (one season only) positive effect on leaf N concentration of both *Vaccinium* species, supporting the hypothesis that soil warming would initially accelerate N cycling and lead to an enhanced N supply due to faster organic matter decomposition. However, our experimental design did not permit us to distinguish between direct temperature-driven effects and indirect nutrient-driven effects of warming on dwarf shrub growth. Dwarf shrub responses to CO₂ enrichment and soil warming were analyzed separately in this study because interactive effects involving these two treatments explained very little of the variation in the response variables. This finding from three years of the combined treatments suggests that, regarding these two specific global change factors, future growth of these three dwarf shrub species can be predicted from independent responses to each shift.

The experimental manipulations led to changes in vegetation composition at the plot scale during the final four years of the long-term treatment. Vascular plant species richness decreased in elevated CO₂ plots with larch but not with pine, while that of non-vascular plants (mosses and lichens) decreased under soil warming. The reduction in vascular plant species richness could not be explained statistically by greater canopy shading from larch trees in elevated CO₂ plots, indicating that our findings were not simply the result of advanced succession under larger trees. However, a trend of greater species loss in plots with taller *V. myrtillus* ramets suggests that increased shading within the understorey canopy and/or increased below-ground competition played a role in the decline. Overall, our results suggest that *V. myrtillus* might increase in dominance under future atmospheric and climate change and that shifts in understorey species composition are likely to occur within the treeline ecotone.

Conclusions from this study pertain to dwarf shrub growth and species composition in an existing understorey layer at treeline. In this environment, below-ground competition, shade, and litter inputs from trees potentially have large influences on understorey plants. Further, the dense heath vegetation means that regeneration from seed is almost non-existent for the three target dwarf shrub species. For predicting possible dwarf shrub expansion, i.e. to higher elevations with available open ground, a greater focus on reproductive output, seed germination success, and establishment would be necessary.

Freezing resistance

Freezing conditions can occur year-round in high elevation environments and are particularly common in temperate regions during the early part of the alpine growing season and in early autumn. At our research site, temperatures below 0°C occurred during every snow-free period of the nine-year experiment. After a freezing event in June 2005 (minimum air temperature -4.7°C at the climate station), one to two weeks after the winter snow cover had disappeared, we observed increased damage to leaf and apical bud tissue under elevated CO₂ in *Vaccinium myrtillus* but no CO₂ effect in *V. gaultherioides* (Rixen *et al.* in prep). In late May 2007, three weeks after winter snow disappearance, freezing conditions (-4.2°C) accompanied by snowfall resulted in

widespread damage to leaf and stem tissue of *Empetrum hermaphroditum* (Rixen *et al.* in prep). There was significantly more damage in plots with soil warming, which remained free of snow, than in unwarmed plots, which were covered by approximately 10 cm of new snow, and the highest damage rate occurred in plots with both warming and elevated CO₂. This same freezing event caused substantial damage to *Larix decidua* needles and newly formed long shoots, with a trend of increased damage in trees growing under elevated CO₂ (Rixen *et al.* in prep). These observations prompted us to conduct an experimental investigation of how CO₂ enrichment and soil warming influence plant sensitivity to early growing season freezing events.

In this study, we exposed newly-formed leaf tissue (approximately two weeks after leaf expansion) to several freezing temperatures and used the electrolyte leakage method to determine the threshold temperature at which damage occurs (lethal temperature 50 [LT50]; Murray *et al.* 1989). We found that CO₂ enrichment led to reduced freezing resistance in half of the species tested. *Larix* and *V. myrtillus* were among these species, consistent with our earlier field observations. Elevated CO₂ acted directly on freezing sensitivity and not via altered phenology. Soil warming showed little to no influence on the phenology and freezing resistance of the sampled species. Advanced phenology occurred in only one of the ten species tested (*V. myrtillus*), while LT50 was shifted in only two species and in opposite directions (freezing resistance decreased in *V. myrtillus* and increased in *Avenella flexuosa*). Warmer temperatures could, however, have a greater impact if earlier snowmelt dates affect species with poor photoperiod control of phenology. Plant responses to the experimental treatments were largely species-specific, and grouping into plant growth forms or functional groups did not contribute to a more general prediction of expected freezing sensitivity under future atmospheric and climate change. In summary, our results suggest that leaf tissue damage caused by episodic early season freezing events will increase in frequency for some species in the coming decades. The resulting shifts in relative freezing resistance among co-occurring species could, in turn, alter competitive interactions among species.

Findings from this chapter have important implications for the growth responses found for trees (Chapter 2) and dwarf shrubs (Chapter 3). *Larix* ring width showed a stronger CO₂-induced stimulation in years with a relatively small maximum snow depth and high early-season temperatures, which in combination led to an early snowmelt date. However, earlier snowmelt can expose trees to stochastic early season freezing events, and the increased susceptibility to freezing damage observed for *Larix* growing under elevated CO₂ might partially offset this growth advantage over the longer term. Similarly, *Vaccinium myrtillus* shoot growth was enhanced by CO₂ enrichment and soil warming, but this experimental freezing study demonstrated that both of these environmental changes can negatively impact this species' resistance to freezing events during the early growing season. Regarding elevated CO₂, we observed significant cumulative growth enhancement for both *Larix* (canopy size, stem basal area) and *V. myrtillus* (total ramet height) despite observations of increased damage from natural freezing events during the nine-year experimental period. This result suggests that growth benefits are

not substantially offset by increased freezing damage under current climatic conditions, but it remains uncertain how this balance will shift with rises in temperature (found to increase *V. myrtillus* freezing sensitivity) and advanced snowmelt date.

Carbon balance

Results from the first season of soil warming indicate that net carbon uptake by plants, soil respiration and DOC leaching respond differently to a 4 K step increase in soil temperature in this alpine treeline ecosystem. While soil respiration rates increased immediately with increased temperature, DOC leaching showed a delayed and much smaller response, probably due to a balancing out of DOC production and degradation. Tracing of ¹³C-depleted CO₂ added for seven years showed that the accelerated CO₂ effluxes from warmed soils were not driven by increased mineralization of recent plant litter and root respiration but by mineralization from older soil organic matter. These soil carbon losses clearly exceeded the estimated carbon accumulation by plants, which showed little response to one growing season of soil warming. These results suggest that soil warming, representative of warmer and drier years, can lead to short-term carbon losses from alpine treeline ecosystems.

Results from a single season provide limited information about how the carbon balance in high elevation treeline ecosystems will be affected by a gradual rise in temperature over many years. Limited or transient soil respiration responses have been observed in *in situ* warming studies with young labile carbon pools that were depleted rapidly (e.g. Melillo *et al.* 2002). In contrast, warming by only 1 K in subarctic peatlands near Abisko, Sweden stimulated soil respiration for at least eight years, with the majority of respired carbon coming from old peat near the bottom of the active layer (Dorrepaal *et al.* 2009). Many temperate high elevation treeline environments have thick organic layers (Körner 2003), even if small compared to the Northern peatlands, which suggests that increased carbon losses during the snow-free season could persist for several years of warming. In our study, soil respiration continued to be strongly stimulated by warming during the 2008-2010 snow-free seasons (F. Hagedorn, unpublished data), whereas increases in carbon gains from enhanced plant productivity remained small in comparison (M. Dawes, unpublished data). Although the duration of this response remains unknown, an increase in carbon losses from treeline ecosystems for at least four active seasons could contribute to positive feedbacks on global warming.

Main Conclusions

1. *Species-specific responses.* From this *in situ* investigation of two tree species and several understorey species, we can conclude that the different responsiveness of individual species to elevated CO₂ and soil warming was related to species identity rather than site conditions. This key result, found for treatment effects on both growth and freezing resistance, indicates that shifts in the species composition and structure of the treeline ecotone are likely to occur in the future.

2. *Contrasting limitations on tree growth.* The lack of growth response to elevated CO₂ in *Pinus* supports the hypothesis that carbon supply does not limit above-ground growth of this species at the high elevation treeline. In contrast, CO₂-induced stimulation of above-ground growth in *Larix* indicates that this species benefits from extra carbon despite low temperatures. Growth enhancement of *Larix* yielded significant effects on tree size after several years of CO₂ enrichment, suggesting that rising CO₂ concentrations could shift the competitive balance of these two treeline species in favour of *Larix*.

3. *Importance of extreme climatic conditions.* Field observations and experimental investigation indicated that some species will be more susceptible to damage from freezing events during the early growing season under higher CO₂ concentrations and temperatures. Tissue loss resulting from such events could partially offset potential growth benefits associated with these environmental changes. At the other extreme, a step increase in temperature, representative of summertime heat waves, can cause strong and immediate stimulation of soil respiration, primarily of old carbon, yielding an increase in carbon losses from treeline ecosystems.

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Monitoring the health of urban Seattle streams through collection and analysis of hydrological data and measurements of aquatic and riparian habitat value.

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Publications

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In review

Dawes, M. A., Hagedorn, F., Handa, I.T., Zumbrunn, T., Hättenschwiler, S., Wipf, S. and Rixen, C. Growth and community responses of alpine dwarf shrubs to *in situ* CO₂ enrichment and soil warming. *New Phytologist*, in review.

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2008	Honor Award for Master's thesis, Washington Chapter American Society of Landscape Architects
2006-2007	Byron and Alice Lockwood, Stanley Gessel, and Riffe Family Endowed Scholarships, University of Washington, College of Forest Resources
2006	Honor Award for group project: Envisioning Seattle's green infrastructure for the next century, Washington Chapter American Society of Landscape Architects
2005-2006	Jay Bee Memorial and Matsutaro Kawaguchi Endowed Scholarships, University of Washington, Department of Landscape Architecture
2000-2003	Richard and Susan Rogel Scholarship, University of Michigan
2000-2003	James B. Angell Scholar, University of Michigan
2001	William J. Branstrom Freshman Prize, University of Michigan

International Scientific Meetings (*published abstracts & oral presentations*)

- 2010 Global Change and the World's Mountains, Mountain Research Initiative (MRI) Conference. Perth, UK.
- 2009 British Ecological Society Annual Meeting, Hatfield, UK (poster).
- 2009 Ecological Society of America Annual Meeting. Albuquerque, New Mexico, USA.
- 2008 & 2009 Swiss Global Change Day, Bern, Switzerland (poster).
- 2007 Annual Greening Rooftops for Sustainable Communities Conference. Minneapolis, Minnesota, USA.

Additional Professional Activities

Scientific Associations: Ecological Society of America, Ecophysiological Ecology section; British Ecological Society; Swiss Botanical Society; Washington Native Plant Society.

Reviewing: Arctic, Antarctic & Alpine Research; Plant Science.

PhD coursework: alpine plant ecology (Prof. Christian Körner), stable isotopes (Dr. Rolf Siegwolf), statistics (Dr. Andy Hector, Dr. Sabine Güsewell), plant carbohydrates (Prof. Felix Keller).

Joint supervision of Master's student thesis research. WSL-SLF, Davos, Switzerland (2008 & 2010).

English language editing work for Prof. Dr. Fritz Schweingruber (2008-present).