

# Growth and community responses of alpine dwarf shrubs to *in situ* CO<sub>2</sub> enrichment and soil warming

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## Summary

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- Rising CO<sub>2</sub> concentrations and the associated global warming are expected to have large impacts on high-elevation ecosystems, yet long-term multifactor experiments in these environments are rare.
- We investigated how growth 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 9 yr of CO<sub>2</sub> enrichment and 3 yr of soil warming at the treeline in the Swiss Alps.
- *Vaccinium myrtillus* was the only species that showed a clear positive effect of CO<sub>2</sub> on growth, with no decline over time in the annual shoot growth response. Soil warming stimulated *V. myrtillus* growth even more than elevated CO<sub>2</sub> and was accompanied by increased plant-available soil nitrogen (N) and leaf N concentrations. Growth of *Vaccinium gaultherioides* and *E. hermaphroditum* was not influenced by warming. Vascular plant species richness declined in elevated CO<sub>2</sub> plots with larch, while the number of moss and lichen species decreased under warming.
- Ongoing environmental change could lead to less diverse plant communities and increased dominance of the particularly responsive *V. myrtillus* in the studied alpine treeline. These changes are the consequence of independent CO<sub>2</sub> and soil warming effects, a result that should facilitate predictive modelling approaches.

## Introduction

Atmospheric CO<sub>2</sub> concentrations are predicted to reach 730–1020 ppm by the year 2100 and, as one consequence of this change, the global mean surface air temperature is expected to increase by 1.8–4.0°C during the same period (Meehl *et al.*, 2007). Elevated CO<sub>2</sub> 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 CO<sub>2</sub> partial pressure, are lower (Körner, 2003). Similarly, ongoing global warming is already documented to have particularly large ecological impacts on high-latitude and high-elevation regions where plants grow close to their low temperature limit (Walther, 2003; Dorrepaal *et al.*, 2009). For these reasons, research on environmental change

in alpine and arctic ecosystems has increased substantially in recent years, and has included both manipulation experiments (see review by Dormann & Woodin, 2002) and observations of natural vegetation change (e.g. Wilson & Nilsson, 2009; Hill & Henry, 2011).

Elevated CO<sub>2</sub> 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 tundra in Alaska (Tissue & Oechel, 1987) indicate that responses of plant growth and biomass to rising CO<sub>2</sub> concentrations are generally small or nonexistent but that co-occurring species often vary in their responses (Dormann & Woodin, 2002). More broadly, CO<sub>2</sub> enrichment studies of various

plant types conducted in relatively natural growth conditions have revealed that other variables, such as climate and availability of nutrients or water, can influence CO<sub>2</sub> effects (e.g. Niklaus & Körner, 2004; Dawes *et al.*, 2011) and that any initial growth stimulation often declines after a few years (Körner, 2006). In ecosystems where nitrogen (N) availability is low, this temporal response pattern can be caused by increasing N limitation over time as a result of increased N immobilization in plant biomass and long-lived soil organic matter and of enhanced microbial activity (Luo *et al.*, 2004). Studies lasting several years are clearly important for determining temporal dynamics of plant responses to CO<sub>2</sub> enrichment and interactions with other environmental factors.

In contrast to the small number of CO<sub>2</sub> manipulation experiments, many relatively long-term warming experiments have been completed in (sub)arctic (Chapin & Shaver, 1985; Parsons *et al.*, 1994; Shevtsova *et al.*, 1997; Aerts *et al.*, 2009) and alpine (Harte & Shaw, 1995; Kudo & 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 availability of soil nutrients, particularly N, as a result of 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 (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 CO<sub>2</sub> enrichment, warming experiments lasting several years are needed to elucidate responses at the individual species, community and ecosystem scales, especially as shifts in competitive interactions and plant–soil feedback processes can alter responses over time (Wookey *et al.*, 2009).

Despite clear predictions that rising CO<sub>2</sub> concentrations will be accompanied by increased temperatures, relatively few studies combining CO<sub>2</sub> enrichment and experimental warming have been conducted within intact systems (Beier, 2004). In a glasshouse study conducted in tussock tundra vegetation (Toolik Lake, AK, USA), CO<sub>2</sub>-induced stimulation of net primary productivity (NPP) lasted only one season when applied alone but was sustained over three growing seasons when combined with a 4°C temperature increase and unaltered water availability (Oechel *et al.*, 1994). However, most field studies of natural vegetation combining elevated CO<sub>2</sub> and warming have been conducted in grassland systems and have yielded conflicting results regarding how the combined changes influence plant productivity, community composition and N cycling (Shaw

*et al.*, 2002; Hovenden *et al.*, 2008; Engel *et al.*, 2009; Dijkstra *et al.*, 2010). While experiments combining CO<sub>2</sub> enrichment and nutrient addition have been conducted in alpine environments (Körner *et al.*, 1997; N. Inauen, pers. comm.), to our knowledge no previous *in situ* studies of high-elevation systems have simultaneously manipulated CO<sub>2</sub> concentration and temperature.

Dwarf shrubs are a major component of arctic and alpine plant communities, and shifts in the 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 determined the effects of 9 yr of free air CO<sub>2</sub> enrichment (FACE) and 3 yr of soil warming on three dominant ericaceous dwarf shrub species, deciduous *Vaccinium myrtillus* (bilberry) and *Vaccinium gaultherioides* (northern bilberry) and evergreen *Empetrum hermaphroditum* (crowberry), growing in the understorey of treeline trees in the Swiss Alps. Studying three co-occurring species allowed us to explore whether individual dwarf shrub species respond differently to the manipulations. We hypothesized that: (1) any initial stimulation of above-ground growth of these dwarf shrub species in response to CO<sub>2</sub> enrichment would decline over time; (2) soil warming would lead to enhanced dwarf shrub growth, reflecting a direct stimulation and/or an indirect benefit from increased soil N availability; (3) soil warming would alleviate temperature or nutrient constraints on the growth response to elevated CO<sub>2</sub>, yielding a positive interactive effect of the two experimental treatments on dwarf shrub growth; and (4) species-specific responses of understorey vegetation to the experimental treatments would lead to shifts in species composition 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 CO<sub>2</sub> enrichment and soil warming experiment covers an area of 2500 m<sup>2</sup> and is situated on a northeast-exposed 25–30° slope at 2180 m asl, 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 to 2009, the mean annual precipitation was 1175 mm and the mean annual air temperature was 2.5°C, with February (mean –5.2°C) being the coldest month and July (mean 10.5°C) the warmest month (WSL climate station at 2090 m asl).

The experiment consists of 40 hexagonal 1.1-m<sup>2</sup> 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) yr 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 understorey vegetation including 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* and *Melampyrum pratense*) and nonvascular species. For the dwarf shrub species targeted in this study, regeneration within the existing heath zone at the treeline occurs mainly by vegetative spread (Körner, 2003).

The CO<sub>2</sub> 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 CO<sub>2</sub> distribution and regulation. Half of these groups were randomly assigned to an elevated CO<sub>2</sub> treatment (mean concentration  $\pm$  1 SD 575  $\pm$  52 ppm from 2001 to 2009) while the remaining groups served as controls and received no additional CO<sub>2</sub> (c. 380 ppm). During daytime hours only, the system released pure CO<sub>2</sub> gas through laser-punched drip irrigation tubes hung vertically around a hexagonal frame surrounding each plot. Interruptions in CO<sub>2</sub> release as a result of adverse weather conditions or technical failure meant that plants received supplementary CO<sub>2</sub> for 73–87% of the seasonal daytime-only treatment periods. The set-up and performance of the CO<sub>2</sub> enrichment facility have been described in detail previously (Hättenschwiler *et al.*, 2002; Handa *et al.*, 2006; Dawes *et al.*, 2011).

In spring 2007, one plot of each tree species identity was randomly selected from each of the 10 CO<sub>2</sub> treatment groups and assigned a soil warming treatment, yielding a balanced design with a replication of five individual plots for each combination of CO<sub>2</sub> concentration, warming treatment and tree species. Soil warming was applied during the 2007–09 growing seasons using heating cables laid on the ground surface underneath the dwarf shrub layer (Hagedorn *et al.*, 2010). As the plots contained trees over 2 m tall and were located on steep, rocky terrain, this method was more feasible and required less disturbance than the use of open top chambers (OTCs) or infrared radiators and additionally allowed greater soil warming than OTCs. 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°C in 2007, 4.4  $\pm$  0.5°C in 2008 and 3.1  $\pm$  0.4°C in 2009 (mean  $\pm$  1 SE;  $n$  = 10). Air temperature was increased at up

to 20 cm above the ground (0.9  $\pm$  0.1 K) but no temperature difference was detected at 50 cm height (Hagedorn *et al.*, 2010). Warming also had a slight drying effect which 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 c. 15% and air humidity (10 cm height) was reduced by c. 10%, irrespective of CO<sub>2</sub> treatment (no effect) and tree species identity (Hagedorn *et al.*, 2010). Nonetheless, soil water potential in both warmed and unwarmed plots was always higher (less negative) than –300 hPa, indicating high water availability. See Fig. S1 in the Supporting Information for details of how the warming treatment influenced soil temperature and moisture.

### Plant growth and plot species composition

During the autumns of 2002 (second season of CO<sub>2</sub> enrichment) to 2009 (final season of study), after seasonal shoot elongation of the dwarf shrubs was complete, we measured the length of the new shoot increment on the longest branch of five to seven randomly selected ramets in each plot. These shoots experience less self-shading than shoots closer to the ground and are therefore less likely to have reduced growth as a result of 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). Shoots produced in 2002 were only measured in half of the plots, with a replication of five for each combination of CO<sub>2</sub> 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 in 2008 and 2009. These measurements are positively correlated with total above-ground biomass per unit area (understorey height) and per ramet (individual species height) at our research site (S. Wipf *et al.*, unpublished). During the peak of the growing season in 2005 and 2009, we recorded all vascular and nonvascular (moss and lichen) species present in each plot to document changes in species composition and richness.

### Leaf morphology

During early August 2006–09, c. 50 leaves of *V. myrtillus* and of *V. gaultherioides* were harvested from each plot to measure leaf traits. *Empetrum 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 h of harvesting and mean leaf area was calculated using IMAGEJ version 1.43 k (measurements not available from 2008; Rasband, 1997–2008). Leaves were subsequently dried at 60°C for at least 24 h and weighed to obtain the average dry leaf mass and specific leaf area (SLA) per plot for each species.

### Soil inorganic N and leaf N concentration

Treatment effects on plant-available soil N were assessed by measuring the soil inorganic N pool size at 0–5 cm depth in August 2004, 2007 and 2009. In each plot, six soil cores with a diameter of 2 cm were taken and the bulked sample was extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub>. We additionally sampled soil water at 3–7 cm soil depth once per month during each vegetation period, using two ceramic suction cups (SoilMoisture Equipment Corp., Santa Barbara, CA, USA) per plot and applying a suction of 400 hPa overnight (Hagedorn *et al.*, 2008). Soil extracts and soil solution were analysed for NH<sub>4</sub><sup>+</sup> by colorimetry using an automated flow injection analysis (FIAS-300; PerkinElmer Inc., Waltham, MA, USA) and for NO<sub>3</sub><sup>−</sup> by ion chromatography (DX-120; Dionex, Sunnyvale, CA, USA). We additionally measured leaf N concentration for the two *Vaccinium* species to estimate treatment effects on plant N status (Marschner, 1995). Dried *Vaccinium* leaves were ground, as one bulk sample per plot for each species, and N concentration (mg N per g dry mass) was measured using a C-N analyser (EA-1110; Carlo Erba, Milan, Italy).

### Plot-level environmental conditions

Light and soil moisture conditions were measured at the plot level to investigate whether understorey plant responses to CO<sub>2</sub> enrichment and soil warming were driven by changes in these environmental variables associated with the treatments (indirect effects). Hemispherical photographs taken in each plot during the seasonal peak of leaf area in 2008 were used to quantify the amount of shade experienced by understorey plants near the end of the experiment when tree canopy size varied most among plots. We defined 'canopy shading' as the percentage of sky obstructed by the tree trunk and canopy, trees in the area surrounding the plot, and topographic elements (see Fig. S2 for details). On average, canopy shading was slightly greater for plots with larch than for those with pine (for a summary of plot conditions, see Table S1). Additionally, stimulation of larch growth under elevated CO<sub>2</sub> meant that tree canopy cover tended to be greatest for elevated CO<sub>2</sub> plots containing a larch tree (Dawes *et al.*, 2011). Seasonal mean soil volumetric water content was calculated for each plot during 2007–2009 to quantify inter-plot variation in soil moisture,

including drying associated with the warming treatment (see Fig. S1 for methods). Snowmelt date in spring and plot topography (slope or ridge) were also estimated for each plot and considered in statistical tests. However, these two parameters did not vary significantly between treatment groups (Table S1) or show a correlation with response variables and are not presented in the results below.

### Statistical analysis

Treatment effects on the growth of each individual dwarf shrub species, on understorey vegetation height, and on soil inorganic N were tested with Type I analysis of (co)variance, using repeated measures linear mixed effects models fitted with REML. To test the CO<sub>2</sub> enrichment effect, we used a model including CO<sub>2</sub> concentration, plot tree species identity 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 3 yr when both CO<sub>2</sub> enrichment and soil warming were applied (2007–09), likelihood ratio tests indicated that none of the interactions between the two treatments contributed significantly to model fits for any of the parameters measured (Table S2). We therefore pooled warmed and unwarmed plots for tests of the CO<sub>2</sub> effect. We used a similar statistical approach to test soil warming effects, with temperature treatment replacing CO<sub>2</sub> treatment in the models. Soil warming effects on understorey vegetation height and total ramet height were not analysed. We included values averaged over 2005 and 2006 as a pre-treatment covariable in tests of shoot growth responses to warming, 2006 values in models involving leaf morphology and N concentration, and 2004 values in soil inorganic N analysis. We used a single model to test effects of CO<sub>2</sub> enrichment and soil warming on species richness in 2009, using values from 2005 as a covariable even though some CO<sub>2</sub> effects might have occurred before that point.

Potential influences of canopy shading (measured in 2008) and soil moisture (measured in 2007–09) on response variables (measurements from 2007 to 2009 only) were tested using linear regression, including pretreatment predictors in tests relevant to the warming treatment. For species richness, the influence of understorey vegetation height and individual dwarf shrub species ramet height (averaged over 2008–09) were also tested. To determine if dwarf shrub shoot growth responses to soil warming were associated with a change in N availability, we regressed shoot increment length against soil inorganic N pool size and against leaf N concentration for each individual treatment year (2007–09), standardizing both variables to pre-warming values.

For all analyses, random effects associated with specific intercepts for the 10 CO<sub>2</sub> treatment groups and 20 warming treatment groups were excluded because they were very small



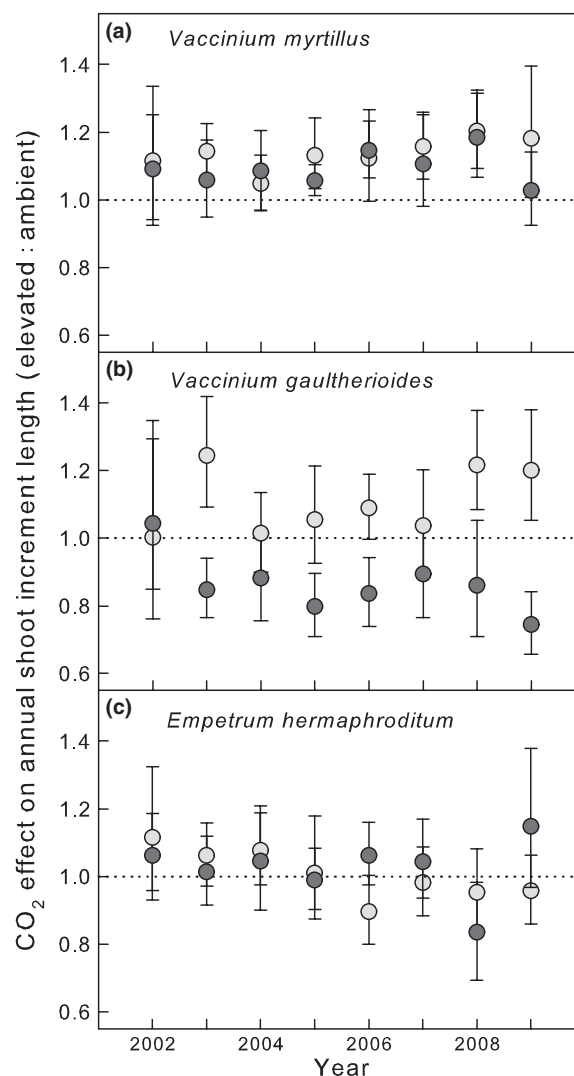
and nonsignificant (Zuur *et al.*, 2009). Therefore, repeated measures tests only included random effects for each individual plot, the unit of measurement, and species richness was analysed without random effects using generalized least squares. Models assuming a normal error distribution were used for all parameters except nonvascular plant species richness, where a Poisson distribution provided a better fit (Venables & Ripley, 2002). Response variables were log-transformed in some cases to improve assumptions of normality and homoscedasticity. For all statistical tests, effects were considered significant at  $P < 0.05$ . Because of relatively low replication and therefore statistical power, we designated  $P$ -values  $\geq 0.05$  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, 2010).

## Results

### Annual shoot growth and total ramet height

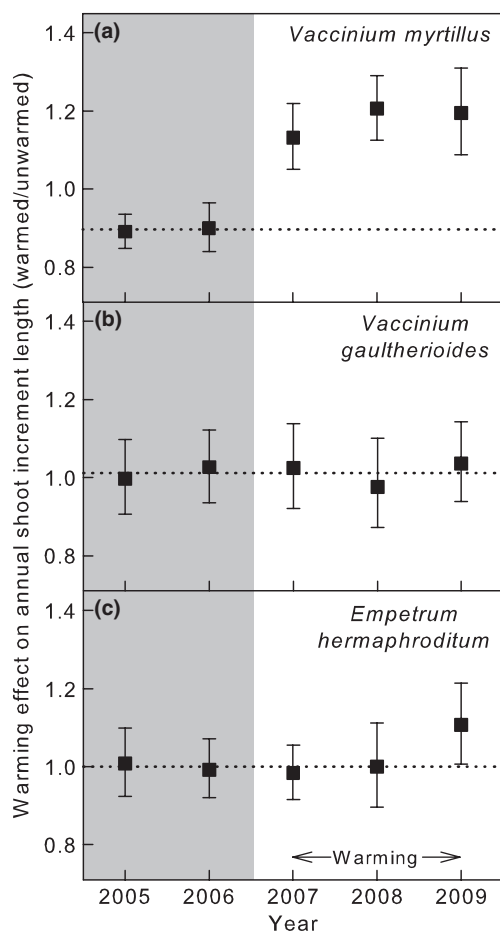
Repeated measures tests of the CO<sub>2</sub> effect on annual shoot growth during 8 yr of treatment yielded a significant stimulation of 12% in *V. myrtillus* ( $F_{1,36} = 7.9$ ,  $P < 0.01$ ), averaged across tree species identities, temperature treatments and years (Fig. 1a). This stimulation was relatively consistent, with no indication of a decline in the signal over time ( $P > 0.9$  for CO<sub>2</sub>  $\times$  year interaction). Plot tree species identity also significantly influenced *V. myrtillus* shoot growth, with longer annual shoot increments in plots with larch than in those with pine, irrespective of CO<sub>2</sub> treatment ( $F_{1,36} = 5.2$ ,  $P = 0.03$ ). For *V. gaultherioides*, the interaction between CO<sub>2</sub> treatment and tree species identity was significant ( $F_{1,36} = 5.9$ ,  $P = 0.02$ ), with a slight negative response to elevated CO<sub>2</sub> in plots with larch and a slight positive response in plots with pine (Fig. 1b). *Empetrum hermaphroditum* shoot growth did not show an effect of CO<sub>2</sub> enrichment or tree species, and no interactions were significant ( $P > 0.16$ ; Fig. 1c). Annual shoot growth of all three species varied significantly among individual treatment years ( $P < 0.01$ ) but did not show a clear trend over time (see Table S3 for ANOVA results). Canopy shading did not significantly influence shoot growth of any species in 2007–09 (Table S4). Further, a harvested subsample 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 Fig. S3 for details). These results indicate that enhanced *V. myrtillus* shoot increment length under elevated CO<sub>2</sub> was caused by direct stimulation rather than by a change in light conditions.

Soil warming had a positive effect on *V. myrtillus* shoot growth ( $F_{1,35} = 12.4$ ,  $P < 0.01$ ; Fig. 2a). Relative to the 2 yr preceding warming, mean annual shoot increment length



**Fig. 1** CO<sub>2</sub> effect on the length of annual shoot increments during 8 yr of enrichment. The CO<sub>2</sub> effect was calculated as the ratio of the mean shoot increment length for all elevated CO<sub>2</sub> plots to the mean shoot increment length for all ambient CO<sub>2</sub> plots ( $n = 5$  for 2002 and  $n = 8$ –10 for all other years). Error bars represent  $\pm 1$  SE of the ratio, estimated according to Gelman & Hill (2007). Plots are separated by tree species identity (light grey circles, under pine; dark grey circles, under larch), and means are pooled across warmed and unwarmed plots for 2007–09. A dotted line is drawn where the elevated to ambient CO<sub>2</sub> ratio is 1.

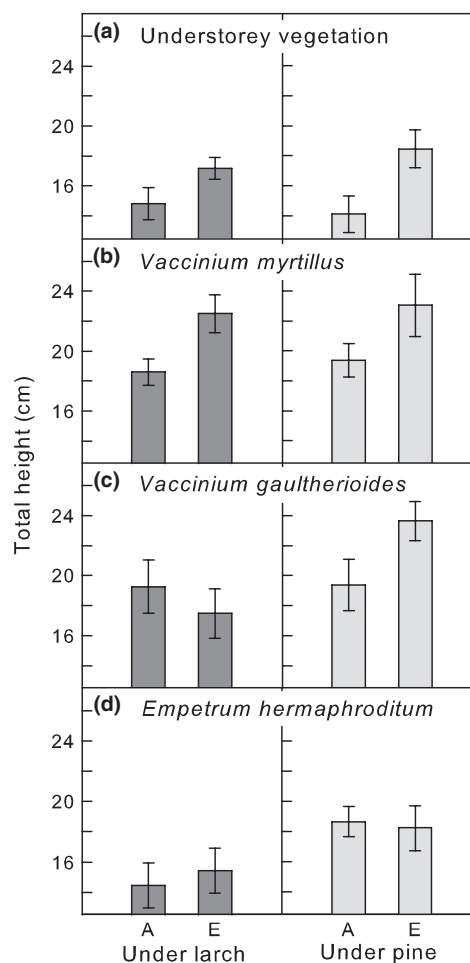
was 31% greater in warmed plots than in unwarmed plots (averaged across tree species identities, CO<sub>2</sub> concentrations and years). Neither *V. gaultherioides* nor *E. hermaphroditum* shoot growth responded significantly to warming, although the latter species showed a trend of stimulation in 2009 (Fig. 2b,c). Shoot increment length measured in 2005 and 2006 influenced growth of each species during the three seasons of warming ( $P < 0.04$ ), and the strength of this relationship did not vary significantly with year or temperature treatment. No interactions between temperature



**Fig. 2** Soil warming effect on the length of annual shoot increments in 2007–09. The warming effect was calculated as the ratio of the mean shoot increment length for all warmed plots to the mean shoot increment length for all unwarmed plots ( $n = 20$  for *Vaccinium myrtillus* and *Vaccinium gaultherioides*;  $n = 15$ – $19$  for *Empetrum hermaphroditum*). Error bars represent  $\pm 1$  SE of the ratio, estimated according to Gelman & Hill (2007). Means are pooled across plot tree species identities and  $\text{CO}_2$  treatment groups. Pre-warming ratios (2005 and 2006) are shown in the shaded region. A dotted line is drawn through the average of these two points, indicating the mean warmed to unwarmed ratio before treatment began.

treatment, tree species and year were significant (see Table S3 for ANOVA results). Soil moisture did not significantly influence shoot growth of any species, implying that slightly reduced soil moisture under warming was not responsible for responses to the treatment (Table S4).

Average understorey vegetation height was positively 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  $\text{CO}_2$  enrichment ( $P \leq 0.01$ ; Fig. 3a,b), irrespective of tree species identity. There was a marginally significant  $\text{CO}_2 \times$  tree species interactive effect on *V. gaultherioides* ramet height ( $F_{1,36} = 4.1$ ,  $P = 0.05$ ), with a positive response



**Fig. 3**  $\text{CO}_2$  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 to 2009 were averaged to calculate the 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 vegetation height for each of the two  $\text{CO}_2$  treatment groups,  $\pm 1$  SE (A, ambient  $\text{CO}_2$ ; E, elevated  $\text{CO}_2$ ;  $n = 10$ ). (b–d) Maximum ramet height of each dwarf shrub species, averaged over each of the two  $\text{CO}_2$  treatment groups,  $\pm 1$  SE ( $n = 10$  for *Vaccinium myrtillus* and *Vaccinium gaultherioides*;  $n = 8$ – $10$  for *Empetrum hermaphroditum*).

in plots with pine but a slight negative response in plots with larch, and ramet height was lower in plots with larch than in those with pine ( $F_{1,36} = 4.3$ ,  $P = 0.05$ ; Fig. 3c). *Empetrum hermaphroditum* ramets were taller in plots with pine than in those with larch ( $F_{1,32} = 5.4$ ,  $P = 0.03$ ) but showed no effect of  $\text{CO}_2$  enrichment (Fig. 3d). Ramet height of *E. hermaphroditum* showed a slight negative relationship with canopy shading ( $P = 0.09$ ), which might have explained the tree species effect, whereas no other height variables were significantly influenced by shading (Table S4). Overall, these results are consistent with patterns observed for shoot increment length during years 2 to 9 of  $\text{CO}_2$  enrichment, and they

demonstrate that sustained responses in shoot growth yielded cumulative effects at the plot level (see Table S3 for dwarf shrub ANOVA results).

### Leaf morphology

*Vaccinium myrtillus* dry mass and area per individual leaf showed a marginally significant positive response to elevated CO<sub>2</sub> (Table 1a; mean values for individual years shown in Table S5). *Vaccinium myrtillus* leaf area but not mass increased with increasing canopy shading, so it is possible that enhanced leaf area was at least partially a response to slightly reduced light conditions in elevated CO<sub>2</sub> plots (Table S4). By contrast, neither leaf mass nor area of *V. gaultherioides* responded significantly to CO<sub>2</sub> enrichment or to the amount of shading (Tables 1b, S4). SLA of both species was unaffected by CO<sub>2</sub> enrichment ( $P > 0.19$ ), meaning that *V. myrtillus* leaf size increased but the area to mass ratio did not change significantly. Greater canopy shading was associated with higher SLA for both species (Table S4), which probably contributed to higher SLA in plots with larch than in those with pine, and *V. myrtillus* consistently had a higher SLA than *V. gaultherioides* (Tables 1, S5). Under soil warming, *V. myrtillus* showed only very limited evidence of a change in leaf morphology (Table 2a). There was a significant temperature  $\times$  year interaction for *V. gaultherioides* mass and area per individual leaf, with both traits slightly reduced under warming in 2007 only (Tables 2b, S5). Soil moisture did not show a significant relationship with any measured leaf parameters (Table S4).

### Soil inorganic N and leaf N concentration

In the soil solution, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were always below the detection limit (0.1 mg N l<sup>-1</sup>). Concentrations of K<sub>2</sub>SO<sub>4</sub>-extractable NO<sub>3</sub><sup>-</sup> were also not detectable, indicating that there was no net nitrification. Soil inorganic N pool size was not significantly influenced by CO<sub>2</sub> treatment, tree species identity, year or any interactive term ( $P > 0.19$ ). Leaf N concentration of the two *Vaccinium* species increased with increasing canopy shading, and concentrations were generally higher in *V. gaultherioides* than in *V. myrtillus* (Tables S4, S5). There was a trend of lower leaf N concentration under elevated CO<sub>2</sub> in both *Vaccinium* species, particularly in 2006 and 2007, but this effect was not statistically significant (marginally significant CO<sub>2</sub>  $\times$  tree species  $\times$  year interaction for *V. myrtillus*; Tables 1, S5). Leaf N concentration of both species varied among the four years and tended to be lower in plots with pine than in those with larch (Table 1).

Soil inorganic N pool size increased strongly with soil warming ( $F_{1,35} = 9.5$ ,  $P < 0.01$ ; Fig. 4), with a more pronounced response in 2009 (140% greater in warmed plots than in unwarmed plots, standardized to 2004 values and pooled across tree species identities and CO<sub>2</sub> concentrations) than in 2007 (+52%). Warming also had a positive influence on leaf N concentration of both *Vaccinium* species (Fig. 4), although this effect declined over the 3 yr for *V. myrtillus* (marginally significant warming  $\times$  year interaction;  $F_{1,35} = 2.8$ ,  $P = 0.07$ ). Increased shoot increment length of *V. myrtillus* was weakly associated with increased leaf N concentration

**Table 1** Results of an analysis of (co)variance repeated measures test of the effect of CO<sub>2</sub> enrichment and tree species on *Vaccinium myrtillus* and *Vaccinium gaultherioides* leaf morphology from 2006 to 2009

	SLA (cm <sup>2</sup> g <sup>-1</sup> ) <sup>a</sup>			Mass per leaf (mg)			Area per leaf (cm <sup>2</sup> ) <sup>a,b</sup>		
	df	F	P	df	F	P	df	F	P
(a) <i>Vaccinium myrtillus</i>									
CO <sub>2</sub> concentration	1, 36	1.78	0.19	1, 36	3.90	0.06	1, 36	8.79	0.01
Tree species	1, 36	9.01	< 0.01	1, 36	5.15	0.03	1, 36	18.90	< 0.01
Year	2, 70	5.34	0.01	3, 108	15.64	< 0.01	2, 71	15.51	< 0.01
CO <sub>2</sub> $\times$ tree species	1, 36	0.53	0.47	1, 36	0.19	0.67	1, 36	0.12	0.73
CO <sub>2</sub> $\times$ year	2, 70	0.03	0.97	3, 108	0.28	0.84	2, 71	0.81	0.45
Tree species $\times$ year	2, 70	2.99	0.06	3, 108	2.09	0.11	2, 71	8.19	< 0.01
CO <sub>2</sub> $\times$ tree $\times$ year	2, 70	0.65	0.53	3, 108	0.09	0.97	2, 71	1.20	0.31
(b) <i>Vaccinium gaultherioides</i>									
CO <sub>2</sub> concentration	1, 36	0.95	0.34	1, 36	0.30	0.59	1, 36	0.01	0.92
Tree species	1, 36	10.61	< 0.01	1, 36	1.64	0.21	1, 36	0.22	0.64
Year	2, 69	13.20	< 0.01	3, 105	5.88	< 0.01	2, 69	24.73	< 0.01
CO <sub>2</sub> $\times$ tree species	1, 36	1.56	0.22	1, 36	2.63	0.11	1, 36	1.60	0.21
CO <sub>2</sub> $\times$ year	2, 69	1.08	0.35	3, 105	1.75	0.16	2, 69	1.54	0.22
Tree species $\times$ year	2, 69	1.88	0.16	3, 105	0.39	0.76	2, 69	1.69	0.19
CO <sub>2</sub> $\times$ tree $\times$ year	2, 69	1.37	0.26	3, 105	0.78	0.51	2, 69	1.41	0.25

<sup>a</sup>Data not available from 2008.

<sup>b</sup>Any missing leaf parts were filled to correct for losses caused by herbivory (< 4% of potential area per individual leaf).

SLA, specific leaf area.

**Table 2** Results of an analysis of covariance repeated measures test of the effect of soil warming on *Vaccinium myrtillus* and *Vaccinium gaultherioides* leaf morphology from 2007 to 2009

	SLA (cm <sup>2</sup> g <sup>-1</sup> ) <sup>a</sup>			Mass per leaf (mg)			Area per leaf (cm <sup>2</sup> ) <sup>a,b</sup>		
	df	F	P	df	F	P	df	F	P
<b>(a) <i>Vaccinium myrtillus</i></b>									
2006 covariable	1, 35	19.11	< 0.01	1, 35	36.39	< 0.01	1, 35	34.25	< 0.01
Temperature	1, 35	0.14	0.71	1, 35	< 0.01	> 0.99	1, 35	0.20	0.66
Tree species	1, 35	0.72	0.40	1, 35	0.02	0.89	1, 35	< 0.01	0.99
Year	1, 35	6.74	0.01	2, 72	17.16	< 0.01	1, 35	5.90	0.02
Temperature × tree species	1, 35	0.43	0.52	1, 35	0.02	0.89	1, 35	0.27	0.61
Temperature × year	1, 35	3.67	0.06	2, 72	1.08	0.35	1, 35	2.20	0.15
Tree species × year	1, 35	4.70	0.04	2, 72	1.12	0.33	1, 35	0.08	0.78
Temperature × tree × year	1, 35	1.45	0.24	2, 72	0.33	0.72	1, 35	0.03	0.86
<b>(b) <i>Vaccinium gaultherioides</i></b>									
2006 covariable	1, 34	70.22	< 0.01	1, 34	58.09	< 0.01	1, 34	47.11	< 0.01
Temperature	1, 34	2.11	0.16	1, 34	0.14	0.71	1, 34	0.06	0.81
Tree species	1, 34	4.61	0.04	1, 34	1.96	0.17	1, 34	0.17	0.68
Year	1, 33	14.15	< 0.01	2, 68	3.52	0.04	1, 33	39.43	< 0.01
Temperature × tree species	1, 34	0.08	0.78	1, 34	0.41	0.52	1, 34	0.70	0.41
Temperature × year	1, 33	0.22	0.65	2, 68	3.96	0.02	1, 33	5.44	0.03
Tree species × year	1, 33	2.54	0.12	2, 68	0.21	0.81	1, 33	5.62	0.02
Temperature × tree × year	1, 33	0.46	0.50	2, 68	0.69	0.51	1, 33	0.07	0.79

<sup>a</sup>Data not available from 2008.<sup>b</sup>Any missing leaf parts were filled to correct for losses caused by herbivory (< 4% of potential area per individual leaf). SLA, specific leaf area.

( $P < 0.05$  in 2007 and 2009) and with increased soil inorganic N in 2009 ( $P = 0.03$ ) but not 2007. By contrast, there was no indication of enhanced shoot growth associated with increased N availability in *V. gaultherioides* or in *E. hermaphroditum* (see Tables S6, S7 for relevant statistical results).

### Understorey vegetation composition

After accounting for 2005 differences, the total number of vascular and nonvascular (moss and lichen) species per plot in 2009 was influenced by both experimental treatments, with a marginally significant CO<sub>2</sub> × tree species interactive effect ( $F_{1,31} = 3.8$ ,  $P = 0.06$ ) and negative soil warming effect ( $F_{1,31} = 3.3$ ,  $P = 0.08$ ). Analysis of vascular species alone indicated that changes in richness over time were influenced by elevated CO<sub>2</sub>, again interacting with tree species identity ( $F_{1,31} = 4.6$ ,  $P = 0.04$ ), but not by warming. Both total and vascular species richnesses were lower in plots with taller *V. myrtillus* ramets ( $P < 0.07$ ) but showed no influence of canopy shading, average understorey vegetation height or soil moisture (Table S4). Separate analysis of plots with each tree species indicated a marginally significant negative CO<sub>2</sub> effect in plots with larch ( $F_{1,15} = 3.9$ ,  $P = 0.07$ ), with a mean loss ( $\pm 1$  SE) of  $1.3 \pm 0.6$  vascular species over the 4 yr in elevated CO<sub>2</sub> plots but no change in ambient CO<sub>2</sub> plots (Fig. 5). Plots with pine showed an opposite but nonsignificant pattern ( $F_{1,15} = 1.1$ ,  $P = 0.30$ ), with somewhat more pronounced vascular species losses in plots with ambient CO<sub>2</sub> concentrations (Fig. 5). Nonvascular species richness

declined overall from 2005 to 2009, with more species lost from warmed plots ( $P = 0.02$ ), especially those also exposed to elevated CO<sub>2</sub>, than from unwarmed plots (Fig. 6; see Table S8 for statistical results). Plots with lower soil moisture were associated with more nonvascular species losses ( $P = 0.07$ ; Table S4). Individual species lost most frequently from the plots are presented in Table S9.

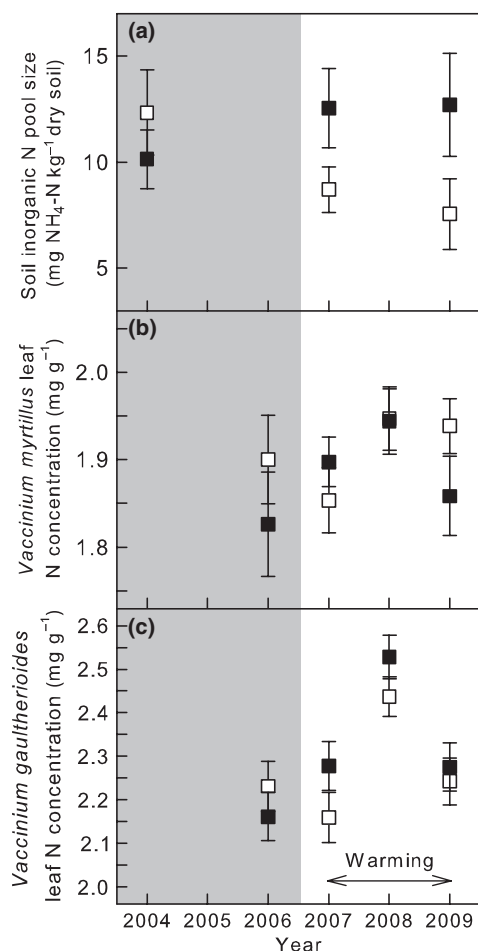
### Discussion

#### Species-specific growth responses to simulated atmospheric and climate change

In this study of three dominant ericaceous dwarf shrub species growing at the treeline in the Swiss Alps, *V. myrtillus* growth was stimulated by both CO<sub>2</sub> enrichment and soil warming and generally showed a stronger response than *V. gaultherioides* or *E. hermaphroditum*.

The CO<sub>2</sub> 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. In contrast to our first hypothesis, the shoot growth response of *V. myrtillus* to elevated CO<sub>2</sub> was sustained over the full experimental period. This result suggests that any increase in competition for nutrients or light that occurred over time with enhanced plant growth and microbial activity did not constrain the longer term above-ground growth response of this species. CO<sub>2</sub> enrichment did not lead to a

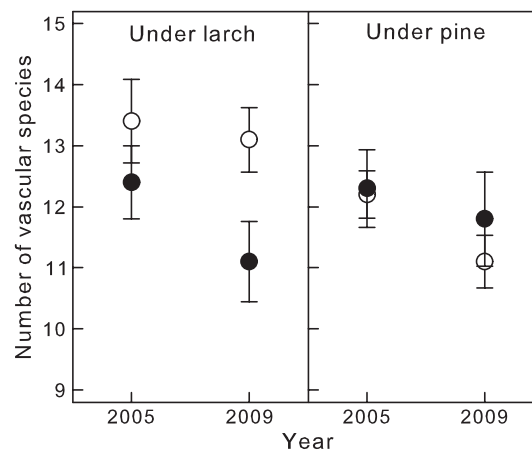




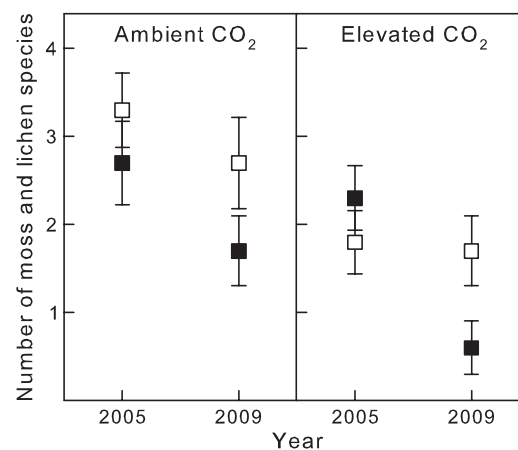
**Fig. 4** Effects of soil warming on nitrogen (N) availability. (a) Mean inorganic N pool size in the soil and (b, c) leaf N concentration of (b) *Vaccinium myrtillus* and (c) *V. gaultherioides*  $\pm$  1 SE for each warming treatment group, pooled across plot tree species identities and CO<sub>2</sub> treatment groups ( $n$  = 20). Leaves and soil were each sampled once per vegetation period. Pre-warming values are shown in the shaded region. Open squares, unwarmed; closed squares, warmed.

significant decline in the soil inorganic N pool or in leaf N concentration, providing evidence that the treatment did not cause N to become limiting.

The relatively strong response of *V. myrtillus* to CO<sub>2</sub> enrichment compared with the other two dwarf shrub species might be related to different leaf traits. For example, higher SLA in *V. myrtillus* compared with *V. gaultherioides* (20–30% in all years measured) and *E. hermaphroditum* (> 50%; Zumbrunn, 2004) means that there is a larger amount of leaf area displayed per unit mass invested (Poorter *et al.*, 2009) in *V. myrtillus* than in the other two species. Efficient light capture and high photosynthetic capacity associated with higher SLA could have led to larger assimilation gains under elevated CO<sub>2</sub> (Roumet & Roy, 1996) in *V. myrtillus* compared with the other two species. For *V. gaultherioides*, the CO<sub>2</sub> effect on annual shoot growth and total ramet height



**Fig. 5** Changes in understory vascular plant species richness from 2005 to 2009. The mean number of species per plot is shown for each CO<sub>2</sub> treatment and tree species combination,  $\pm$  1 SE (pooled across temperature treatments;  $n$  = 10). Open circles, ambient CO<sub>2</sub>; closed circles, elevated CO<sub>2</sub>.



**Fig. 6** Changes in understory nonvascular (moss and lichen) plant species richness from 2005 to 2009. The mean number of species per plot is shown for each CO<sub>2</sub> and temperature treatment combination,  $\pm$  1 SE (pooled across tree species identities;  $n$  = 10). Open squares, unwarmed; closed squares, warmed.

depended on which tree species grew in the experimental plot. This response could not be explained by differences in light conditions (canopy shading), snowmelt date, plot topography, soil moisture, leaf N concentration (slightly higher in plots with larch than in those with pine) or soil inorganic N pool size (Tables S1, S4). Reasons for the shoot growth response of *V. gaultherioides* remain unclear, but differences in litter production and quality or in below-ground competition, for example the availability of nutrients other than N, might have played a role. In general, lower *V. gaultherioides* ramet height in plots with larch than in those with pine could indicate less favourable growth conditions for this species in the understory of larch trees.

Consistent with our findings, species-specific responses were observed in a 3-yr mesocosm CO<sub>2</sub> 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 CO<sub>2</sub> enrichment. However, whereas we observed a tree species-specific CO<sub>2</sub> response in *V. gaultherioides* (group *V. uliginosum* agg.) and no effect in *E. hermaphroditum*, at the Abisko site *V. uliginosum* showed no CO<sub>2</sub> 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 CO<sub>2</sub> for a single season in a glasshouse study in low-elevation heathlands of the Netherlands (Arp *et al.*, 1998). The consistent growth responses to CO<sub>2</sub> enrichment observed for *V. myrtillus* across multiple studies under various growth conditions indicates an inherent CO<sub>2</sub> responsiveness of this species and suggests that *V. myrtillus* growth and abundance might increase in a future CO<sub>2</sub>-enriched atmosphere.

Deciduous *V. myrtillus* was also the only species to show a significant positive shoot growth response to the warming treatment, with an average stimulation over twice the size of the mean CO<sub>2</sub> effect. *Vaccinium gaultherioides*, which is also deciduous, showed no response to warming, suggesting that factors other than leaf type had a greater influence on the responses of individual species. In the Alps, *V. myrtillus* has a lower elevational distribution compared with *V. gaultherioides* and *E. hermaphroditum*, both of which extend to > 3000 m asl (Landolt *et al.*, 2010). *Vaccinium 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. uliginosum* (no growth response) in a 5-yr study in Abisko, Sweden (Hartley *et al.*, 1999). Positive shoot growth responses were similarly observed for dwarf shrubs after the second and third seasons of warming by OTCs in the Swedish subarctic heath (Parsons *et al.*, 1994), although there all three species that also occur in our experiment responded positively. Finally, warming by OTCs at a temperate alpine site in northern Japan had no effect on vegetative growth of *V. uliginosum* (Kudo & Suzuki, 2003), whereas *Empetrum nigrum* var. *japonicum* shoot elongation was strongly stimulated.

Different responses to warming for the same species might be attributable to genetic differences between regions, especially for *E. nigrum* and *V. uliginosum*, which are highly heterogeneous species complexes (Bell & Tallis, 1973; Jacquemart, 1996). Lower atmospheric pressure (and therefore lower CO<sub>2</sub> partial pressure), contrasting day–night solar radiation and temperatures during summer, and generally higher precipitation in temperate alpine environments

compared with 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 warming by heating cables on the ground surface (Rustad *et al.*, 2001). Further, warming by OTCs is confounded to some extent with shelter effects, that is, 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 led to a strong increase in soil mineral N content that was still evident after three growing seasons. This result supports our prediction that warming would at least initially accelerate N cycling and lead to an enhanced N supply (Melillo *et al.*, 2002). In a study in open birch (*Betula pubescens* ssp. *tortuosa*) forest in northern Sweden including the same dwarf shrub species as our study, N mineralization rates were doubled compared with controls in plots with 5°C soil warming in the second year of treatment, although no effect was observed in the fifth year (Hartley *et al.*, 1999). Corresponding to the larger inorganic N pool in the soil, the leaf N concentration of both *Vaccinium* species showed a short-term increase in warmed plots in our study. Warming effects on *V. myrtillus* leaf N concentration were only apparent in the first year of treatment when the growth response was smallest. It is possible that greater stimulation of shoot growth in 2008 and 2009 diluted the soil warming-induced increase in total N uptake, yielding an overall larger N pool in leaf biomass but no effect on concentrations (Weih & Karlsson, 2001). Hartley *et al.* (1999) observed no effect of soil warming on foliar N concentrations in *V. myrtillus* or *V. uliginosum* in any year of their study, despite increased N mineralization rates. By contrast, 8 yr of warming by tents and then OTCs in another experiment in northern Sweden had a large positive effect on leaf and shoot N concentration of *V. myrtillus* (73%) but a negative effect on that of *V. uliginosum* (19%) (Richardson *et al.*, 2002). We observed weak but positive correlations between *V. myrtillus* shoot increment length and soil/leaf N, suggesting that increased N availability might have been partially responsible for stimulation of shoot growth but that other mechanisms (i.e. direct effects of temperature on photosynthesis) were probably also important.

In contrast to our third hypothesis, we found no evidence of a positive CO<sub>2</sub> × warming interactive effect on dwarf shrub growth, suggesting that responses to CO<sub>2</sub> enrichment were not constrained by low N availability or low temperature. This result is in contrast to the positive CO<sub>2</sub> × warming interactive effect on NPP observed for tussock tundra vegetation in Alaska (Oechel *et al.*, 1994). Although no other combined warming and CO<sub>2</sub> enrichment studies have been conducted at high elevation, an alpine grassland community

showed no effect of CO<sub>2</sub> enrichment on biomass production even when combined with 40 kg ha<sup>-1</sup> a<sup>-1</sup> of NPK fertilization, indicating that nutrient limitation was not the reason for no biomass response to elevated CO<sub>2</sub> (Körner *et al.*, 1997). Similarly, a FACE × N addition experiment on glacier forefield vegetation showed strong stimulation by fertilization over 3 yr but no positive CO<sub>2</sub> effect or interaction between treatments (N. Inauen, pers. comm.).

Independent growth responses of *V. myrtillus* to elevated CO<sub>2</sub> and soil warming suggest that this species will have a competitive advantage over the co-occurring dwarf shrub species in the future. However, at our research site both treatments 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 CO<sub>2</sub> concentrations and soil warming

The experimental treatments led to changes in vegetation composition at the plot scale during the final 4 yr of the 9-yr study, with a decline in the number of vascular and non-vascular species in the plots. The observed trend of greater species loss in plots with taller *V. myrtillus* ramets, although not in those with greater canopy shading, 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 *V. myrtillus* was also enhanced in elevated CO<sub>2</sub> plots shared with this tree species. Three years of experimental warming had no detectable effect on vascular plant composition, consistent with results from a 5-yr soil warming treatment in Abisko, Sweden (Hartley *et al.*, 1999). By contrast, 9 yr of warming in glasshouses near Toolik Lake, Alaska led to a decline in species richness (Chapin *et al.*, 1995). Given that the response of *V. myrtillus* shoot growth to warming was more pronounced than that to CO<sub>2</sub> 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 (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 the observed correlation with soil moisture in our study suggests

that drying associated with the warming treatment was at least partially responsible for the loss of these species. Overall, negative effects of CO<sub>2</sub> enrichment and soil warming on species richness indicate that the ongoing environmental change could lead to less diverse plant communities at the studied alpine treeline.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Time series of soil warming effects on soil conditions.

**Fig. S2** Hemispherical photography methods.

**Fig. S3** Dwarf shrub shoot allometric relationships.

**Table S1** Summary of conditions for plots in each of the eight treatment groups

**Table S2** Results of likelihood ratio tests used to justify separate analyses of CO<sub>2</sub> enrichment and soil warming effects

**Table S3** Repeated measures analysis of (co)variance results for dwarf shrub annual shoot growth and total ramet height

**Table S4** Results of linear regressions relating response variables to canopy shading and soil moisture

**Table S5** Mean values of dwarf shrub leaf traits in individual years, averaged across CO<sub>2</sub> and soil warming treatment groups

**Table S6** Repeated measures analysis of (co)variance results for soil inorganic nitrogen (N) and leaf N concentrations

**Table S7** Results of linear regressions relating shoot growth to soil inorganic nitrogen (N) and leaf N concentrations

**Table S8** Analysis of covariance and analysis of deviance results for species richness

**Table S9** Vascular, lichen and moss species most frequently lost from the experimental plots

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