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SPECIAL TOPIC: IN HONOR OF CHRISTIAN KÖRNER

An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study

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Abstract We evaluated the impacts of elevated CO_2 in a treeline ecosystem in the Swiss Alps in a 9-year free-air CO_2 enrichment (FACE) study. We present new data and synthesize plant and soil results from the entire experimental period. Light-saturated photosynthesis (A_{max}) of ca. 35-year-old *Larix decidua* and *Pinus uncinata* was stimulated by elevated CO_2 throughout the experiment. Slight down-regulation of photosynthesis in *Pinus* was consistent with starch accumulation in needle tissue. Above-ground growth responses differed between tree species, with a 33 % mean annual stimulation in *Larix* but no response in *Pinus*. Species-specific CO_2 responses also occurred for abundant dwarf shrub species in the understorey, where *Vaccinium myrtillus* showed a sustained shoot growth enhancement (+11 %) that was not apparent for *Vaccinium gaultherioides* or *Empetrum hermaphroditum*. Below ground, CO_2 enrichment did not stimulate fine root or mycorrhizal mycelium growth, but increased CO_2 effluxes from the soil (+24 %) indicated that enhanced C assimilation was partially offset by greater respiratory losses. The dissolved organic C (DOC) concentration in soil solutions was consistently higher under elevated CO_2 (+14 %), suggesting accelerated soil organic matter turnover. CO_2 enrichment hardly affected the C–N balance in plants and soil, with unaltered soil total or mineral N concentrations and little impact on plant leaf N concentration or the stable N isotope ratio. Sustained differences in plant species growth responses suggest future shifts in species composition with atmospheric change. Consistently increased C fixation, soil respiration and DOC production over 9 years of CO_2 enrichment provide clear evidence for accelerated C cycling with no apparent consequences on the N cycle in this treeline ecosystem.

Keywords Carbon cycling · Dwarf shrub · Global change · Nitrogen · Treeline conifer

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Introduction

Global environmental changes profoundly affect the structure and functioning of ecosystems, with important implications for ecosystem services provided to human societies (Chapin et al. 2009; Isbell et al. 2011). Some changes have abrupt, at times catastrophic consequences for ecosystems, such as an increased magnitude and frequency of extreme weather events (Jentsch et al. 2007) or changes in the El Niño Southern Oscillation (Holmgren et al. 2006), while others, such as rising atmospheric CO_2 concentrations, induce more subtle, but in the longer term still considerable, alterations in ecosystem processes. The past few years have been tremendously fruitful in terms of CO_2 research, as many large-scale free-air CO_2 enrichment (FACE) experiments have been running for close to or even longer than a decade and can provide novel information about if and how responses change over time. Understanding which plant and soil processes show a sustained effect of increased C input or flux under elevated CO_2 over several years can now be addressed for a number of ecosystem types (see reviews by Körner 2006; Leakey et al. 2009; Norby and Zak 2011). High-elevation and high-latitude ecosystems are expected to be particularly sensitive to ongoing atmospheric and climate change, yet relatively few greenhouse or open-air field studies in (sub)arctic and alpine regions have included manipulation of atmospheric CO_2 concentrations, and none of these experiments have spanned more than 4 years (Oechel et al. 1994; Gwynn-Jones et al. 1997; Körner et al. 1997; Kostiainen et al. 2004; Inauen et al. 2012). To our knowledge, no previous CO_2 enrichment experiment has investigated the alpine treeline ecotone, defined here as the altitudinal zone merging tree-dominated forest and dwarf shrub-dominated heath (Körner 2012).

Studies of C_3 plants growing in near-natural conditions have found that elevated CO_2-induced stimulation of the light-saturated rate of photosynthetic C uptake (A_{max}) is usually sustained over time (Leakey et al. 2009). Plant growth does not mirror leaf-level photosynthesis, however, and a great deal of CO_2 research has focussed on understanding where plants allocate additional C taken up in a CO_2-enriched atmosphere (Körner 2006). In several experiments, prolonged exposure to elevated CO_2 has led to increased C allocation to below-ground sinks, including plant roots (Norby et al. 2004) but also mycorrhizal associations and the heterotrophic soil food web via rhizodeposition and/or increased fine root turnover (Pollierer et al. 2007). CO_2-induced shifts in plant-soil fluxes can have important implications for C storage at the ecosystem level (Jackson et al. 2009; Drake et al. 2011). In addition, these changes can influence the cycling of N, a limiting resource for plant growth in many natural ecosystems. In situations where increased productivity occurs initially in response to elevated CO_2, a negative biogeochemical feedback can occur over the longer term if greater N sequestration into plant and soil organic matter is not met with sufficient replenishment via mineralization [progressive N limitation (PNL); Luo et al. 2004; Norby et al. 2010]. However, plant physiological adjustments such as increased plant N-use efficiency (Crous et al. 2008), accelerated N mineralization (Finzi et al. 2007; Phillips et al. 2011; Schleppi et al. 2012), and enhanced below-ground C allocation to increase plant nutrient acquisition through stimulated root and mycorrhizal activity (Treseder 2004), have been reported to prevent or at least delay the onset of PNL in some ecosystems with low N availability.

In addition to facilitating assessments of how plant and soil responses change over several years, FACE experiments provide the opportunity to compare responses of multiple naturally co-occurring species and investigate community dynamics. Studies involving multiple species also provide valuable information about how plant species identity in itself or species-specific responses to altered conditions influence ecosystem processes such as nutrient cycling (Andresen et al. 2010) or interactions with other trophic levels such as herbivores (Zvereva and Kozlov 2006). Important achievements have been made in this respect for grassland-type ecosystems (Zavaleta et al. 2003; Langley and Megonigal 2011), where different responses of individual species have led to shifts in species composition. However, few CO_2 enrichment experiments in ecosystems with woody plants have included more than one tree species in a well-replicated design, making it difficult to determine if different responses are due to species identity or site conditions (but see Seiler et al. 2009).

The long-term (2001–2009) FACE study in the Swiss Central Alps allowed us to evaluate the impacts of elevated CO_2 in an undisturbed, multi-species treeline ecosystem for the first time. Unlike other FACE studies with woody plants, low growing season temperatures at the treeline impose direct constraints on plant growth, particularly for upright trees, and additionally limit decomposition and mineralization processes, resulting in low availability of mineral N (Körner 2012). Some key goals in establishing this treeline FACE experiment were to compare growth responses of two dominant tree species growing at treelines in the Central Alps (e.g. Handa et al. 2005), to assess changes in the growth and abundance of different understorey plant species (Hättenschwiler and Zumbrunn 2006; Dawes et al. 2011a) and to determine how elevated CO_2 influenced biogeochemical processes such as soil C and N cycling (e.g. Hagedorn et al. 2008). On a broader level, we aimed to understand how soil responses and those of individual plants might lead to changes in the biodiversity and function of the treeline ecotone.

In this synthesis paper, we review responses to CO_2 enrichment over 9 years by including three categories of results: data presented in detail in previous publications, which are mentioned here only briefly; datasets including published results from early treatment years as well as more recent data, which are included together in new, multi-year analyses; and observations that are presented here for the first time. Reviewing these responses helps us to address: (1) which plant and soil processes showed an effect of increased C input/flux under elevated CO_2 and if responses changed over 9 years of enrichment, (2) whether elevated CO_2 altered the C–N balance in plants and soil and if there was evidence of increasing CO_2-induced N limitation over the course of the study, and (3) how differences in tree species identity or CO_2 effects on the two tree species influenced soil processes and understorey plants.

Materials and methods

Site and experimental design

The study site was located at Stillberg, Davos in the Central Alps, Switzerland (9°52′E, 46°46′N). The CO_2 enrichment experiment covered an area of 2,500 m^2 and was situated on a north-east-exposed 25°–30° slope at 2,180 m a.s.l. (Hättenschwiler et al. 2002). This elevation is slightly above the current treeline in the region, although the treeline position probably lags significantly behind recent rapid increases in temperature (Körner 2012). The site was part of 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). From 1975 to 2009, the mean annual precipitation was 1,133 mm and the mean annual air temperature was 2.1 °C, with February (mean −5.0 °C) as the coldest month and July (mean 10.0 °C) as the warmest month (WSL climate station at 2,090 m a.s.l., ca. 100 below the study site). During this 34-year period, mean annual and summer air temperatures increased by 1.3 and 2.0 °C, respectively (Rixen et al. 2012). 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 (Bednorz et al. 2000). The site has a low N availability, indicated by extractable NO_3^- concentrations in the soils close to the detection limit, a dominance of dissolved organic N (DON) in soil waters (Hagedorn et al. 2008) and atmospheric N inputs of <5 kg ha^{-1} $year^{-1}$ at sites above 2,000 m a.s.l. in the Swiss Alps (Schmitt et al. 2005). Soil water potential was always higher (less negative) than −300 hPa in all experimental plots, indicating high water availability (Dawes et al. 2011a).

The experiment consisted of 40 hexagonal 1.1-m^2 plots, 20 with a *Pinus mugo* ssp. *uncinata* Ramond individual in the centre and 20 with a *Larix decidua* L. individual in the centre. At the end of the CO_2 enrichment period in 2009, these trees were 39 (*Pinus*) and 37 (*Larix*) years old but were <3.5 m tall and had a stem basal diameter of <10 cm. Reproductive output was very low, with fewer than three strobili produced on only a few individuals of each species during the study period (M. Dawes, unpublished data). The study trees were sparsely distributed and did not form a closed canopy; thus, each plot contained a single tree surrounded by a dense cover of understorey vegetation including the dominant dwarf shrub species *Vaccinium myrtillus* L., *Vaccinium gaultherioides* Bigelow (group *Vaccinium uliginosum* agg.) and *Empetrum nigrum* ssp. *hermaphroditum* (Hagerup) Böcher. A total of 27 herbaceous species and seven non-vascular species (mosses and lichens) constituted 10–60 % of plant cover in the plots, with *Avenella flexuosa*, *Gentiana punctata*, *Homogyne alpina*, *Leontodon helveticus* and *Melampyrum pratense* as the most common and abundant of these species (see Dawes et al. 2011a for details on species composition). The species composition and soil characteristics at our research area are typical for treeline sites in the Central Alps with similar siliceous bedrock.

The CO_2 enrichment experiment started after snowmelt in early June 2001, at which point the 40 plots were assigned to ten groups of four neighbouring plots (two *Larix* and two *Pinus* trees per group) in order to facilitate the logistics of CO_2 distribution and regulation. Half of these groups were randomly assigned to an elevated CO_2 treatment (575 ± 52 p.p.m. mean concentration ± 1 SD for 2001–2009) while the remaining groups served as controls and received no additional CO_2 (ca. 380 p.p.m.), resulting in a split-plot experimental design. In order to keep a reasonable financial budget for CO_2 consumption, the CO_2 enrichment treatment was applied during daytime hours only throughout each growing season (ca. beginning of June to end of September). Continuous (24 h) CO_2 enrichment might slightly suppress plant dark respiration, but available data are conflicting (Long et al. 2004) and any overall effects on the plant C budget would be very small and negligible for plant growth responses. From 2001 to 2006, the system released pure CO_2 gas through laser-punched drip irrigation tubes hung vertically around a hexagonal frame surrounding each plot. From 2007 to 2009, additional tubes were woven into the tree crown to provide more efficient CO_2 delivery to the entire canopy as trees continued to grow both taller and wider. Interruptions in CO_2 release due to adverse weather conditions (saving CO_2 during periods of very low plant

photosynthetic activity) or technical failure meant that plants received supplementary CO_2 for 73–87 % of the seasonal daytime-only treatment periods. The setup and performance of the CO_2 enrichment facility have been described in detail previously (Hättenschwiler et al. 2002; Dawes et al. 2011b).

Above-ground plant measurements

The photosynthetic rate (A_{max}) of intact shoots in both tree species was measured under light-saturating conditions (natural sunlight, >1,200 µmol photons m^{-2} s^{-1}) during August or September in 2001–2005 and 2009, before the onset of leaf senescence in *Larix*, using the LI-COR 6400 gas exchange equipment (LI-COR, Lincoln, NE, USA) with a LI-6400-05 conifer cuvette (details for 2001–2003 in Hättenschwiler et al. 2002; Handa et al. 2005). Shoots formed in the current year were used in 2001–2005 whilst 1-year-old shoots were used for 2009, but this difference did not significantly affect the photosynthetic rate or treatment effects. Each tree was measured at 380 p.p.m. (ambient) and subsequently at 550 p.p.m. (elevated) cuvette CO_2 concentrations. Comparing A_{max} of the two CO_2 treatment groups when measured at the same cuvette concentration allowed us to test for possible photosynthetic down-regulation of trees grown under elevated CO_2 (Bader et al. 2010). The dry mass of all needles enclosed in the cuvette was measured to express A_{max} as µmol g^{-1} s^{-1}.

The chemical composition of current-year tree needles was measured on material sampled during early to mid-August of each year of the experiment, after needle maturation but before senescence of *Larix* needles began (details for 2001–2003 in Hättenschwiler et al. 2002; Handa et al. 2005). Tree needle chemistry measurements (expressed as % needle dry mass) included N concentrations from all treatment years and non-structural carbohydrates (NSC; sum of starch, sucrose, glucose and fructose) from 2001 to 2005 and 2009 (following methods described in Hoch and Körner 2003 and Handa et al. 2005). *Vaccinium* leaf chemistry was measured in detail throughout the second growing season of treatment (Asshoff and Hättenschwiler 2005), and material for August 2006–2009 was analysed for total C and N concentrations (Dawes et al. 2011a). The N concentration and stable N isotopic composition ($\delta^{15}N$) of tree and dwarf shrub leaves was measured in all plots for 2006–2009 using an automated elemental analyser–continuous flow isotope ratio mass spectrometer [Euro EA 3000, HEKAtech, Germany, interfaced with a Delta-S (Thermo Finnigan, Bremen, Germany)] to estimate effects of CO_2 enrichment on plant-available N in the ecosystem (Norby et al. 2010).

We collected two microcores from each tree stem in autumn 2009, sampling near the base of the tree using a 2-mm-diameter increment puncher with a cutting length of 35 mm (TREPHOR, Università degli Studi di Padova, S. Vito di Cadore, Italy; Rossi et al. 2006). Microcores extended back to 1997, thus including 4 years before CO_2 enrichment started (details in Dawes et al. 2011b). The length of new shoots formed on tree mid-canopy lateral branches was measured for each growing season of CO_2 enrichment and for the pre-treatment year 2000 (Handa et al. 2006; Dawes et al. 2011b). Cumulative effects of CO_2 enrichment on tree growth [total height, leaf canopy area, stem basal area and total (summed) shoot length] were assessed in the final 2 years of enrichment (Dawes et al. 2011b).

During autumn 2002–2009, after seasonal shoot elongation of the dwarf shrubs was complete, we measured the new shoot increment on the longest branch of five to seven randomly selected ramets in each plot (details in Dawes et al. 2011a). While the dominant dwarf shrub species at the site primarily reproduce by vegetative spread, changes in reproductive output could help explain where extra C assimilated under elevated CO_2 was allocated. Therefore, in August 2007 and 2008, we counted the berries in each plot produced by *V. myrtillus* and *E. hermaphroditum*. Very few *V. gaultherioides* berries were produced in the experimental plots and berry production was not quantified for this species. Counts were scaled up to the full plot area (berries 1.1 m^{-2}) using cover estimates of each species. In addition, ca. ten berries per species were sampled from each plot, dried at 60 °C and weighed to estimate average berry biomass. The amount of shade created by the tree canopy in each plot was quantified using hemispherical photographs taken during the peak of leaf area in 2008 (details in Dawes et al. 2011a) to determine if dwarf shrub responses were due to altered light conditions associated with tree growth responses rather than direct effects of elevated CO_2.

Fine root and mycelium production

Fine root standing biomass was determined from soil cores (2 cm in diameter) sampled from the ca. 10-cm-deep organic soil layer during five sampling periods distributed over the 2001–2004 vegetation periods (two cores per plot on each date, 0–10 cm depth; Handa et al. 2008) and in September 2009 after the final season of CO_2 enrichment (six cores per plot, 0–5 cm depth). Additionally, root growth was measured from ingrowth cores that were established at the beginning of the first experimental year and harvested on the same 2001–2004 dates as soil cores for standing biomass (details in Handa et al. 2008). After each sampling event, cores were washed and roots were separated from soil particles using a 0.5-mm sieve. Roots were separated by size class, including <0.1 and 0.1–2 mm

diameter (split made at 0.15 mm in 2009), and washed roots were dried at room temperature and subsequently weighed. Due to slightly different sampling techniques, only data from 2009 were analysed statistically for this overview paper.

Fungal mycelium production in the upper 5 cm of the soil was estimated using sand-filled (pure silica sand, 0.36–2.0 mm, 99.6 % SiO_2) ingrowth mesh bags (50-µm mesh, nylon cloth; Sintab Produkt, Oxie, Sweden), which allow the ingrowth of mycelium but not of roots. The mycelium biomass in these bags is mainly produced by ectomycorrhizal fungi (Wallander et al. 2001). In the beginning of July 2007, three cylindrical bags (23 mm diameter, 50 mm height) were installed per plot into 5-cm-deep holes made with a 2-cm-diameter soil corer. One bag from each plot was harvested in September 2007, 2008 and 2009. The bags were kept at −20 °C until freeze dried, after which the mycelium was extracted according to methods by Wallander et al. (2001) and weighed.

Soil sampling

Soils from the Oa horizon (0–5 cm depth) in each plot were sampled with a soil corer (2 cm in diameter, six cores per plot) during mid-August throughout the experimental period (details in Hagedorn et al. 2008). Total soil C and N concentrations and $\delta^{15}N$ were measured for bulked samples taken from each plot in 2003, 2005 and 2009 (see plant isotope section above for lab methods). Samples from 2004, 2007 and 2009 were extracted with 0.5 M K_2SO_4 to determine extractable NO_3^- and NH_4^+ concentrations (details in Dawes et al. 2011a). In 2003, 2004, 2007 and 2009, soil gravimetric water content was determined by weighing soils before and after drying at 105 °C for 48 h. In addition to collecting soil cores, we sampled soil solution at 3–7 cm soil depth (within the Oa horizon) once per month during each vegetation period for 2002–2009, using two ceramic suction cups (SoilMoisture Equipment, Santa Barbara, CA) per plot and applying a suction of 400 hPa overnight (Hagedorn et al. 2008). Soil solution was analysed for NH_4^+ and NO_3^-, as well as dissolved organic C (DOC) and DON.

Soil respiration was measured two to 11 times per year for 2002–2009, with sampling dates distributed evenly over each vegetation period (Hagedorn et al. 2010). Briefly, measurements were made near the centre of each plot in a permanently installed PVC collar (10 cm diameter, 5 cm height) pressed to a depth of 2 cm into the organic layer between dwarf shrubs. Respiration rates were determined by measuring the increase in CO_2 concentrations at ambient CO_2 levels in a LI-COR 6400-09 soil chamber connected to a LI-COR-820 portable system used for data collection (LI-COR).

Statistical analysis

Data analysed specifically for this overview paper included parameters presented for the first time (e.g. berry production, leaf $\delta^{15}N$) and variables measured over several vegetation periods where recent years have not been analysed previously (e.g. photosynthesis, soil respiration). For all analyses, we used statistical methods consistent with earlier publications and reflecting the split-plot experimental design (e.g. Dawes et al. 2011b). Effects of CO_2 treatment and tree species presence (*Larix* or *Pinus* in the plot centre, dwarf shrub and soil parameters only) were tested with type I analysis of (co)variance using linear mixed effects models fitted with the restricted maximum likelihood estimation method. When data from multiple years were available, repeated-measures analysis was completed with treatment year included as a categorical variable. For above-ground plant variables, models were fitted for each species separately, as previous analyses established that responses were generally species specific (e.g. Handa et al. 2005; Dawes et al. 2011a). Response variables were log or square-root transformed in some cases to improve assumptions of normality and homoscedasticity.

Additional defoliation (applied in spring 2002; Handa et al. 2005) and soil warming (2007-ongoing; Hagedorn et al. 2010) treatments were applied in a crossed manner with the CO_2 enrichment treatment during parts of the 9-year experimental period. The influence of these additional treatments and potential interactions with effects of CO_2 enrichment have been described in earlier publications (Handa et al. 2005, 2006 regarding defoliation; Hagedorn et al. 2010, Dawes et al. 2011a, b regarding warming). For this paper focusing on responses to CO_2 enrichment, we excluded plots exposed to the crossed treatment (yielding $n = 5$) when there were indications of an interactive effect with CO_2 treatment or a strong independent effect. For all other cases, we pooled across defoliation or warming treatments to maintain consistent replication across treatment years ($n = 10$). For each parameter included in this overview paper, information about replication, years included in analysis, and previous publications describing methods and results is provided in Table S1 of the Electronic Supplementary Material (ESM). For all statistical tests, effects were considered significant at $P < 0.05$ and marginally significant at $P \geq 0.05$ but <0.10. All analyses were performed using R version 2.14.0 (R Development Core Team 2008–2010), and mixed models were fitted using the nlme package (Pinheiro et al. 2008).

Results

Leaf-level plant responses

Photosynthetic CO_2 uptake (A_{max} per unit dry needle mass) of both tree species was significantly higher at elevated CO_2 concentrations over all years measured (2001–2005 and 2009), with a mean stimulation of 41 % in *Larix* ($F_{1,18} = 99.38$, $P < 0.001$) and 31 % in *Pinus* ($F_{1,18} = 39.77$, $P < 0.001$) over the 6 years of measurement (Fig. 1; Table 1). Unsurprisingly, A_{max} was more than twice as high for deciduous *Larix* as for evergreen *Pinus* (Table 1). While net CO_2 assimilation of both species remained strongly stimulated through the end of the experiment, there was a decrease in effect size after the first 2 years in *Pinus* (from 38 to 27 %) but not in *Larix*. There was a marginally significant down-regulation of photosynthesis in *Pinus* individuals growing under elevated CO_2, by an average of 10 % over all years measured ($F_{1,17} = 4.33$, $P = 0.053$ for CO_2 effect and non-significant $CO_2 \times$ year interaction), whereas no such effect was observed for *Larix* ($P > 0.39$ for CO_2 effect and $CO_2 \times$ year interaction).

For both tree species, there was a significant increase in NSC under elevated CO_2 (*Larix*, $F_{1,18} = 8.44$, $P = 0.009$; *Pinus*, $F_{1,18} = 9.54$, $P = 0.006$) that was mainly due to an increase in starch (*Larix*, $F_{1,18} = 3.66$, $P = 0.072$; *Pinus*, $F_{1,18} = 12.13$, $P = 0.003$) rather than simple sugars (Fig. 1; Table 1). There was a significant $CO_2 \times$ year interaction for *Pinus* total NSC ($F_{5,53} = 2.50$, $P = 0.042$) and a similar but non-significant trend for *Larix* ($F_{5,56} = 1.80$, $P = 0.128$). Analysis of individual years revealed that *Larix* needles experienced a significant increase in starch in 2001 (marginal) and 2002 but not in any later year, whereas starch in *Pinus* needles was enhanced by elevated CO_2 (at least marginally significant) in all years except 2003, with an average increase of 53 % (Table 1).

Statistical models including all years of the study showed that elevated CO_2 did not have a significant impact on *Larix* needle N concentrations ($F_{1,18} = 1.06$, $P = 0.318$) but led to slightly lower concentrations in *Pinus* ($F_{1,18} = 7.36$, $P = 0.014$; Fig. 1; Table 1). The CO_2 effect varied somewhat from year to year in *Pinus* ($CO_2 \times$ year interaction: $F_{8,79} = 2.68$, $P = 0.012$) but did not become more or less pronounced over time (Table 1). This CO_2 effect was primarily due to dilution from increased NSC, as results were no longer significant when N was expressed as a percentage of NSC-free leaf mass (data not shown). *Larix* needle N concentrations were ca. twice as great as those of current-year *Pinus* needles, consistent with its deciduous leaf type and higher rate of photosynthesis. Elevated CO_2 led to significantly lower

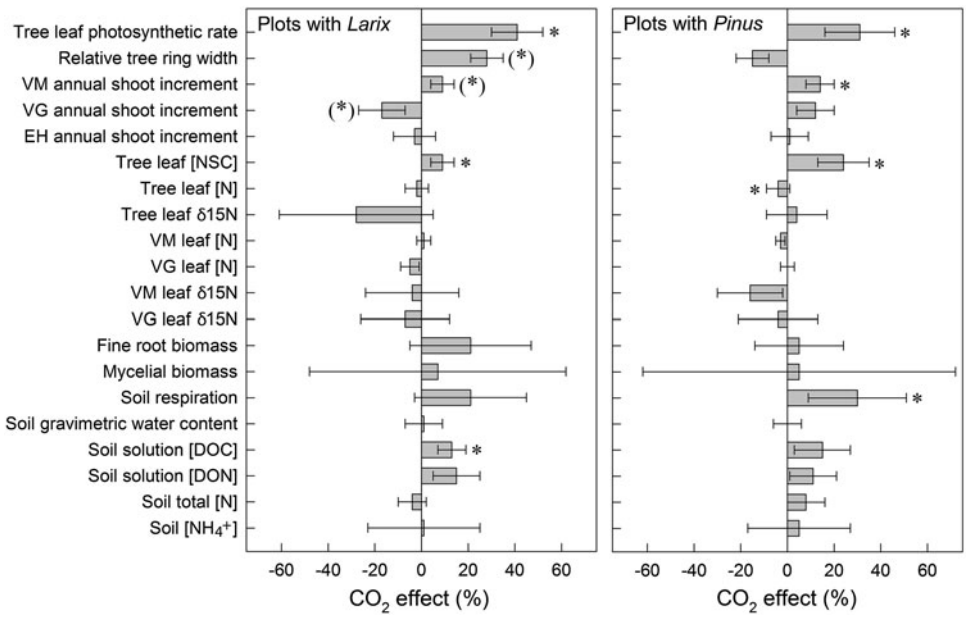

Fig. 1 Overview of plant and soil responses to CO_2 enrichment for plots with *Larix decidua* (*left*) or *Pinus uncinata* (*right*). For each parameter, the mean CO_2 effect size ± 1 SE over all years where data were available (see "Materials and methods" and Table S1 for years, replication and relevant publications) is shown as a percentage, with zero indicating no response. These averages show overall patterns but are not derived from the statistical tests applied. Instead, significant (* $P < 0.05$) and marginally significant [(*) $P < 0.10$] results of the repeated-measures analyses are shown. For relative tree ring width, values for each tree were standardized to ring width averaged over 1997–2000 (pre-treatment). *VM Vaccinium myrtillus*, *VG Vaccinium gaultherioides*, *EH Empetrum hermaphroditum*, *NSC* non-structural carbohydrates, *DOC* dissolved organic C, *DON* dissolved organic N

Table 1 Needle-level tree responses to CO_2 enrichment

Year	Replication	*Larix decidua*		*Pinus uncinata*	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
A_{max} (μmol CO_2 g^{-1} s^{-1})					
2001	10	0.094 ± 0.005	0.127 ± 0.006	0.027 ± 0.001	0.037 ± 0.001
2002	4–5	0.114 ± 0.008	0.170 ± 0.006	0.023 ± 0.004	0.032 ± 0.005
2003	5	0.107 ± 0.002	0.145 ± 0.010	0.029 ± 0.002	0.036 ± 0.001
2004	4–5	0.077 ± 0.003	0.118 ± 0.023	0.052 ± 0.010	0.072 ± 0.017
2005	4–5	0.122 ± 0.021	0.161 ± 0.006	0.079 ± 0.008	0.094 ± 0.014
2009	8–10	0.084 ± 0.005	0.122 ± 0.005	0.022 ± 0.001	0.027 ± 0.002
ANOVA	CO_2	$F_{1,18} = 99.38$*		$F_{1,18} = 39.77$*	
	Year	$F_{5,43} = 11.07$*		$F_{5,42} = 36.98$*	
	CO_2 × year	$F_{5,43} = 0.29$		$F_{5,42} = 0.47$	
Sugar (% of needle dry mass)					
2001	10	12.1 ± 0.4	13.1 ± 0.4	5.1 ± 0.3	5.7 ± 0.1
2002	5	12.5 ± 0.4	12.9 ± 0.4	5.2 ± 0.3	5.4 ± 0.4
2003	4–5	11.6 ± 0.7	11.7 ± 0.3	4.7 ± 0.2	4.3 ± 0.3
2004	6–7	10.4 ± 0.5	10.5 ± 0.5	4.6 ± 0.3	4.1 ± 0.2
2005	6–7	12.2 ± 0.5	12.7 ± 0.4	5.0 ± 0.5	5.0 ± 0.4
2009	8–10	10.8 ± 0.2	11.0 ± 0.3	6.7 ± 0.2	6.3 ± 0.2
ANOVA	CO_2	$F_{1,18} = 2.30$		$F_{1,18} = 0.19$	
	Year	$F_{5,56} = 13.27$*		$F_{5,53} = 13.97$*	
	CO_2 × year	$F_{5,56} = 0.45$		$F_{5,53} = 1.41$	
Starch (% of needle dry mass)					
2001	10	6.2 ± 0.6	8.3 ± 0.9	5.3 ± 0.6	8.6 ± 0.6
2002	5	3.2 ± 0.4	5.0 ± 0.3	4.8 ± 1.0	7.1 ± 0.8
2003	4–5	9.9 ± 0.8	10.6 ± 1.2	7.1 ± 0.8	9.6 ± 1.4
2004	6–7	4.1 ± 0.3	5.2 ± 0.8	4.8 ± 0.5	6.8 ± 0.7
2005	6–7	3.5 ± 0.2	4.0 ± 0.8	3.7 ± 0.5	7.4 ± 1.0
2009	8–10	1.8 ± 0.2	1.8 ± 0.1	3.3 ± 0.4	4.4 ± 0.4
ANOVA	CO_2	$F_{1,18} = 3.66$(*)		$F_{1,18} = 12.13$*	
	Year	$F_{5,56} = 88.94$*		$F_{5,53} = 26.52$*	
	CO_2 × year	$F_{5,56} = 1.53$		$F_{5,53} = 1.62$	
N (% of needle dry mass)					
2001	10	2.30 ± 0.07	2.11 ± 0.09	1.21 ± 0.03	1.16 ± 0.02
2002	5	1.91 ± 0.14	2.06 ± 0.11	0.90 ± 0.06	0.84 ± 0.07
2003	5	2.00 ± 0.04	2.13 ± 0.09	1.29 ± 0.03	1.18 ± 0.02
2004	3	2.42 ± 0.16	2.22 ± 0.05	1.29 ± 0.05	1.31 ± 0.13
2005	3	2.18 ± 0.12	1.99 ± 0.13	1.04 ± 0.08	0.99 ± 0.01
2006	8–10	2.24 ± 0.05	2.18 ± 0.04	1.13 ± 0.02	1.04 ± 0.02
2007	8–10	2.43 ± 0.04	2.39 ± 0.06	1.17 ± 0.02	1.14 ± 0.02
2008	6–10	2.46 ± 0.07	2.51 ± 0.08	1.57 ± 0.03	1.64 ± 0.02
2009	8–10	2.21 ± 0.09	2.18 ± 0.06	1.10 ± 0.04	1.03 ± 0.04
ANOVA	CO_2	$F_{1,18} = 1.06$		$F_{1,18} = 7.36$*	
	Year	$F_{8,96} = 10.30$*		$F_{8,79} = 134.28$*	
	CO_2 × year	$F_{8,96} = 1.67$		$F_{8,79} = 2.68$*	

Means ± 1 SE are shown for each year, as well as repeated-measures ANOVA results [*$P < 0.05$, (*) $P < 0.10$]. For light-saturated photosynthesis (A_{max}), trees grown under ambient CO_2 concentrations were measured at cuvette CO_2 concentrations of 380 p.p.m. and those grown under elevated CO_2 were measured at 550 p.p.m. Data from 2001 are presented in Hättenschwiler et al. (2002) and data for 2002–2003 are presented in Handa et al. (2005)

leaf N concentrations in the two *Vaccinium* dwarf shrub species throughout the second growing season of treatment (Asshoff and Hättenschwiler 2005), again partially due to an increase in starch, but there was no significant treatment effect in 2006–2009 (Fig. 1; Dawes et al. 2011a). N concentrations were generally greater in *V. gaultherioides* than in *V. myrtillus*, and both species had significantly greater concentrations in plots with *Larix* than with *Pinus* (Dawes et al. 2011a). For the two tree species and two deciduous dwarf shrub species, $\delta^{15}N$ in leaf material for 2006–2009 showed no significant difference between CO_2 treatments ($P > 0.271$; Figs. 1, 2). $\delta^{15}N$ was generally lower in *Pinus* needles than *Larix* needles, whilst values for the dwarf shrubs were between those of the two tree species. $\delta^{15}N$ of *V. myrtillus* was lower in plots with *Pinus* compared to plots with *Larix* ($F_{1,36} = 7.97$, $P = 0.008$; Fig. 2). In all 4 years, $\delta^{15}N$ signatures of dwarf shrub leaves were correlated with those of tree needles sampled from the same plot and on approximately the same date ($r^2 = 0.38$–0.59 and $P < 0.001$ for each dwarf shrub species and year; Fig. S1 of ESM).

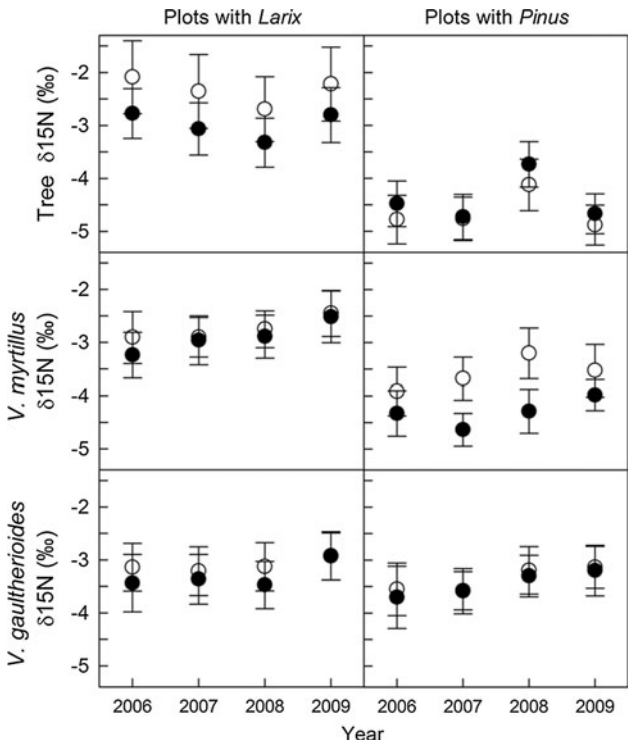

Fig. 2 Leaf $\delta^{15}N$ values from the two tree species *L. decidua* ($n = 10$) and *P. uncinata* ($n = 8$) and the two deciduous dwarf shrub species VM ($n = 10$) and VG ($n = 10$), sampled in early August 2006–2009. Mean values ± 1 SE are shown for each CO_2 and tree species combination (*open circles* ambient CO_2, *filled circles* elevated CO_2). CO_2 effects on $\delta^{15}N$ were not significant for any species. For abbreviations, see Fig. 1

Above-ground growth and reproductive output

Larix showed 33 % wider annual tree rings averaged over all 9 years, which resulted in a cumulative enhancement in stem growth of the same magnitude (Figs. 1, 3). Consistent with this result, there was a trend of increased annual lateral shoot growth in *Larix* throughout the study as well as enhanced tree canopy size, basal area and total shoot production near the end of CO_2 enrichment (Dawes et al. 2011b). The positive CO_2 effect on *Larix* ring width varied somewhat from year to year and declined in the last 2 years of the experiment. In contrast to *Larix*, CO_2 enrichment did not significantly influence *Pinus* ring width (Figs. 1, 3), and all other above-ground growth parameters were similarly unresponsive for this species (Dawes et al. 2011b). Due to the expanding tree canopy, it is likely that initial above-ground growth benefits for *Larix* but not *Pinus* contributed to cumulative differences between species observed after 9 years.

The three dominant ericaceous dwarf shrub species growing in the plot understorey also differed in their above-ground growth response to elevated CO_2 (Dawes et al. 2011a). Annual shoot increment growth of *V. myrtillus*, the most abundant species in the plots, was enhanced by CO_2 enrichment, with a relatively consistent stimulation in plots shared with each tree species and across all treatment years (mean +11 %; Figs. 1, 4). Independent from this CO_2 effect, shoot increments were slightly larger in plots with *Larix* than in those with *Pinus* (mean +8 %

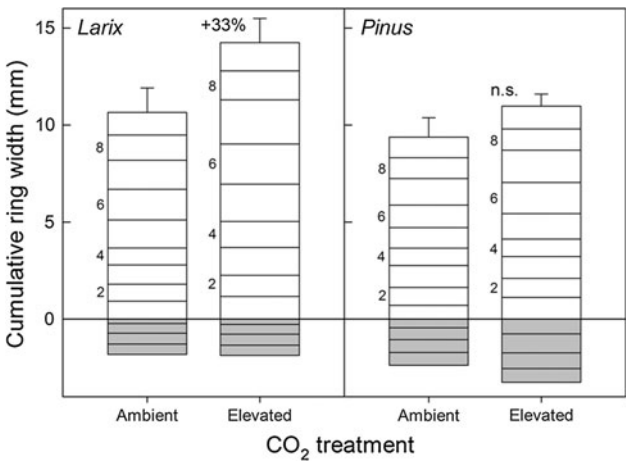

Fig. 3 Mean tree ring width for each CO_2 treatment and tree species combination, summed over the experimental period ($n = 10$ for *L. decidua* and 8–9 for *P. uncinata*). The *zero line* indicates the start of CO_2 enrichment in 2001, and pre-treatment years 1997–2000 are shown in *grey*. *Numbers* indicate the year when a specific tree ring was formed after treatment began. *Error bars* represent +1 SE of the mean cumulative (9-year) ring width for each treatment combination. Averaged over all individual years, *Larix* ring width was enhanced by 33 % whereas *Pinus* did not show a significant growth response. Data are presented in Dawes et al. (2011b)

across all years). CO_2 enrichment also influenced *V. gaultherioides* shoot growth but the direction of response depended on tree species identity, with a slight positive response in plots with *Pinus* and a slight negative response in plots with *Larix* (Figs. 1, 4). *E. hermaphroditum* shoot growth did not show an effect of CO_2 enrichment or plot tree species (Figs. 1, 4). There was no correlation between the amount of canopy shading and shoot growth of any species, demonstrating that the observed treatment effects were not simply due to altered light conditions resulting from tree growth responses (Dawes et al. 2011a).

Measurements of berry mass in the later years of the experiment indicated that, in addition to longer shoots, *V. myrtillus* produced slightly larger berries under elevated CO_2, irrespective of plot tree species identity ($F_{1,32} = 5.44$, $P = 0.026$). Additionally, berries were larger in plots with *Pinus* than in those with *Larix* for both elevated and ambient CO_2 treatments ($F_{1,32} = 10.95$, $P = 0.002$; Fig. 4). In contrast to *V. myrtillus*, *E. hermaphroditum* berry mass was not influenced by CO_2 treatment or plot tree species identity ($P > 0.55$; Fig. 4). As with shoot growth, there was no correlation between berry mass and canopy shading for either species ($r^2 < 0.04$). In contrast, berry abundance showed a negative correlation with canopy shading in general, so it was not possible to discern direct treatment effects.

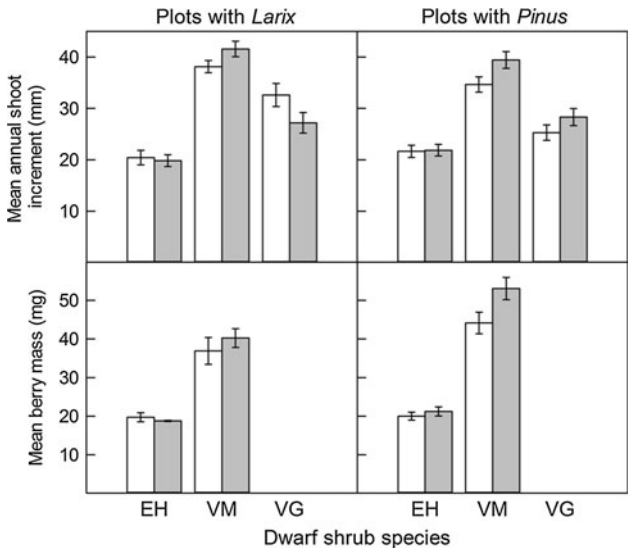

Fig. 4 Vegetative and reproductive production in the major dwarf shrub species EH, VM and VG. *Left panels* Plots shared with a *L. decidua* individual, *right panels* plots shared with a *P. uncinata* individual. *Top panels* annual shoot increment, averaged over 2003–2009 (n = 8–10); *bottom panels* mass per berry, averaged over 2007 and 2008 (n = 2–10). VG berries occurred in very few plots each year and production was not quantified. In both panels, *bars* represent the multi-year mean for each CO_2 and plot tree species combination, ±1 SE (*open bars* ambient CO_2, *shaded bars* elevated CO_2). Please refer to the text for results of repeated-measures analyses. Detailed shoot growth data are presented in Dawes et al. (2011a). For abbreviations, see Fig. 1

Root and mycorrhizal growth

Measurements of fine root production and standing biomass for 2001–2004 showed no significant effect of CO_2 enrichment, both for total fine roots (<2 mm diameter) and for individual size classes broadly based on plant functional groups (Figs. 1, 5; Handa et al. 2008). A harvest in autumn 2009 similarly indicated no significant response to elevated CO_2 in total fine root biomass or in <0.15-mm or 0.15- to 2-mm-diameter size classes individually ($F_{1,34} < 0.02$, $P > 0.898$ for all statistical tests; Figs. 1, 5). Although not statistically significant, there was a trend of higher root biomass under elevated CO_2 in plots with *Larix* in the larger size class on most sampling dates (mean +30 %; Fig. 5). Biomass of 0.15- to 2-mm-diameter roots, and consequently total fine root biomass, was also greater in plots with *Pinus* than with *Larix* in 2009 ($F_{1,34} = 9.66$, $P = 0.004$), although there was no consistent trend over sampling dates in earlier years (Handa et al. 2008). There was no CO_2 treatment effect on the biomass of mycelia colonizing sterile sand sampled in 2007–2009, irrespective of which tree species was present ($F_{1,16} = 0.09$, $P = 0.763$; Fig. 1). Mycelial colonization increased between the first and second sampling dates (ca. 300 and 700 g m^{-2} after one and two vegetation periods, respectively) but showed no difference between the second and third sampling dates (overall year effect: $F_{2,23} = 6.79$, $P = 0.005$).

Soil responses

In 2003, 2004, 2007 and 2009 when the gravimetric water content of soils was measured, there was no significant effect of CO_2 enrichment or tree species identity and no interactive effects were significant ($P > 0.13$; Fig. 1). Soil moisture varied significantly among the 4 years ($F_{3,65} = 15.2$, $P < 0.001$) although water availability was consistently quite high in all plots (lowest mean for all plots ± 1 SE = 54.4 ± 1.5 % in 2003, highest mean = 64.6 ± 1.2 % in 2004). Elevated CO_2 did not have a significant influence on C or N concentrations or $\delta^{15}N$ in bulk soil samples, irrespective of plot tree species or treatment year ($F_{1,36} < 0.16$, $P > 0.698$; soil N; Fig. 1). Concentrations of K_2SO_4-extractable NO_3^- were always below the detection limit (0.1 mg N l^{-1}), and NH_4^+ concentrations were not significantly affected by CO_2 treatment, tree species identity, or any interactive term (Fig. 1; Dawes et al. 2011a). DOC in soil solution co-varied with plot-level bulk soil C concentration and increased significantly under elevated CO_2 ($F_{1,35} = 4.34$, $P = 0.045$; Fig. 1), with the largest

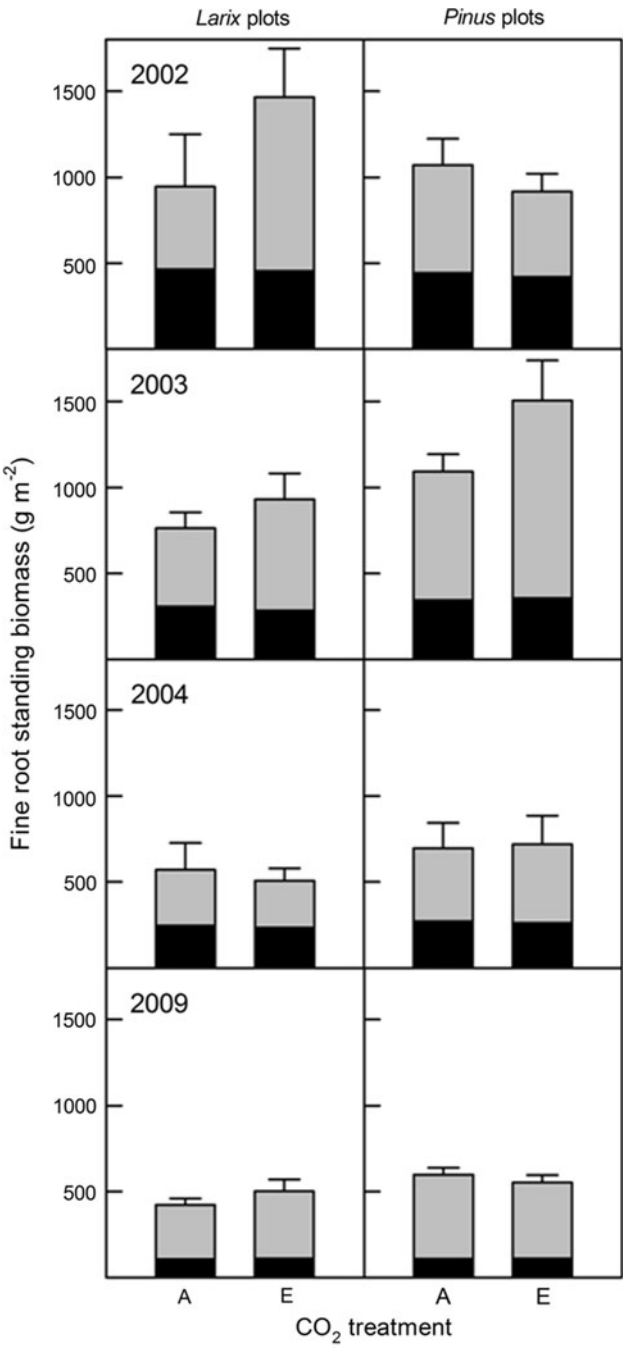

Fig. 5 Mean fine root standing biomass for ambient (*A*) and elevated (*E*) CO_2 treatments, based on soil cores sampled in September or October of each year shown (2002, $n = 6$; 2003, $n = 7$–9; 2004, $n = 5$; 2009, $n = 9$–10). *Left panels* Plots shared with a *L. decidua* individual,*right panels* plots shared with a *P. uncinata* individual. *Black bars* represent roots <0.1 mm in diameter and *grey bars* represent roots ≥0.1 but <2 mm (split made at 0.15 mm for 2009). *Error bars* show +1 SE of the mean total (<2 mm) fine root standing biomass. A sampling depth of 5 cm in 2009 compared to 10 cm in 2002–2004 contributed to lower biomass in 2009 than in earlier years. Detailed data for 2002–2004 are presented in Handa et al. (2008)

effect size occurring in 2005 (Hagedorn et al. 2008) but generally a consistent response over all years (CO_2 × year interaction: $F_{4,1245} = 0.90$, $P = 0.508$; Fig. 1). DON and DOC were strongly correlated, and DON showed an increase of similar magnitude in response to elevated CO_2, although greater variability meant that this effect was not statistically significant ($F_{1,36} = 1.92$, $P = 0.175$; Fig. 1). DOC:DON ratios were not affected by CO_2 enrichment. Both DOC and DON were generally higher in plots with *Larix* than with *Pinus* (marginally significant; $F_{1,36} > 3.48$, $P < 0.071$). NH_4^+ and NO_3^- concentrations in soil solution were below the detection limit (0.1 mg N l^{-1}).

CO_2 enrichment stimulated soil CO_2 effluxes from the plots throughout the experimental period ($F_{1,35} = 6.52$, $P = 0.015$; Fig. 1). Averaged over the two tree species and all measurement dates (2002–2009), respiration was 107 ± 13 mg CO_2 m^{-2} h^{-1} in ambient CO_2 plots and 132 ± 13 mg CO_2 m^{-2} h^{-1} in elevated CO_2 plots (24 % increase). This effect did not vary significantly from year to year (CO_2 × year interaction: $F_{7,1127} = 1.48$, $P = 0.169$), although it was most pronounced in 2003–2007 (data not shown). Plot tree species was not significant as a main effect, indicating similar soil respiration rates under *Larix* and *Pinus* in all years, and the CO_2 × tree species interaction was not statistically significant. However, separate models for plots with each species revealed that the positive CO_2 effect was more pronounced in plots with *Pinus* ($F_{1,18} = 5.46$, $P = 0.031$) than with *Larix* ($F_{1,17} = 1.52$, $P = 0.235$; Fig. 1), and the interannual variation in the CO_2 effect was significant in plots with *Pinus* only (CO_2 × year interaction: $F_{7,560} = 2.16$, $P = 0.036$).

Discussion

Consistent responses over 9 years of CO_2 enrichment

Overall, plant responses to elevated CO_2 showed little change over the experimental period. Both tree species showed sustained photosynthetic stimulation, irrespective of their growth responses. The allocation of extra C to above-ground growth was significant for *Larix*, with enhanced annual ring width through at least the seventh treatment year, whereas *Pinus* showed no response (Fig. 3). Tree needle traits were hardly affected by CO_2 enrichment, with the exception of an accumulation of starch in *Pinus* needle tissue that probably contributed to the observed slight down-regulation of photosynthesis in this species through end-product inhibition (Table 1; Long et al. 2004). Greater sink capacity in *Larix* than in *Pinus*, as

suggested by down-regulation only in the latter species, is supported by the capacity for indeterminate growth in *Larix* through the production of long shoots but only determinate growth in *Pinus*. This difference, as well as the 'early pioneer' species traits associated with *Larix* (Tinner and Kaltenrieder 2005), probably contributed to contrasting above-ground growth responses to elevated CO_2 (Handa et al. 2006). Species-specific growth responses extended to the three dwarf shrubs examined, where *V. myrtillus* showed a slight but consistent positive response in shoot growth and berry mass whilst the other species showed low or no responsiveness (Fig. 4). *V. myrtillus* has a lower elevational distribution than *V. gaultherioides* or *E. hermaphroditum*, and adaptation to conditions with a higher partial pressure of CO_2 might explain its greater responsiveness to elevated CO_2 in our study where *V. myrtillus* grew close to its upper elevation limit (Körner 2003). Species-specific above-ground growth responses were similarly observed over multiple years in a late successional alpine grassland (Schäppi and Körner 1996) and for glacier forefield vegetation grown in mesocosms in Central Switzerland (Inauen et al. 2012), although in both studies most species showed no CO_2 response or even a negative effect. Regarding trees in particular, three *Quercus* species in a fire-regenerated Florida scrub-oak ecosystem differed in their responsiveness to 11 years of elevated CO_2 (Seiler et al. 2009), whereas species-level measurements in other studies have focussed on a single dominant tree species.

The relatively long duration of our study allowed us to detect how season to season variation in climate conditions, especially temperature, influenced above-ground plant growth responses to CO_2 enrichment. For example, *Larix* ring width showed a stronger CO_2-induced stimulation in years with a relatively early snowmelt date (Dawes et al. 2011b), but these conditions simultaneously exposed trees to stochastic early season freezing events. *Larix* individuals exposed to elevated CO_2 showed greater sensitivity to freezing temperatures (Martin et al. 2010), and it is possible that the decline in the CO_2 effect on ring width in 2008–2009 was due to more severe damage to new shoots of CO_2-enriched trees in 2007 (Rixen et al. 2012). CO_2 enrichment similarly led to more severe freezing damage to buds and leaves of *V. myrtillus* (Martin et al. 2010; Rixen et al. 2012). Unlike *Larix*, however, consistent enhancement of *V. myrtillus* shoot growth over several years of enrichment suggests that effects of freezing damage and generally low growing season temperatures did not noticeably counteract growth benefits of CO_2 enrichment, at least for the duration of this study.

Below-ground C pools generally showed rather low responsiveness to CO_2 enrichment, and results were again relatively consistent over time. Fine root data provided no evidence of increased allocation to below-ground productivity, although conclusive statements about biomass allocation await an extensive excavation of coarse roots. Similarly, unresponsiveness in (ectomycorrhizal) mycelium biomass during the final 3 treatment years suggests that C allocation to this below-ground sink did not increase, although large variation between plots might have masked subtle CO_2 effects (Fig. 1). Similar to our results, CO_2 enrichment did not increase fine root productivity in a lowland mature deciduous forest after 5–7 years (Bader et al. 2009), or in a nutrient-poor post-burn scrub oak system after 6 years (Brown et al. 2009). In contrast, a sustained increase in fine root productivity was observed under elevated CO_2 over 12 years in a young closed-canopy *Pinus taeda* forest (Duke FACE; Jackson et al. 2009), and the production of mycorrhizal root tips and rhizomorphs was stimulated by elevated CO_2 over a 5-year period in the same experiment (Pritchard et al. 2008). Consistent with our findings, however, no CO_2 effect on total external mycorrhizal mycelium production was detected in the Duke FACE experiment (Parrent and Vilgalys 2007) or in other field studies where this parameter was measured (Kasurinen et al. 2005; Godbold et al. 2006). In our study, the late-successional dwarf shrub community means that there is little or no below-ground space available that has not yet been fully explored by plants. These 'steady-state' below-ground conditions might explain why fine root responses remained small despite pronounced growth stimulation in *Larix* above ground, where the open canopy facilitated tree expansion (Körner 2006; Handa et al. 2008).

In contrast to other below-ground responses, soil respiration and DOC increased significantly in CO_2-enriched plots, and both of these responses were largely maintained throughout the experimental period. New plant-derived C typically constitutes a large fraction of CO_2 respired from soil (Högberg et al. 2001; Hagedorn et al. 2010), indicating a greater below-ground flux of recent assimilates under elevated CO_2 even though allocation to below-ground pools apparently was not affected. Similar to our results, stimulated soil respiration was observed for at least 6 years in lowland forest FACE sites where increased plant productivity was observed (King et al. 2004). Notably, increases in soil respiration even became more pronounced over 12 years in a temperate *P. taeda* plantation where there was a sustained plant-productivity response (Jackson et al. 2009). Collectively, these longer-term studies show that enhanced photosynthetic C uptake is often at least partially offset by increased C losses, contributing to an increased C flux rather than C storage. In addition to larger CO_2 effluxes from soil in elevated-CO_2 plots, we observed a sustained increase in soil solution DOC concentrations. Greater inputs of labile throughfall DOC (Hagedorn et al. 2008) and an increased accumulation of starch in fine roots

possibly followed by increased root exudation (Handa et al. 2008) might have contributed directly to the observed effect on DOC. Unlike soil respiration, however, contributions of recent plant-derived C to soil-solution DOC were <30 % in 2007–2009 (F. Hagedorn, unpublished data). Therefore, inputs of recent assimilates might not have been solely responsible for the 14 % average annual increase in DOC. It is possible that CO_2 enrichment additionally led to an accelerated mobilization of soil organic matter through stimulated microbial activity, an indirect consequence of greater plant C inputs. Such a 'priming' effect, which yields increased soil organic matter turnover rates and counteracts an increased storage of C in soils, has been observed in long-term low-elevation forest FACE experiments (Langley et al. 2009; Drake et al. 2011; Zak et al. 2011; Schleppi et al. 2012).

Little change in N availability under elevated CO_2

Throughout the 9-year experimental period, we observed no changes in soil C:N, inorganic N pool size, or DOC:DON in soil solution. Additionally, there was no long-term reduction of leaf N concentrations in *Vaccinium* or *Larix*, and the slight reduction in *Pinus* needles was a dilution effect related to starch accumulation. These results suggest that the CO_2-induced growth stimulation in plants was not large enough to alter the ecosystem balance between C and N or cause a progressive reduction in soil N availability to plants. In a young *Liquidambar styraciflua* forest, a stronger decline over time in litterfall $\delta^{15}N$ values under elevated CO_2 was interpreted as a sign of PNL even though N mineralization and leaf N concentration were not affected significantly (Norby et al. 2010). Although we only measured $\delta^{15}N$ during the last 4 years of treatment, values in leaf tissues showed no difference between CO_2 treatments and no CO_2-induced decline over time, providing support that N did not become increasingly limiting to photosynthetic and growth processes in a CO_2-enriched atmosphere, at least during the duration of our experiment (Figs. 1, 2). Finally, reduced N availability in soils under elevated CO_2 has been found to trigger a greater plant C allocation to the root-mycorrhizal system (Treseder 2004), but we observed no increase in fine root or mycorrhizal productivity.

Similar to our findings, estimates of N availability in an alpine grassland system based on ion exchange resin bags indicated no CO_2 treatment effect over 4 years (Körner et al. 1997), and CO_2 enrichment even led to increased NO_3^- concentrations in resin bags and soil solution at the Swiss Canopy Crane FACE site (Schleppi et al. 2012). In our treeline study, the overall minor changes in leaf chemistry, in combination with small litter inputs compared to the thick organic layer with large soil organic matter stocks, suggest that any litter-driven negative feedback on N cycling could take multiple decades to occur and was therefore not captured in our 9-year study. Edge effects associated with the relatively small plot size, required for logistical reasons, might also have contributed to the apparent lack of change in N dynamics. Plant roots probably extended outside the CO_2-enriched plot area, especially roots of trees as they grew larger during the experiment. Therefore, N acquired from the surrounding, ambient CO_2 area might have diluted any subtle changes in the C–N balance of plant tissue.

Importance of changes in species interactions

The species-specific responses we observed for tree and dwarf shrub species suggest that changes in competitive interactions and, ultimately, species composition are likely to occur at the treeline ecotone. If other global changes, such as warming or altered precipitation patterns, do not favour *Pinus* over *Larix*, results from this study indicate that *Larix* could become increasingly dominant in the treeline ecotone under future atmospheric conditions. Our findings suggest that changes in tree species dynamics could influence soil processes as well as understorey plants. For example, concentrations of DOC and, to a lesser extent, DON in soil solution were greater in plots with *Larix* than with *Pinus*, suggesting a higher rate of soil organic matter turnover, and therefore nutrient cycling, in plots with the deciduous tree species. Additionally, a somewhat larger CO_2-induced increase in soil respiration in plots with *Pinus* (+30 %) compared to *Larix* (+21 %; Fig. 1) indicates a greater below-ground C flux under *Pinus* and demonstrates that different plant species' responses to atmospheric change can have important consequences for below-ground processes.

Tree species also had clear influences on dwarf shrub properties. Needles of deciduous *Larix* had higher N concentrations than *Pinus* needles (Table 1), and these differences were reflected in the nutrient concentrations of *V. myrtillus* and *V. gaultherioides* growing in the understorey of each tree species. Similarly, considerably lower needle $\delta^{15}N$ values in *Pinus* compared to *Larix* needles were mirrored in leaves of the two *Vaccinium* species to some extent (Fig. 2), and the isotopic signature for the tree and dwarf shrubs growing in a given plot were clearly correlated (Fig. S1). These patterns suggest a close link between dwarf shrub and tree nutrient relations and lower N availability in plots with *Pinus* than *Larix*. For tree and dwarf shrub leaf N concentration, differences between species and effects of tree species identity on dwarf shrubs were greater than CO_2-induced changes in individual species. These species differences in leaf nutritional quality

had clear influences on insect herbivores in this treeline ecosystem. In a study of the alpine grasshopper *Miramella alpina* conducted in 2002, differences in leaf chemistry, especially N concentration, between host plants *V. myrtillus* and *V. gaultherioides* contributed to different relative consumption rates and relative growth rates of nymphs, and plant species effects were generally of equal or greater magnitude than effects of CO_2 enrichment (Asshoff and Hättenschwiler 2005). Differences in nutrient conditions may also have contributed to larger shoot increments but smaller berries of *V. myrtillus* in plots with *Larix* than with *Pinus*, further demonstrating the strong influence tree species identity can have on the heath vegetation.

Conclusion

In this first in situ experiment of how the alpine treeline ecotone may change under future atmospheric CO_2 concentrations, we found that many responses observed in the first 3 years of enrichment were largely maintained for the remaining duration of the experiment. While experimental step increases in CO_2 can never fully represent the expected gradual increase, results from 9 years strengthen our earlier findings. Temperature and extreme climate events modified plant responses to elevated CO_2, demonstrating that interactions with climate will probably shape how the treeline ecotone changes in the coming decades. In contrast, CO_2 enrichment had little impact on the C–N balance in plants and soil over 9 years, suggesting that any potential biogeochemical feedbacks on N availability will take much longer to occur in this cool climate with slow-growing species. Finally, observations that plant species differences are sometimes more influential than changes in growth or physiology experienced by an individual species highlights the importance of species identity for projections about the influence of atmospheric CO_2 enrichment. Shifts in species composition and biodiversity resulting from species-specific responses to elevated CO_2 could therefore have important consequences for the structure and function of treeline ecosystems.

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