

# Soil warming and CO<sub>2</sub> enrichment induce biomass shifts in alpine tree line vegetation

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## Abstract

Responses of alpine tree line ecosystems to increasing atmospheric CO<sub>2</sub> concentrations and global warming are poorly understood. We used an experiment at the Swiss tree line to investigate changes in vegetation biomass after 9 years of free air CO<sub>2</sub> enrichment (+200 ppm; 2001–2009) and 6 years of soil warming (+4 °C; 2007–2012). The study contained two key tree line species, *Larix decidua* and *Pinus uncinata*, both approximately 40 years old, growing in heath vegetation dominated by dwarf shrubs. In 2012, we harvested and measured biomass of all trees (including root systems), above-ground understorey vegetation and fine roots. Overall, soil warming had clearer effects on plant biomass than CO<sub>2</sub> enrichment, and there were no interactive effects between treatments. Total plant biomass increased in warmed plots containing *Pinus* but not in those with *Larix*. This response was driven by changes in tree mass (+50%), which contributed an average of 84% (5.7 kg m<sup>-2</sup>) of total plant mass. *Pinus* coarse root mass was especially enhanced by warming (+100%), yielding an increased root mass fraction. Elevated CO<sub>2</sub> led to an increased relative growth rate of *Larix* stem basal area but no change in the final biomass of either tree species. Total understorey above-ground mass was not altered by soil warming or elevated CO<sub>2</sub>. However, *Vaccinium myrtillus* mass increased with both treatments, graminoid mass declined with warming, and forb and nonvascular plant (moss and lichen) mass decreased with both treatments. Fine roots showed a substantial reduction under soil warming (–40% for all roots <2 mm in diameter at 0–20 cm soil depth) but no change with CO<sub>2</sub> enrichment. Our findings suggest that enhanced overall productivity and shifts in biomass allocation will occur at the tree line, particularly with global warming. However, individual species and functional groups will respond differently to these environmental changes, with consequences for ecosystem structure and functioning.

**Keywords:** dwarf shrub, European larch, free air CO<sub>2</sub> enrichment, global change, *Larix decidua*, mountain pine, *Pinus uncinata*

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## Introduction

As boundary ecosystems between mountain forests and alpine tundra, high-elevation tree lines are expected to be especially sensitive to global change (Smith *et al.*, 2009; Körner, 2012). In particular, increases in atmospheric CO<sub>2</sub> concentration and the associated global warming could alter plant growth and species composition at tree line, with important consequences for ecosystem structure and functioning. While knowledge about how other ecosystem types might respond to these environmental changes has advanced rapidly in recent years (e.g. Dieleman *et al.*, 2012), it remains unclear if these findings apply to alpine tree line eco-

systems in the temperate zone. The environment at temperate tree lines is characterized by a long snow cover duration, low and sometimes even freezing temperatures during the growing season, and low availability of soil nutrients (particularly N) due to low-temperature constraints on decomposition and mineralization (Körner, 2012). Integration of tree line ecosystems into large-scale modelling efforts is additionally difficult because relatively little is known about biomass distribution at tree line among different tree species and plant growth forms or about allocation of biomass to above- and below-ground structures (Bolliger *et al.*, 2008; Devi *et al.*, 2008; Elkin *et al.*, 2013).

Despite clear evidence from observational investigations that high-elevation ecosystems are impacted by ongoing environmental change (Cannone *et al.*, 2007; Dullinger *et al.*, 2012), we are aware of only three other experiments that have applied CO<sub>2</sub> enrichment in this

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setting. A late-successional sedge community (Körner *et al.*, 1997) and a mix of glacier forefield pioneer species (Inauen *et al.*, 2012) were studied at alpine sites in the Central Swiss Alps (Furka Pass, 2440 m a.s.l.), and a subalpine *Picea abies* forest community was investigated in a model ecosystem study (Hättenschwiler & Körner, 1998). These three experiments showed no enhanced above-ground plant productivity after multiple years of CO<sub>2</sub> enrichment but some evidence of greater allocation to below-ground biomass.

In general, CO<sub>2</sub> research from near-natural environments at lower elevations has suggested no or only moderate plant productivity responses to CO<sub>2</sub> enrichment when total plant biomass and cover have reached a steady state (Körner, 2006; Norby & Zak, 2011). Further, many experiments spanning several years have shown initially positive plant growth responses to elevated CO<sub>2</sub> that decline over time, particularly in forests (Körner, 2006; Leuzinger *et al.*, 2011; Norby *et al.*, 2010; but see McCarthy *et al.*, 2010). Increased allocation to below-ground productivity has been reported in some longer-term CO<sub>2</sub> enrichment studies, including enhanced fine root production in a system where no sustained above-ground growth stimulation was observed (Norby *et al.*, 2004). This shift in allocation has been interpreted as one of several ways in which plants achieve greater N uptake to balance extra carbon assimilated under elevated CO<sub>2</sub> (Luo *et al.*, 2004; Norby *et al.*, 2010). However, knowledge from field studies about below-ground plant productivity responses to CO<sub>2</sub> enrichment is often limited to fine root dynamics, especially for trees, due to obvious difficulties in assessing root system growth *in situ* and to the destructive nature of major below-ground sampling efforts.

Compared to CO<sub>2</sub> enrichment field experiments, there is a slightly longer and more widespread history of warming studies in mid-latitude alpine environments (e.g. Kudo & Suzuki, 2003; Kudernatsch *et al.*, 2008). Meta-analyses of warming studies in subarctic, arctic and alpine tundra ecosystems have reported enhanced plant growth or reproductive output with increased temperatures but have also indicated large heterogeneity across species or growth form, location and experimental duration (Arft *et al.*, 1999; Dormann & Woodin, 2002; Walker *et al.*, 2006; Elmendorf *et al.*, 2012). Additionally, in contrast to the general trend observed in CO<sub>2</sub> enrichment studies, the magnitude of warming effect size on tundra vegetation has been found to increase linearly with experimental duration (Elmendorf *et al.*, 2012). Despite the rich literature about impacts of climate change in tundra environments, high-elevation studies in the temperate zone have been vastly under-represented in synthesis efforts. Further, few experimental warming studies have been conducted in high-elevation

environments including trees. This research gap is critical because the upper elevational limit of trees (i.e. alpine tree line) is thought to be primarily caused by low temperature inhibiting tree growth processes despite an adequate carbon supply (Körner, 2012). In older trees, heating of individual *Pinus mugo* ssp. *uncinata* branches (Lenz *et al.*, 2013) and of the apical shoot of *Picea abies* (Petit *et al.*, 2011) has shown enhanced growth of the individual warmed tissue, and a field study warming entire *Picea glauca* seedlings with passive open-top chambers resulted in increased height growth (Danby & Hik, 2007). Findings from these previous experimental studies suggest that above-ground tree growth processes are indeed limited by low temperature at the alpine tree line, yet the consequences of warmer growing conditions on entire tree biomass are largely unknown.

Meta-analyses including a wider spectrum of ecosystem types and regions have indicated overall enhanced above-ground plant productivity with experimental warming (Rustad *et al.*, 2001; Dieleman *et al.*, 2012), including in tree species from temperate and boreal regions (Way & Oren, 2010). Biomass of coarse or fine roots has often been unresponsive to experimental warming, however, yielding a lower proportion of below-ground biomass in some cases (Way & Oren, 2010; Dieleman *et al.*, 2012). This altered biomass allocation may be caused, at least partially, by increased N availability with warming, in that plants do not need to invest as much into below-ground structures involved in nutrient acquisition (Melillo *et al.*, 2011).

CO<sub>2</sub> emissions from anthropogenic sources and long-term temperature increases are linearly related (Stocker *et al.*, 2013), and understanding potential interactive effects between these factors is essential for improving predictions of how ecosystems will be impacted by global change (Dieleman *et al.*, 2012). However, simultaneous manipulation of CO<sub>2</sub> level and temperature is challenging in high-elevation and high-latitude ecosystems due to logistical and financial constraints, especially at sites with trees, and few such experiments exist. In two separate closed-top chamber studies of boreal forest ecosystems, radial growth of 20-year-old *Pinus sylvestris* was stimulated by CO<sub>2</sub> enrichment but not by air warming (Kilpelainen *et al.*, 2005) and neither CO<sub>2</sub> enrichment nor air warming altered the growth of 40-year-old *Picea abies* at natural low nutrient availability (Kostiainen *et al.*, 2009; Sigurdsson *et al.*, 2013). Whereas no interactions between CO<sub>2</sub> enrichment and warming were reported in these studies of trees, a positive CO<sub>2</sub> × warming interactive effect on NPP was observed over 3 years for arctic tussock tundra vegetation in Alaska, potentially due to increased ecosystem sink strength at higher temperatures (Oechel *et al.*, 1994). Clearly, it remains uncertain if and how these

two global change factors will interact to influence different plant groups or species in cold ecosystems in alpine and arctic locations.

To our knowledge, the Stillberg experiment located near Davos, Switzerland, is the only existing CO<sub>2</sub> enrichment study of an alpine tree line ecosystem (Hättenschwiler *et al.*, 2002). The study is additionally unique for simultaneously manipulating CO<sub>2</sub> concentration and soil temperature for two key high-elevation tree species, *Larix decidua* and *Pinus uncinata*, in a replicated factorial experimental design (Hagedorn *et al.*, 2010). After 9 years of free air CO<sub>2</sub> enrichment (2001–2009) and 6 years of soil warming (2007–2012), including 3 years of the treatments applied in combination (2007–2009), we conducted a complete final harvest of the experiment. With this harvest effort, we were able to evaluate cumulative effects of multiple years of CO<sub>2</sub> enrichment, soil warming and the combined treatments on plant biomass and to provide novel comparative above- and below-ground biomass measures for two major tree line tree species and the associated understorey plant community. Additionally, we measured tree stem radial growth on entire stem discs to study tree above-ground growth responses to the treatments over the entire 12 years of the experiment.

We hypothesized that (i) both treatments led to an increase in total tree and understorey plant biomass. We expected positive biomass responses to the combined treatment to be more than additive if the soil warming treatment alleviated constraints on the CO<sub>2</sub> growth response associated with low temperature. In addition, we hypothesized that (ii) CO<sub>2</sub> enrichment and soil warming had opposing effects on the root mass fraction (RMF) of the trees, with CO<sub>2</sub> enrichment increasing RMF due to an increased nutrient demand associated with a sustained photosynthetic enhancement of both *Larix* and *Pinus* (Streit *et al.*, 2014). In contrast, evidence of increased nitrogen availability during the initial 3 years of soil warming (Dawes *et al.*, 2011a) led us to expect a reduced RMF in trees growing in warmed soils.

## Materials and methods

### Site and experimental setup

The study site was located at Stillberg, Davos in the Central Alps, Switzerland (9° 52' E, 46° 46' N, 2180 m a.s.l.), on a NE-exposed 25–30° slope slightly above the current tree line in the region (Barbeito *et al.*, 2012). The site was situated 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). From 1975 to 2012, the mean annual precipitation was 1155 mm and the mean annual air temperature was 2.1 °C. For the same period, the main growing season

months (June–August) had a mean precipitation of 444 mm and a mean air temperature of 9.2 °C. Soil types are sandy Ranker and Podzols (Lithic Cryumbrepts and Typic Cryorthods), derived from siliceous Paragneis parent material and dominated by a 5–20 cm thick organic Humimor layer (Bednorz *et al.*, 2000).

Experimental plots for the study were established in spring 2001 when a free air CO<sub>2</sub> enrichment (FACE) experiment was initiated (Hättenschwiler *et al.*, 2002). A total of 40 plots were created, each 1.1 m<sup>2</sup> in area, 20 with a *Larix decidua* L. (European larch) individual in the centre and 20 with a *Pinus mugo* ssp. *uncinata* Ramond (mountain pine; referred to as *Pinus uncinata* elsewhere in this paper) individual in the centre. These trees were ca. 40 years old in 2012 with average heights of 1.5 m (*Pinus*) and 2.6 m (*Larix*). The trees were sparsely distributed (not more than one neighbouring tree within 80 cm of each tree in the experiment) so did not form a closed canopy, and a dense cover of understorey vegetation dominated by ericaceous dwarf shrubs surrounded the tree stem in each plot. The plots were assigned to 10 groups of four neighbouring plots (two plots with each tree species 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 (ambient concentration +200 ppm), whereas the remaining groups received no supplementary CO<sub>2</sub>. CO<sub>2</sub> enrichment was supplied during daytime hours only throughout each snow-free period (approximately beginning of June to end of September) from 2001 to 2009. The setup and performance of the FACE system have been described in detail previously (Hättenschwiler *et al.*, 2002; Handa *et al.*, 2006; Dawes *et al.*, 2011b).

In spring 2007, one plot of each tree species was randomly selected from each of the 10 CO<sub>2</sub> treatment groups and assigned a warmed soil treatment, yielding a balanced design with a replication of five individual plots for each combination of CO<sub>2</sub> concentration, soil warming treatment and tree species. Warming was accomplished using 420-W heating cables laid on the ground surface underneath the dwarf shrub layer, with a distance of 5 cm between neighbouring cables (details about the heating system are given in Hagedorn *et al.*, 2010). The warming treatment was applied during the entire snow-free period in 2007–2011 and from 2 June to 8 August in 2012 (Table S1). Soil warming increased the growing season mean soil temperature at 5 cm depth by an average of 3.6 °C over the six seasons of heating (Table S1). Increased air temperatures were detected within the dwarf shrub canopy (0.9 °C at 20 cm above ground) but no temperature difference was detected at 50 cm height (Hagedorn *et al.*, 2010). Soil warming had a slight drying effect on the soil organic layer during the first 3 years of treatment, but this effect was not apparent in later years (Table S1). The soil matric water potential at 5 cm depth was always above –300 hPa in all plots, indicating overall very moist soil conditions (Dawes *et al.*, 2014).

### Understorey above-ground biomass

Understorey vegetation from the entire plot area was clipped at the ground surface at the beginning of August in 2012. The harvested vegetation was separated into individual species for

dwarf shrubs (excluding dead ramets) and into plant functional groups of graminoids, forbs and nonvascular plants (mosses and lichens) for the remaining species. This material was dried and weighed to obtain the dry mass of each species or functional group (expressed as  $\text{g m}^{-2}$ ). All plant samples were dried at 70 °C for 48 h or until weights stabilized.

#### *Fine root mass*

To estimate fine root mass, we sampled soils down to 20 cm depth in early August of 2012 after understorey vegetation was removed. For 0–5 cm soil depth (Oe + Oa horizons), we took 8 cores, each 5 cm in diameter, evenly distributed across the area of each plot; for 5–10 cm depth (Oa horizon), we took 6 cores, each 4.4 cm in diameter; and for 10–20 cm depth (Oa and mineral soil), we took 6 cores, each 2 cm in diameter. Samples from each soil layer were bulked at the plot level, and all fine roots were separated from the soil samples using a 4 and a 2 mm sieve. Roots were then washed using a 50  $\mu\text{m}$  mesh and were separated by size class (<0.5 and 0.5–2 mm in diameter) for the 0–5 and 5–10 cm depths where almost all roots occurred. Samples were then dried and weighed, and mass values were scaled up to  $\text{g m}^{-2}$  from the sampling area. Accurate separation of fine roots into individual plant species or functional types was not possible, and thus, mass data represented plot-level estimations of all species combined.

#### *Tree biomass and basal area increments*

Tree material above the ground surface was harvested at the beginning of August in 2012. All needles and branches (<1 cm in diameter for *Larix* and <1.5 cm for *Pinus*, including new shoots formed in 2012) were removed from the main stem and oven-dried. Needles were then separated from the branches, and the dry mass of each compartment was measured. The main stem material was cut into ca. 15 cm long sections with a circular saw (minimal mass loss), dried and weighed. The entire coarse root system of each tree (i.e. roots > ca. 2 mm in diameter) was excavated in mid-August (after soil core sampling) by carefully removing all surrounding soil and stones and was subsequently dried and weighed. All coarse roots were located less than 50 cm below the ground surface and could be accessed easily. Data from all tree compartments were combined to calculate the following biomass data for each tree: wood mass (main stem and branches); needle mass (all needles were formed in 2012 for deciduous *Larix*; needles formed in 2012 and in previous years were bulked for evergreen *Pinus*); above-ground mass (main stem, branches and needles); below-ground mass (coarse root system); and total tree biomass (above and below ground). We additionally calculated the leaf mass fraction (LMF, total needle mass divided by total tree mass) and RMF (coarse root system mass divided by total tree mass) for each tree.

Three to four stem discs were cut from the lower section of each tree stem (bottom 5–20% of total tree height) for detailed tree ring measurements. Each disc was progressively sanded to a grit of 400 and scanned at a resolution of 1200 dpi (Epson Expression 10000 XL, Seiko Epson, Nagano, Japan). Tree-ring

width was measured along 12 equally spaced radii on each disc using the software WINDENDRO (version 2008 g; Regent Instruments Inc., Québec City, Québec, Canada). Ring width measurements were averaged at the disc level and then used to calculate annual basal area increments of each disc for each year from 2000 to 2012. We used basal area values averaged over the three to four discs for each tree in statistical analyses.

#### *Total plant biomass per land area unit*

Tree density in the experimental area (pooled across *Larix decidua* and *Pinus uncinata*) was one tree per 1.14  $\text{m}^2$  in 2005 (P. Bebi, unpublished data), indicating that the experimental plot area of 1.1  $\text{m}^2$  was realistic for the actual tree density at the site. We therefore estimated total plant biomass on a land area basis ( $\text{g m}^{-2}$ ) by combining tree above-ground and coarse root mass (scaled down from  $\text{g 1.1 m}^{-2}$  to  $\text{g m}^{-2}$ ), understorey above-ground mass and fine root mass.

#### *Statistical analysis*

We assessed treatment effects on plant biomass and tree stem radial growth (basal area) with linear mixed-effects models fitted with restricted maximum likelihood (REML). The random effects structure of all statistical models reflected the experimental design, where individual plots (i.e. one tree) were nested within 20 soil warming treatment groups nested within 10  $\text{CO}_2$  treatment groups. For all biomass response variables (tree compartments, understorey above-ground parts, fine roots and total plant biomass), we included soil warming treatment (unwarmed or warmed),  $\text{CO}_2$  level (ambient or elevated), plot tree species (*Larix* or *Pinus*) and all interactions between these variables as fixed effects in statistical models and tested for significance using Type I conditional *F* tests (Pinheiro & Bates, 2000). Results were never sensitive to the order in which fixed factors were fitted in the models. All 20 plots containing a *Larix* tree were included in analyses ( $n = 5$  for each  $\text{CO}_2$  and warming treatment combination). Three *Pinus* trees died during the  $\text{CO}_2$  enrichment period: one ambient  $\text{CO}_2$ , unwarmed; one ambient  $\text{CO}_2$ , warmed; and one elevated  $\text{CO}_2$ , unwarmed. These plots were excluded from analyses of tree biomass and total plant biomass ( $n = 4$  for these treatment groups) but were included for analyses of understorey above-ground and fine root biomass ( $n = 5$ ; results did not change if these plots were excluded).

In models for tree and total plant biomass, we included tree basal area after the 2006 growing season (BA2006), immediately before the soil warming treatment was initiated, as a covariate to account for prewarming differences in tree size. Although using BA2006 as a covariate was most appropriate for testing effects of soil warming and interactive effects between the  $\text{CO}_2$  and warming treatments, doing so could underestimate effects of elevated  $\text{CO}_2$  on biomass if substantial  $\text{CO}_2$ -induced gains occurred in 2001–2006. We therefore additionally applied a second model for each biomass variable where basal area after the 2000 growing season (BA2000) was used as a covariate to account for pre- $\text{CO}_2$  enrichment differences in tree size, and  $\text{CO}_2$ , plot tree species and  $\text{CO}_2 \times \text{plot}$



tree species (but not soil warming) were included as fixed effects. No pretreatment information was available for biomass allocation ratios of the trees (LMF and RMF).

Estimates of cover for each plant species present in each experimental plot were made in 2005, thereby providing information about understorey plant composition at that prewarming time point. The percentage cover of each species was classified as <1%, 1–4%, 5–14%, 15–24%, 25–49%, 50–74% or 75–100%. We used the mid-point of each cover category as a covariate in analyses of the above-ground biomass of different understorey species and plant functional groups, acknowledging that doing so would not capture any CO<sub>2</sub> effects that occurred before 2005. Fine root mass measured using soil cores sampled in autumn of 2002–2004 (data presented in Handa *et al.*, 2008) did not differ between CO<sub>2</sub> and soil warming treatment groups and showed no relationship with fine root mass in 2012. Therefore, we completed fine root mass analyses of different size classes and depths without considering these earlier data.

**Tree stem basal area growth rate.** Flexible nonlinear models, such as those using a power law, are preferred for modelling plant growth because relative growth rate (RGR) frequently slows as plants increase in size (e.g. Evans, 1972; Philipson *et al.*, 2012). However, nonlinear models could not be fit successfully with our tree basal area data set. We therefore modelled tree stem radial growth as the log of basal area through time using linear mixed-effects models, where the slope represents RGR (Paine *et al.*, 2012). To evaluate the degree to which RGR differed between species and treatment groups, we analysed the log of tree basal area using separate models for two key periods in the 12-year experiment. For the CO<sub>2</sub> enrichment period (beginning of 2001 to end of 2009), models included CO<sub>2</sub> level, tree species, treatment year (continuous variable) and all interactions as fixed effects. For the soil warming period (beginning of 2007 to final harvest in 2012, including the 2007–2009 period with combined CO<sub>2</sub> and warming treatments), we included warming treatment and all associated interactions as additional fixed effects. We applied a residual auto-correlation structure (auto-regressive model of order 1, corAR1) to account for violation of independence of residuals from repeated basal area measurements on a given tree.

For all statistical analyses, we log-transformed response variables where necessary to meet assumptions of normality and homoscedasticity of the residuals. In addition, we accounted for cases where the residual variance differed between levels of the treatments or between tree species using the VarIdent argument (Pinheiro *et al.*, 2008). We considered fixed effects significant at  $P < 0.05$ . Due to relatively low replication and therefore statistical power, we additionally designated  $P$ -values  $\geq 0.05$  but  $< 0.10$  as marginally significant. All analyses were performed using R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria), and mixed-effects models were fitted using the NLME package (Pinheiro *et al.*, 2014). All estimates of treatment differences reported in the main article reflect statistical analyses by showing model estimates for a standardized 2006 (prewarming treatment) or 2000

(pre-CO<sub>2</sub> treatment) tree stem basal area for total plant biomass and tree biomass compartments and model estimates for a standardized 2005 cover value for understorey plants. However, information provided about different biomass contributions in this tree line ecosystem in general was derived from raw data, which is provided in Tables S2 and S3.

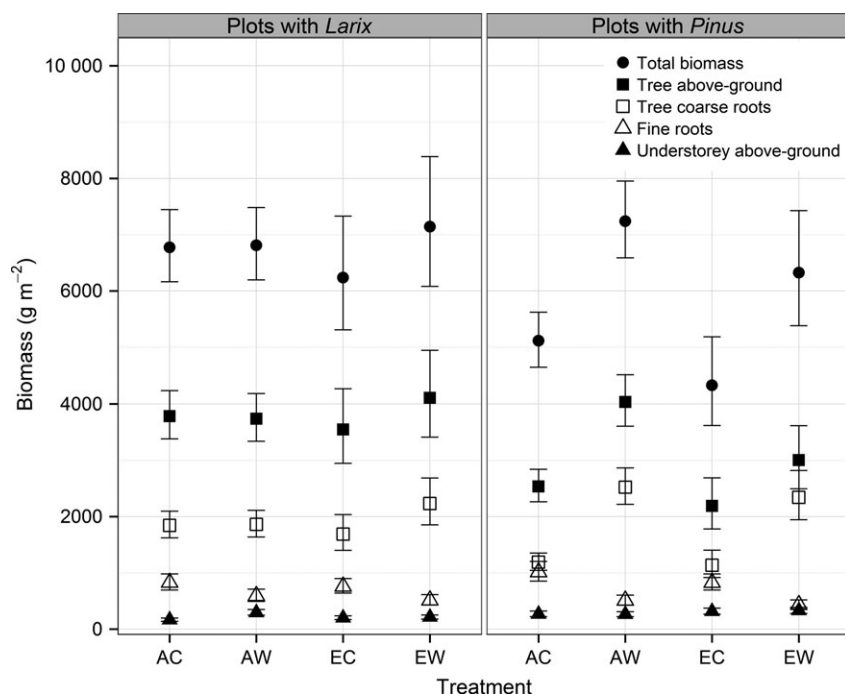
## Results

### Total plant biomass per unit of land area

Total plant biomass summed to  $6.67 \pm 0.42 \text{ kg m}^{-2}$  (mean of all plots  $\pm 1$  SE), with 54% ( $3.65 \pm 0.28 \text{ kg m}^{-2}$ ) in tree above-ground mass, 30% ( $2.04 \pm 0.16 \text{ kg m}^{-2}$ ) in tree coarse root mass, 4% ( $0.26 \pm 0.02 \text{ kg m}^{-2}$ ) in above-ground understorey mass and 12% ( $0.71 \pm 0.05 \text{ kg m}^{-2}$ ) in fine root mass (Tables S2 and S3). As trees were by far the largest contributors to plot-level biomass, tree stem basal area at the end of 2006 (BA2006; immediately before the soil warming treatment was initiated), strongly influenced total plant biomass ( $F_{1,12} = 175.65$ ,  $P < 0.001$ ). Accounting for this prewarming variability in tree basal area, total plant biomass increased in warmed plots ( $F_{1,8} = 14.23$ ,  $P = 0.006$ ), mainly in plots containing *Pinus* (warming  $\times$  tree species interaction:  $F_{1,12} = 17.61$ ,  $P = 0.001$ ; Fig. 1). The total plant biomass response to warming was driven by responses in tree above-ground and tree coarse root mass, as neither total understorey above-ground mass nor fine root mass showed increases with soil warming (see below). Irrespective of plot tree species and warming treatment, CO<sub>2</sub> enrichment (after 2006) did not significantly influence total plant biomass ( $F_{1,8} = 0.07$ ,  $P = 0.795$ ; Fig. 1). When tree basal area at the end of 2000, just before the FACE experiment was initiated, was used as a covariate in models testing effects of CO<sub>2</sub> enrichment and plot tree species, the pretreatment covariate again had a significant effect on biomass ( $F_{1,24} = 5.24$ ,  $P = 0.031$ ). In this second analysis, mean total plant biomass was  $7492 \text{ g m}^{-2}$  ( $\pm 1$  SE: 6454–8696) in elevated CO<sub>2</sub> plots with *Larix* compared to  $5659 \text{ g m}^{-2}$  ( $\pm 1$  SE: 4848–6605) in ambient CO<sub>2</sub> plots with *Larix*, but this difference was not statistically significant due to large variability ( $F_{1,8} = 1.09$ ,  $P = 0.327$ ). Total plant biomass was greater in plots with *Larix* than in those with *Pinus* in statistical models accounting for prewarming differences in tree size (marginally significant:  $F_{1,12} = 4.69$ ,  $P = 0.051$ ; Fig. 1).

### Understorey above-ground biomass

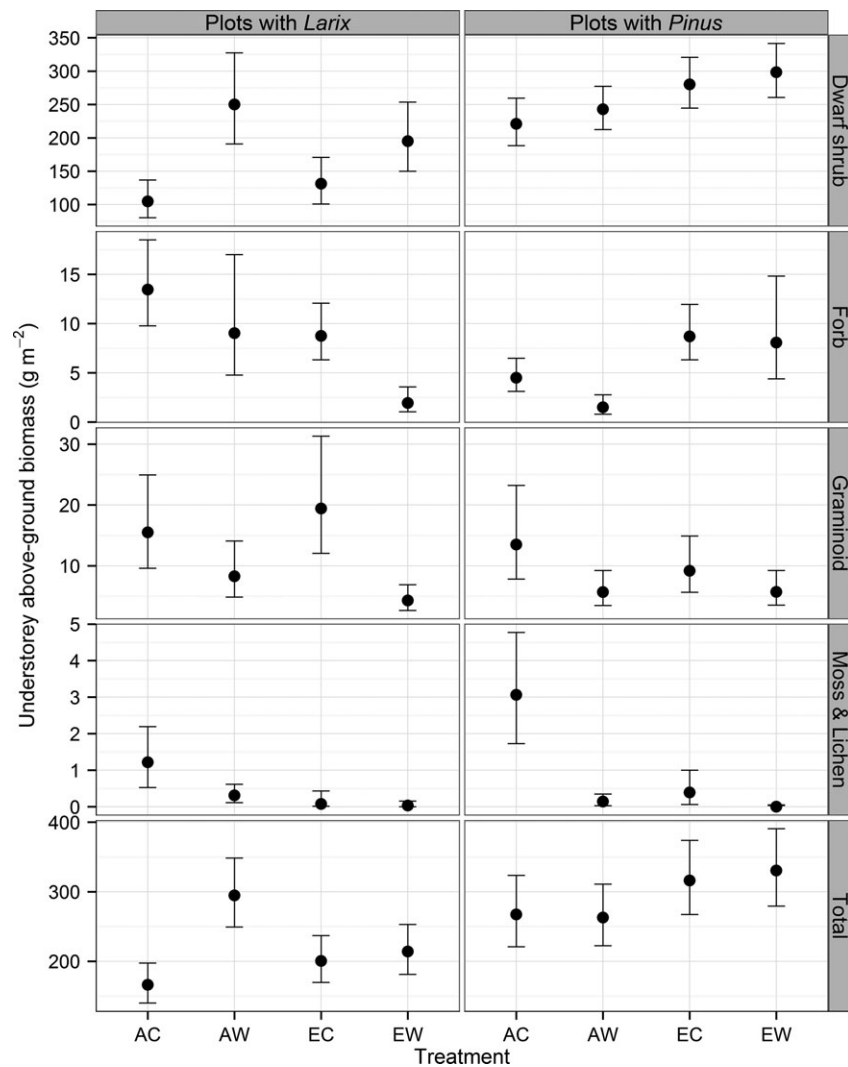
Averaged over all plots, dwarf shrub species contributed  $239 \pm 19 \text{ g m}^{-2}$  (88%) of  $271 \pm 17 \text{ g m}^{-2}$  of total



**Fig. 1** Plant biomass per unit of land area for each combination of CO<sub>2</sub> level (A = ambient, E = elevated) and soil warming treatment (C = unwarmed soil, W = warmed soil) in plots containing a *Larix decidua* (left) or *Pinus uncinata* (right) tree ( $n = 4-5$ ). Total plant biomass (filled circles) is shown, as well as contributions from tree above-ground parts (filled squares), understorey above-ground parts (filled triangles), tree coarse roots (open squares) and fine roots (<2 mm diameter) bulked for all plant species (open triangles). The figure reflects statistical analyses by showing model estimates ( $\pm 1$  SE) for a standardized 2006 (prewarming treatment) tree stem basal area (947 mm<sup>2</sup>, mean of all trees) for total plant biomass and tree biomass components and model estimates for a standardized 2005 total (sum of all species) vegetation cover (105%, mean of all plots) for understorey biomass. Significant fixed effects in statistical models included the following: warming ( $P < 0.05$ ), plot tree species ( $P < 0.10$ ) and warming  $\times$  plot tree species interaction ( $P < 0.05$ ) for total plant biomass; warming ( $P < 0.05$ ) and warming  $\times$  plot tree species interaction ( $P < 0.05$ ) for tree above-ground and tree coarse root mass; plot tree species ( $P < 0.10$ ) for tree above-ground mass; plot tree species ( $P < 0.05$ ) for understorey above-ground mass; warming ( $P < 0.05$ ) for fine root mass.

understorey above-ground biomass, and *V. myrtillus* was the greatest contributor in most plots (mean  $121 \pm 9$  g m<sup>-2</sup> or 45%; Figs 2 and 3, Table S2). Taking into account cover estimates from 2005 (included as a covariate in statistical models of understorey biomass), total above-ground mass of understorey vegetation was not significantly altered by soil warming, elevated CO<sub>2</sub> or the combined treatments, but the relative contributions of individual species and functional groups changed (Fig. 2). The total mass of all dwarf shrubs increased with warming in plots with a *Larix* individual (marginally significant warming  $\times$  tree species interaction:  $F_{1,14} = 3.55$ ,  $P = 0.081$ ) but showed no effect of CO<sub>2</sub> enrichment ( $F_{1,8} = 2.19$ ,  $P = 0.177$ ; Fig. 2). For the three dominant dwarf shrub species, *V. myrtillus* above-ground mass was enhanced by soil warming ( $F_{1,8} = 6.79$ ,  $P = 0.031$ ) and by CO<sub>2</sub> enrichment after 2005 (marginally significant:  $F_{1,8} = 4.23$ ,  $P = 0.074$ ), whereas neither *Vaccinium gaultherioides* nor *Empetrum hermaphroditum* mass was

significantly affected by the treatments (Fig. 3). In contrast to dwarf shrub mass, graminoid ( $F_{1,8} = 6.80$ ,  $P = 0.031$ ), forb ( $F_{1,8} = 4.77$ ,  $P = 0.060$ ; marginally significant) and nonvascular (moss and lichen) species ( $F_{1,8} = 9.49$ ,  $P = 0.015$ ) mass were all lower in warmed than in unwarmed plots (Fig. 2). Forbs additionally experienced a negative CO<sub>2</sub> effect, but only in plots with *Larix* (CO<sub>2</sub>  $\times$  tree species interaction:  $F_{1,14} = 8.52$ ,  $P = 0.011$ ), and mosses and lichens showed a slight overall decline in plots that had received supplementary CO<sub>2</sub> (marginally significant:  $F_{1,8} = 3.87$ ,  $P = 0.085$ ; Fig. 2). The interaction between CO<sub>2</sub> and warming treatments was not significant for any of the species or functional groups tested. Plots with *Pinus* had a greater total above-ground understorey mass ( $F_{1,14} = 6.24$ ,  $P = 0.026$ ) and dwarf shrub mass ( $F_{1,14} = 8.97$ ,  $P = 0.010$ ) than plots with *Larix*, largely due to tree species effects on the mass of *Vaccinium myrtillus* ( $F_{1,14} = 16.43$ ,  $P = 0.001$ ) and *V. gaultherioides* ( $F_{1,14} = 14.23$ ,  $P = 0.002$ ), whereas plot tree species did



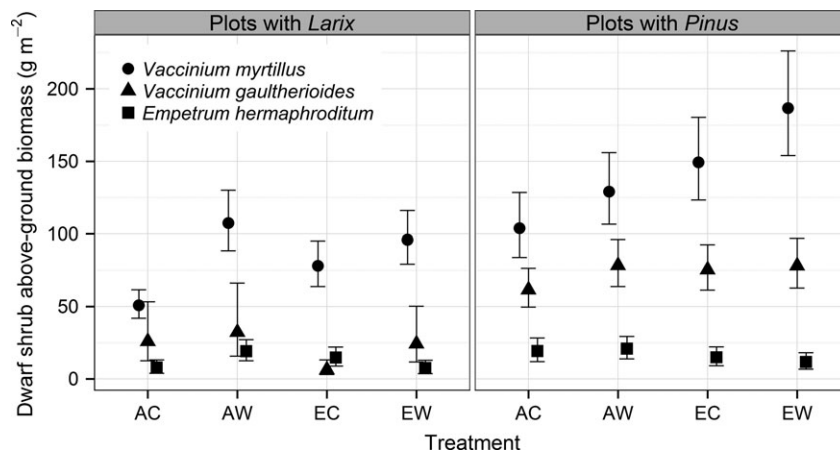
**Fig. 2** Total plot understorey above-ground biomass and contributions from different functional groups (each shown in a separate panel): dwarf shrub, graminoid, forb and nonvascular (moss and lichen). Different scales are used for each panel to highlight treatment differences. Values reflect statistical analyses by showing model predictions ( $\pm 1$  SE) for a standardized vegetative cover value (%) of each functional group in the prewarming year 2005. Estimates for each combination of CO<sub>2</sub> level (A = ambient, E = elevated), soil warming treatment (C = unwarmed, W = warmed) and plot tree species (*Larix decidua*, *Pinus uncinata*) are shown ( $n = 5$ ), and values are expressed per unit of land area. Significant fixed effects in statistical models included the following: plot tree species ( $P < 0.05$ ) and warming  $\times$  plot tree species interaction ( $P < 0.10$ ) for dwarf shrub mass; warming ( $P < 0.05$ ) for graminoid mass; warming ( $P < 0.10$ ) and CO<sub>2</sub>  $\times$  plot tree species interaction ( $P < 0.05$ ) for forb mass; and warming ( $P < 0.05$ ) and CO<sub>2</sub> ( $P < 0.10$ ) for moss and lichen mass.

not affect the mass of other functional groups (Figs 2 and 3).

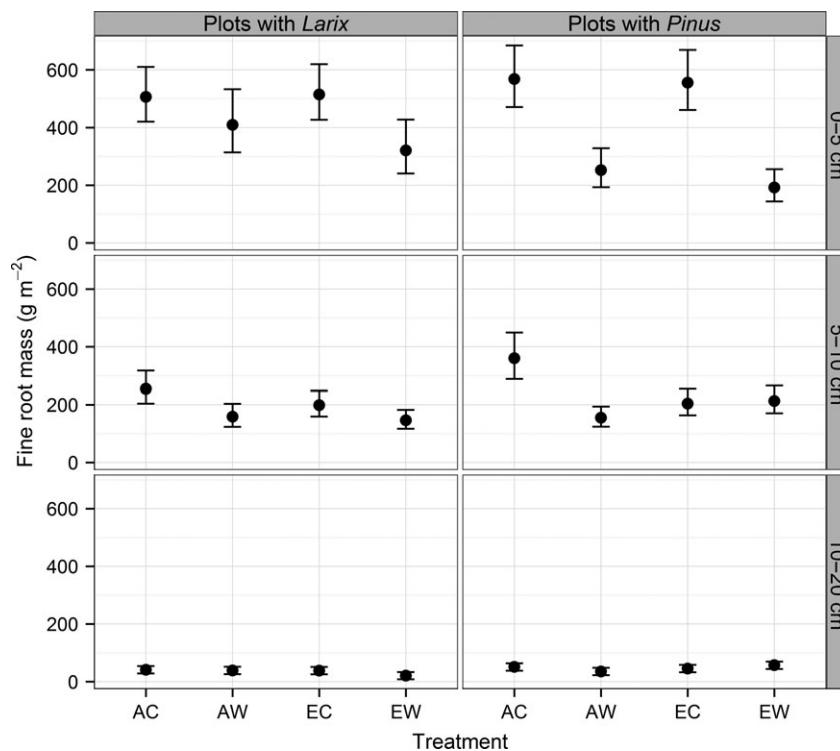
#### Fine root biomass

Fine root mass measurements from soil core samples revealed that the majority of roots were in the uppermost 5 cm of the organic layer ( $61 \pm 3\%$  of total fine root mass; mean pooled across warming treatments, CO<sub>2</sub> levels and plot tree species). An average of  $33 \pm 2\%$  of roots occurred at 5–10 cm depth and only

$6 \pm 1\%$  occurred at 10–20 cm depth (Fig. 4). Total fine root mass ( $< 2$  mm diameter, 0–20 cm depth) showed a substantial reduction in plots with warmed soil ( $F_{1,8} = 38.38$ ,  $P < 0.001$ ), with  $555 \pm 55$  g m<sup>-2</sup> compared to  $889 \pm 55$  g m<sup>-2</sup> in unwarmed plots, pooled across CO<sub>2</sub> levels and plot tree species (Fig. 1). This effect was apparent at depths of 0–5 cm ( $F_{1,8} = 25.25$ ,  $P = 0.001$ ) and 5–10 cm ( $F_{1,8} = 6.84$ ,  $P = 0.031$ ), both within the organic layer, although in the shallower depth class it occurred primarily in plots with *Pinus* (warming  $\times$  tree species interaction:  $F_{1,14} = 5.63$ ,



**Fig. 3** Above-ground biomass of the three dominant dwarf shrub species: *Vaccinium myrtillus* (circles), *Vaccinium gaultherioides* (triangles) and *Empetrum hermaphroditum* (squares). Values reflect statistical analyses by showing model predictions ( $\pm 1$  SE) for a standardized vegetative cover value (%) of each individual species in the prewarming year 2005. Estimates for each combination of CO<sub>2</sub> level (A = ambient, E = elevated), soil warming treatment (C = unwarmed, W = warmed) and plot tree species (*Larix decidua*, *Pinus uncinata*) are shown ( $n = 5$ ), and values are expressed per unit of land area. Significant fixed effects in statistical models included the following: warming ( $P < 0.05$ ), plot tree species ( $P < 0.05$ ) and CO<sub>2</sub> ( $P < 0.10$ ) for *V. myrtillus*; plot tree species ( $P < 0.05$ ) for *V. gaultherioides*.



**Fig. 4** Mass of fine roots (<2 mm diameter) at soil depths of 0–5 cm (top panels), 5–10 cm (middle panels) and 10–20 cm (bottom panels). Mean values  $\pm 1$  SE for each combination of CO<sub>2</sub> level (A = ambient, E = elevated), soil warming treatment (C = unwarmed, W = warmed) and plot tree species (*Larix decidua*, *Pinus uncinata*) are shown ( $n = 4$ –5), and values are expressed per unit of land area. Significant fixed effects ( $P < 0.05$ ) in statistical models included the following: warming for 0–5 cm and 5–10 cm soil depths; warming  $\times$  plot tree species interaction for 0–5 cm depth.

$P = 0.033$ ; Fig. 4). There was no change in fine root mass (total or at individual depths) in plots previously exposed to elevated CO<sub>2</sub>, and interactive effects

involving CO<sub>2</sub> were not statistically significant. There were no treatment effects at 10–20 cm depth where only a small fraction of fine roots grew (Fig. 4). We did



not detect differences in the vertical distribution of fine roots associated with soil warming, CO<sub>2</sub> enrichment or tree species, suggesting an overall decline in fine root mass with warming rather than a redistribution to deeper soil depths.

Averaged across all plots and pooled over the top 10 cm of organic soil,  $32 \pm 2\%$  of the total fine root mass consisted of roots <0.5 mm in diameter. Reductions in fine root mass with warming occurred both for roots <0.5 mm in diameter ( $F_{1,8} = 34.41$ ,  $P < 0.001$ ) and for roots 0.5–2 mm in diameter ( $F_{1,8} = 16.85$ ,  $P = 0.003$ ; Fig. S1). Warming-induced decreases were stronger in plots with *Pinus* than with *Larix* (warming  $\times$  tree species interaction), both for roots <0.5 mm in diameter ( $F_{1,13} = 11.42$ ,  $P = 0.005$ ) and for roots 0.5–2 mm in diameter ( $F_{1,12} = 9.63$ ,  $P = 0.009$ ; Fig. S1). In plots with *Larix*, roots <0.5 mm additionally experienced a decline in elevated CO<sub>2</sub> plots (warming  $\times$  tree species  $\times$  CO<sub>2</sub> interaction:  $F_{1,15} = 5.52$ ,  $P = 0.033$ ; Fig. S1). Neither size class showed a significant overall difference in mass associated with the different plot tree species.

#### Tree biomass and biomass allocation

Accounting for pretreatment differences in tree size (by fitting BA2006 as a covariate in statistical models), soil warming had a significant positive effect on all tree biomass variables tested: total, above-ground, needle, wood and coarse root mass. Treatment group estimates and linear mixed-effects model results are given in Table 1. Additionally, there was a warming  $\times$  tree species interactive effect for each compartment, indicating that overall soil warming effects were primarily due to increased biomass of *Pinus* but not *Larix* trees (Table 1). Trees that grew under elevated CO<sub>2</sub> did not have significantly enhanced biomass compared to trees exposed to ambient CO<sub>2</sub> for any of the compartments analysed (Table 1), irrespective of soil warming treatment or tree species, even in models where BA2000 was used as a covariate instead of BA2006 (Table 2). *Larix* trees that were exposed to elevated CO<sub>2</sub> tended to have greater mass of all compartments compared to trees that experienced ambient CO<sub>2</sub> (by ca. +50%), but these differences were not statistically significant (Table 2). Relative to tree size in 2006, *Larix* trees had greater total, above-ground and wood mass than *Pinus* trees, whereas needle mass was greater for the evergreen species *Pinus* and coarse root mass did not differ between tree species (Table 1).

Tree biomass allocation to needles (LMF) did not show a significant effect of soil warming, CO<sub>2</sub> enrichment or the interaction between these treatments (Fig. 5). Unsurprisingly, LMF was greater for evergreen *Pinus* ( $13.0 \pm 0.8\%$ , averaged across CO<sub>2</sub> and warming

treatments) than for deciduous *Larix* ( $6.3 \pm 0.2\%$ ;  $F_{1,11} = 78.19$ ,  $P < 0.001$ ; Fig. 5). Root mass fraction (RMF) was enhanced for trees growing in warmed plots ( $F_{1,8} = 6.96$ ,  $P = 0.030$ ), and a warming  $\times$  tree species interaction ( $F_{1,13} = 5.77$ ,  $P = 0.032$ ) revealed that this effect mainly applied to *Pinus* trees ( $42 \pm 2\%$  in warmed plots compared to  $34 \pm 1\%$  in unwarmed plots; Fig. 5). Overall, *Pinus* trees had a slightly greater RMF ( $38 \pm 2\%$ ) compared to *Larix* trees ( $33 \pm 5\%$ ;  $F_{1,13} = 11.40$ ,  $P = 0.005$ ; Fig. 5). Trees that grew under elevated CO<sub>2</sub> did not differ significantly in RMF from those exposed to ambient CO<sub>2</sub>, and there were no significant interactive effects involving CO<sub>2</sub> level. Biomass relationships can change with increasing tree size and may indirectly lead to treatment effects (e.g. Gebauer *et al.*, 1996), but we found that biomass allocation ratios showed no relationship with absolute tree biomass or with tree height (data not shown).

#### Tree stem radial growth

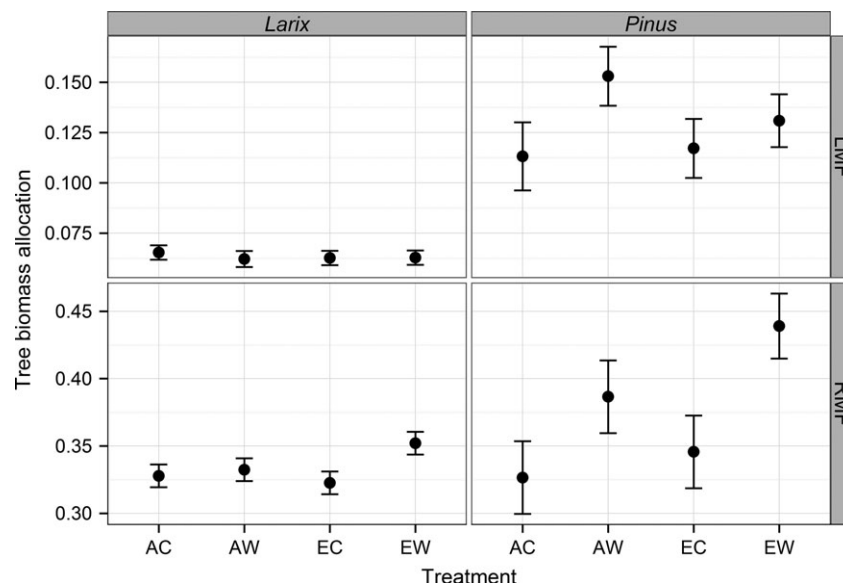
Increases in (log) tree stem basal area over time indicated species-specific positive RGR responses to the two experimental treatments. For the 2001–2009 period (analysis of CO<sub>2</sub> and tree species effects), trees exposed to elevated CO<sub>2</sub> did not show an overall significant difference in basal area compared to trees grown under ambient CO<sub>2</sub>, including at the beginning of the experiment (CO<sub>2</sub> effect on model intercept:  $F_{1,8} = 2.80$ ,  $P = 0.133$ ; Fig. 6a). However, elevated CO<sub>2</sub> had a positive effect on RGR (CO<sub>2</sub>  $\times$  year interaction:  $F_{1,329} = 6.34$ ,  $P = 0.012$ ), primarily for *Larix* trees (trend of CO<sub>2</sub>  $\times$  tree species  $\times$  year interaction:  $F_{1,329} = 2.35$ ,  $P = 0.127$ ; Fig. 6a). For the 2007–2012 period where we additionally analysed effects of soil warming and associated interactive effects, trees already exposed to elevated CO<sub>2</sub> for 6 years had a larger basal area than those growing under ambient CO<sub>2</sub> conditions, but this difference did not continue to increase (positive CO<sub>2</sub> effect on model intercept ( $F_{1,8} = 5.67$ ,  $P = 0.044$ ) but no CO<sub>2</sub>  $\times$  year interactive effect ( $F_{1,214} = 0.83$ ,  $P = 0.364$ ); Fig. 6b). *Pinus* trees in warmed plots had an increased RGR compared to those in unwarmed plots, whereas *Larix* RGR was not responsive to soil warming (marginally significant warming  $\times$  tree species  $\times$  year interaction;  $F_{1,214} = 3.80$ ,  $P = 0.053$ ; Fig. 6b). There were no interactive effects between CO<sub>2</sub> and soil warming treatments. RGR was lower for *Pinus* than for *Larix* (tree species  $\times$  year interaction) during both 2001–2009 ( $F_{1,329} = 48.38$ ,  $P < 0.001$ ) and 2007–2012 ( $F_{1,214} = 7.93$ ,  $P = 0.005$ ). However, *Pinus* trees had a greater stem basal area than *Larix* at the beginning of the experiment, and this initial difference persisted throughout the 2001–2009 period ( $F_{1,25} = 12.81$ ,  $P = 0.001$ ; Fig. 6a).

**Table 1** Tree total biomass and biomass of tree needles, woody above-ground parts and coarse roots. Mean values  $\pm 1$  SE intervals for each CO<sub>2</sub> level (ambient, elevated), soil warming treatment (unwarmed, warmed) and tree species (*Larix decidua*, *Pinus uncinata*) are listed ( $n = 4-5$ ). Values reflect statistical analyses by showing model estimates ( $\pm 1$  SE) for each compartment for a standardized tree stem basal area in the prewarming year 2006 (947 mm<sup>2</sup>, mean of all trees). Results from the linear mixed-effects models are shown to the right of biomass estimates.

Tree compartment	Tree species	CO <sub>2</sub> treatment	Warming treatment	-1 SE (g m <sup>-2</sup> )	Estimate (g m <sup>-2</sup> )	+1 SE (g m <sup>-2</sup> )	Fixed effect	df	F	P
Total	<i>Larix</i>	Ambient	Unwarmed	5521	6197	6956	BA2006	1, 12	144.06	<0.001
			Warmed	5493	6163	6916	Temp	1, 8	22.21	0.002
		Elevated	Unwarmed	4767	5761	6961	CO <sub>2</sub>	1, 8	0.00	0.981
			Warmed	5777	6977	8426	Tree	1, 12	4.47	0.056
	<i>Pinus</i>	Ambient	Unwarmed	3650	4097	4600	Temp $\times$ CO <sub>2</sub>	1, 8	0.18	0.683
			Warmed	6373	7149	8019	Temp $\times$ Tree	1, 12	29.75	<0.001
		Elevated	Unwarmed	2955	3651	4512	CO <sub>2</sub> $\times$ Tree	1, 12	1.24	0.288
			Warmed	4899	5919	7152	Temp $\times$ CO <sub>2</sub> $\times$ Tree	1, 12	0.65	0.436
			Unwarmed	294	379	489	BA2006	1, 12	27.07	0.000
			Warmed	231	297	382	Temp	1, 8	5.89	0.041
Needles	<i>Larix</i>	Ambient	Unwarmed	290	370	472	CO <sub>2</sub>	1, 8	0.00	0.979
			Warmed	339	431	548	Tree	1, 12	9.34	0.010
	<i>Pinus</i>	Ambient	Unwarmed	287	376	491	Temp $\times$ CO <sub>2</sub>	1, 8	0.00	0.995
			Warmed	824	1075	1404	Temp $\times$ Tree	1, 12	6.90	0.022
		Elevated	Unwarmed	333	445	595	CO <sub>2</sub> $\times$ Tree	1, 12	0.73	0.410
			Warmed	608	776	989	Temp $\times$ CO <sub>2</sub> $\times$ Tree	1, 12	2.02	0.181
Wood	<i>Larix</i>	Ambient	Unwarmed	3335	3739	4191	BA2006	1, 12	163.58	<0.001
			Warmed	3351	3755	4209	Temp	1, 8	4.69	0.062
		Elevated	Unwarmed	2978	3550	4233	CO <sub>2</sub>	1, 8	0.01	0.931
			Warmed	3419	4075	4856	Tree	1, 12	41.76	<0.001
	<i>Pinus</i>	Ambient	Unwarmed	2112	2369	2656	Temp $\times$ CO <sub>2</sub>	1, 8	0.03	0.879
			Warmed	2941	3296	3695	Temp $\times$ Tree	1, 12	15.79	0.002
		Elevated	Unwarmed	1637	1989	2417	CO <sub>2</sub> $\times$ Tree	1, 12	2.75	0.123
			Warmed	2122	2529	3016	Temp $\times$ CO <sub>2</sub> $\times$ Tree	1, 12	0.59	0.459
Coarse roots	<i>Larix</i>	Ambient	Unwarmed	1777	2028	2316	BA2006	1, 12	68.12	<0.001
			Warmed	1793	2046	2335	Temp	1, 8	20.99	0.002
		Elevated	Unwarmed	1531	1857	2254	CO <sub>2</sub>	1, 8	0.07	0.802
			Warmed	2025	2454	2975	Tree	1, 12	0.41	0.532
	<i>Pinus</i>	Ambient	Unwarmed	1144	1308	1496	Temp $\times$ CO <sub>2</sub>	1, 8	0.56	0.474
			Warmed	2429	2774	3168	Temp $\times$ Tree	1, 12	19.92	0.001
		Elevated	Unwarmed	1001	1247	1555	CO <sub>2</sub> $\times$ Tree	1, 12	0.39	0.546
			Warmed	2123	2575	3124	Temp $\times$ CO <sub>2</sub> $\times$ Tree	1, 12	0.63	0.444

**Table 2** Tree total biomass and biomass of tree needles, woody above-ground parts and coarse roots. Mean values  $\pm 1$  SE intervals for each CO<sub>2</sub> level (ambient, elevated) and tree species (*Larix decidua*, *Pinus uncinata*) are listed ( $n = 8$ –10). Values reflect statistical analyses by showing model estimates ( $\pm 1$  SE) for each compartment for a standardized tree stem basal area in the pre-CO<sub>2</sub> enrichment year 2000 (329 mm<sup>2</sup>, mean of all trees). Results from the linear mixed-effects models are shown to the right of biomass estimates.

Tree compartment	Tree species	CO <sub>2</sub> treatment	–1 SE (g)	Estimate (g)	+1 SE (g)	Fixed effect	df	F	P
Total	<i>Larix</i>	Ambient	4183	5031	6052	BA2000	1, 24	4.67	0.041
		Elevated	6076	7259	8673	CO <sub>2</sub>	1, 8	1.39	0.272
	<i>Pinus</i>	Ambient	4150	5034	6106	Tree	1, 24	0.68	0.418
		Elevated	4547	5520	6701	CO <sub>2</sub> $\times$ Tree	1, 24	1.24	0.277
Needles	<i>Larix</i>	Ambient	222	285	364	BA2000	1, 24	15.71	0.001
		Elevated	355	450	569	CO <sub>2</sub>	1, 8	0.99	0.349
	<i>Pinus</i>	Ambient	466	605	784	Tree	1, 24	4.89	0.037
		Elevated	523	679	882	CO <sub>2</sub> $\times$ Tree	1, 24	0.83	0.371
Wood	<i>Larix</i>	Ambient	2648	3164	3781	BA2000	1, 24	1.98	0.172
		Elevated	3741	4445	5283	CO <sub>2</sub>	1, 8	1.00	0.347
	<i>Pinus</i>	Ambient	2089	2514	3026	Tree	1, 24	6.87	0.015
		Elevated	2086	2513	3027	CO <sub>2</sub> $\times$ Tree	1, 24	2.41	0.133
Coarse roots	<i>Larix</i>	Ambient	1345	1632	1980	BA2000	1, 24	5.23	0.031
		Elevated	2010	2419	2910	CO <sub>2</sub>	1, 8	2.21	0.175
	<i>Pinus</i>	Ambient	1454	1782	2186	Tree	1, 24	0.00	0.978
		Elevated	1798	2207	2709	CO <sub>2</sub> $\times$ Tree	1, 24	0.38	0.542



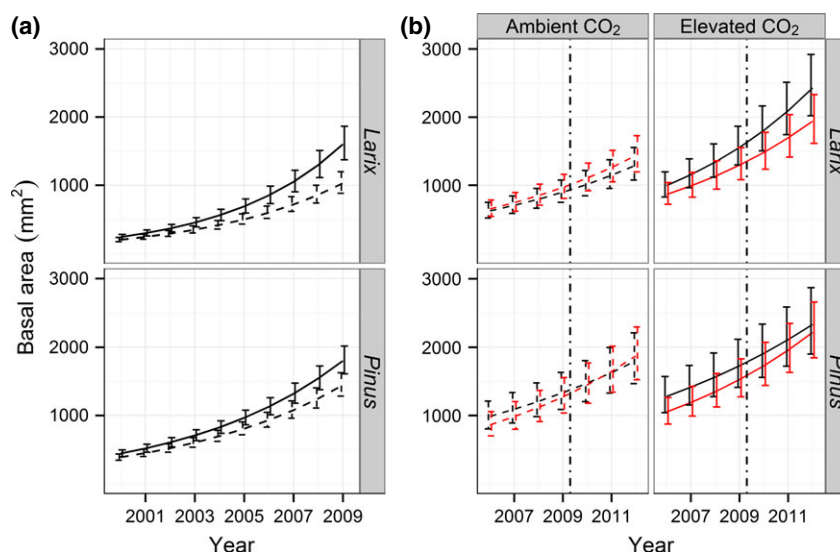
**Fig. 5** Leaf mass fraction (LMF, needle mass divided by total tree mass; top panels) and root mass fraction (RMF, coarse root mass divided by total tree mass; bottom panels) of trees. Mean values  $\pm 1$  SE for each combination of CO<sub>2</sub> level (A = ambient, E = elevated), soil warming treatment (C = unwarmed, W = warmed) and plot tree species (*Larix decidua*, *Pinus uncinata*) are shown ( $n = 4$ –5). Significant fixed effects ( $P < 0.05$ ) in statistical models included the following: tree species for LMF; warming, tree species and warming  $\times$  tree species interaction for RMF.

## Discussion

### Enhanced tree line vegetation biomass after six years of soil warming

Our 12-year field experiment showed that higher summer soil temperatures led to shifts in biomass production

among plant species and functional groups at the alpine tree line. Enhanced biomass of *Pinus* trees exposed to warmed soil (50% for total tree mass and over 100% for coarse root mass alone) was the most pronounced response to either soil warming or CO<sub>2</sub> enrichment, and this response drove an increase in total plant biomass on a land area basis. The roots of most trees extended a



**Fig. 6** Increases in tree stem basal area through time, with mean values  $\pm 1$  SE (estimated from statistical models) shown for individual treatment groups and years. *Larix decidua* is shown in top panels and *Pinus uncinata* is shown in bottom panels. In (a), basal area during the CO<sub>2</sub> enrichment period (end of 2000 (i.e. beginning of 2001) to end of 2009) is shown for trees exposed to ambient (dashed lines) and elevated (solid lines) CO<sub>2</sub> ( $n = 8$ – $10$ ). In (b), basal area during the soil warming period [end of 2006 (i.e. beginning of 2007) to harvest in summer 2012], including the 2007–2009 period when both CO<sub>2</sub> enrichment and soil warming were applied, is shown for trees treated with ambient (left panels) or elevated (right panels) CO<sub>2</sub> and to unwarmed (black lines) or warmed (red lines) soil ( $n = 4$ – $5$ ). The dashed vertical lines in (b) indicate the end of the CO<sub>2</sub> enrichment treatment (after the 2009 growing season). Significant fixed effects in statistical models included the following: CO<sub>2</sub>  $\times$  year interaction ( $P < 0.05$ ), tree species ( $P < 0.05$ ) and tree species  $\times$  year interaction ( $P < 0.05$ ) for the CO<sub>2</sub> enrichment period (a); CO<sub>2</sub> ( $P < 0.05$ ), tree species  $\times$  year interaction ( $P < 0.05$ ) and warming  $\times$  tree species  $\times$  year interaction ( $P < 0.10$ ) for the soil warming period (b).

considerable distance outside of the 1.1 m<sup>2</sup> plot area by the time of the harvest, and it is therefore feasible that the growth response of *Pinus* would have been even greater if the entire root system had been warmed to the same extent. Similar to our findings, *Picea abies* in a boreal forest showed an increase in cumulative stem volume production by 115% after 6 years of 5 °C soil warming in summer combined with advanced soil thawing and snowmelt during spring (Strömgren & Linder, 2002). We intentionally did not manipulate snowmelt dynamics in our experiment, and therefore, the observed growth enhancement of *Pinus* was due to warmer soils alone without a potential additional effect of an advanced or longer growing season. Our findings regarding *Pinus* support observational studies showing that tree lines have advanced to higher elevations in a number of regions during the past century as warming has occurred (Harsch *et al.*, 2009; Hagedorn *et al.*, 2014).

Our warming treatment only increased soil temperatures and air temperatures near the ground surface, yet *Pinus* growing in warmed plots had an increased above-ground biomass and a greater RGR of stem basal area (Table 1 and Fig. 6). In their soil warming study of boreal *Picea abies*, Strömgren & Linder (2002) attributed above-ground growth stimulation to increased nitrogen mineralization. Low nutrient availability is not widely

considered to be a main factor determining tree line position at a global scale (Körner, 2012). However, results from a few observational studies of plant and/or soil nitrogen along altitudinal gradients (Sullivan *et al.*, 2014; Thébault *et al.*, 2014) and N addition experiments (Sveinbjörnsson *et al.*, 1992; Susiluoto *et al.*, 2010) have suggested that low nitrogen availability plays a role in tree growth limitation for some species and tree line sites. In our study, mineral nitrogen contents in the soil increased during the initial 3 years of soil warming (Dawes *et al.*, 2011a), which might have contributed to overall enhanced growth of *Pinus*. However, *Pinus* trees might have shown a larger growth response, and *Larix* might have also had enhanced growth, if air at canopy height was additionally warmed. In support of this possibility, seedlings of *Larix decidua* and *Pinus uncinata* exposed to partial cooling of either roots or shoots in a climate chamber experiment were found to have considerably decreased growth relative to seedlings growing completely at warmer temperatures (Hoch, 2013). Nonetheless, soil temperature is typically more buffered than air temperature (Pregitzer *et al.*, 2000), and thus, soil temperatures in natural settings can remain below temperature thresholds critical for tree growth (ca. 6–8 °C; Alvarez-Uria & Körner, 2007) even when the air and especially leaf surfaces reach high daytime



temperatures. Therefore, soil temperature could have a particularly strong influence on tree growth at the tree line (Körner, 2012).

The lack of a response to soil warming by *Larix* in what is often assumed to be a low-temperature-limiting environment for tree growth was surprising. A study using an elevational transect near tree line in the Central Swiss Alps, spanning 900 m in elevation and representing a 4 °C temperature gradient, revealed decreasing tree-ring width of *Larix decidua* with increasing elevation despite a lack of genetic differentiation along the transect, suggesting that this species can respond to higher temperatures with increased growth (King *et al.*, 2013). At our tree line site, *Larix* needles and new shoots were frequently damaged by freezing events during the growing season (Rixen *et al.*, 2012), so it is possible that extreme air temperatures influence the growth of this species at tree line locations more than mean growing season temperatures. Additionally, in excavating the tree root systems, we found that *Larix* coarse roots grew deeper in the soil (ca. uppermost 20 cm) than *Pinus* coarse roots (ca. uppermost 10 cm), irrespective of treatment. Therefore, it is possible that a smaller proportion of *Larix* (coarse) roots experienced the warming treatment, which might have contributed to the lack of growth response of this species.

In the understorey layer, we found differences between functional groups in response to soil warming, with increased dwarf shrub biomass (driven by the +35% response of *V. myrtillus*) but reduced mass of herbaceous plants (−35%) and of mosses and lichens (−85%). Above-ground understorey vegetation made only a minor contribution to total plant biomass in this tree line site where trees were planted densely 40 years ago. However, trees are generally sparser at unplanted tree line locations in the same region as our study site (Hefti & Bühler, 1986), and changes in the community composition of low-stature vegetation could have large impacts on the structure of such ecosystems. Despite the relatively dense tree cover, we found no correlation between understorey biomass and variables representing tree size (height, total biomass, leaf mass; data not shown). Thus, we would expect similar responses of low-stature plants in 'natural' tree lines and in tundra ecosystems just above the tree line.

Michelsen *et al.* (2012) found that two decades of open-top chamber (OTC) warming of tree line heath vegetation in subarctic Sweden (Abisko) led to a doubling in shrub cover and a considerable reduction in moss and lichen cover. More broadly, a meta-analysis of the International Tundra Experiment (ITEX), with most sites in subarctic and arctic regions, indicated that warming by OTCs in locations with relatively high summer temperatures and/or wet soils (as at our site)

led to enhanced productivity of shrubs but a tendency of reduced abundance of graminoids, mosses and lichens (Elmendorf *et al.*, 2012). Therefore, our results suggest that understorey vegetation within temperate alpine tree line ecosystems will follow the general patterns predicted for arctic tree line and tundra sites under climate warming, despite differences in daylight distribution, terrain and species composition between these regions.

#### *Low and species-specific biomass responses to nine years of CO<sub>2</sub> enrichment*

In contrast to tree responses to soil warming, stem basal area measurements revealed that *Larix* (but not *Pinus*) trees exposed to elevated CO<sub>2</sub> had a greater RGR of basal area during the CO<sub>2</sub> enrichment period from 2001 to 2009 (Fig. 6a). However, *Larix* trees in plots treated with elevated CO<sub>2</sub> no longer had an enhanced RGR during the 2007–2012 period (including 3 years of enrichment; Fig. 6b) and did not have significantly increased biomass above or below ground in 2012, although there was a clear trend in this direction (Table 2). These results suggest both a decline in the growth response to CO<sub>2</sub> after the first 6 years of enrichment and a lack of carry-over effects in years after enrichment stopped. Our results of CO<sub>2</sub>-induced enhanced stem radial growth in *Larix* that declined during the last few years of enrichment confirm preliminary findings from ring width measured on microcores sampled when the experiment was ongoing (Handa *et al.*, 2006; Dawes *et al.*, 2011b). A declining growth response to elevated CO<sub>2</sub> over time has similarly been found in other studies of trees (e.g. Norby *et al.*, 2010). In our tree line experiment, this response pattern could not be attributed to photosynthetic downregulation (Streit *et al.*, 2014) and there was no evidence of increasing nitrogen limitation under elevated CO<sub>2</sub> (Dawes *et al.*, 2013). Instead, *Larix* trees that were growing under elevated CO<sub>2</sub> were more severely damaged by a freezing event during the early growing season in 2007 than those growing at ambient CO<sub>2</sub> (Rixen *et al.*, 2012). This effect might have contributed to smaller CO<sub>2</sub>-induced RGR gains in the final years of enrichment and ultimately reduced the CO<sub>2</sub> effect on biomass.

There were few indications of CO<sub>2</sub>-induced increases in understorey vegetation biomass, where no enhancement was observed for any functional group and *Vaccinium myrtillus* was the only individual dwarf shrub species to show a moderate positive response. We even observed negative biomass responses to CO<sub>2</sub> enrichment in forb above-ground mass in plots with *Larix* (ca. −50%) and in moss and lichen mass irrespective of the tree species present in the plot (ca. −80%).

Similarly, alpine glacier forefield species showed a 35% decrease in above-ground biomass when exposed to elevated CO<sub>2</sub> for 3 years (Inauen *et al.*, 2012). In our study, the decline in biomass of functional groups that made only a minor contribution to total plant biomass could indicate that elevated CO<sub>2</sub> led to increased competition within the understorey layer, particularly from *V. myrtillus*.

The unresponsiveness of total plant biomass to elevated CO<sub>2</sub> in a tree line ecosystem is consistent with findings from previous studies of alpine and montane ecosystems in the Alps (Körner *et al.*, 1997; Hättenschwiler & Körner, 1998; Inauen *et al.*, 2012). More broadly, there was no productivity response after several years of CO<sub>2</sub> enrichment by heath vegetation in a subarctic birch forest (Olsrud *et al.*, 2010), by mature deciduous temperate forest trees (Bader *et al.*, 2013) or by vegetation in a desert ecosystem (Newingham *et al.*, 2013). The long-term steady state of total biomass on a land area basis in all of these study systems might explain the lack of CO<sub>2</sub>-induced productivity enhancement (Körner, 2006).

#### *Lack of interaction between soil warming and CO<sub>2</sub> enrichment*

We found no interactive effects between CO<sub>2</sub> enrichment and soil warming, which implies that these two environmental changes will have independent effects on tree line vegetation. This result additionally suggests that direct low soil temperature limitations on growth processes did not control biomass responses to CO<sub>2</sub> enrichment. As observed for *Pinus* in our tree line study, *Picea abies* trees in a boreal forest showed no response to elevated CO<sub>2</sub>, even when air was warmed (Sigurdsson *et al.*, 2013). Conversely, similar to *Larix* in our study, boreal *Pinus sylvestris* showed a significant enhancement of tree-ring width under elevated CO<sub>2</sub> but no such change with warmer air and no interactive effects (Kilpelainen *et al.*, 2005). Results from these previous studies suggest that the lack of a positive CO<sub>2</sub> × warming interactive effect on tree growth in our experiment was not simply because soil was warmed instead of air, but it remains unclear whether such an interaction might have occurred if temperatures in the entire system were increased.

A meta-analysis including various ecosystem types indicated that combining experimental warming and CO<sub>2</sub> enrichment often yielded results that were less than additive, possibly because reduced root biomass resulting from increased nutrient availability in warmed soils can make plants more susceptible to periodic droughts (Dieleman *et al.*, 2012). It is therefore notable that in our experiment, where soil moisture

was permanently high, we did not observe any antagonistic effects of elevated CO<sub>2</sub> and soil warming. However, we acknowledge that potential interactive effects operating over longer timescales, such as altered nutrient availability, would not necessarily have been captured in our study. Understanding longer-term dynamics between CO<sub>2</sub> enrichment and increased temperatures remains as an important goal for global change research in various ecosystem types (Dieleman *et al.*, 2012).

#### *Shifts in biomass allocation under soil warming but not with CO<sub>2</sub> enrichment*

We found that soil warming led to an increased RMF, at least for *Pinus* trees. This finding was contrary to our hypothesis that trees growing in warmed plots would experience improved nitrogen availability and therefore invest less biomass into below-ground structures. Instead, our results suggest that warming directly ameliorated low-temperature limitations on (coarse) root growth and that this change had a stronger effect on tree growth allocation than altered nitrogen availability over 6 years of warming. On the typically steep slopes at the alpine tree line, these larger root systems could improve tree stability in the face of natural hazards such as snow avalanches, shallow landslides and rock-fall (Schönenberger, 1978; Mao *et al.*, 2014).

In contrast, we found a considerable (ca. 40%) decline in (bulk) fine root mass with soil warming, especially in plots with *Pinus* despite the increased RMF of this tree species. Fine roots are primarily responsible for nutrient uptake, and the warming treatment in our study led to increased mineral N in the soil (Dawes *et al.*, 2011a). Therefore, reduced investment into nutrient acquisition by vegetation as a whole in warmed plots could explain this negative response of fine root mass. Similarly, 6 years of soil warming in a temperate lowland forest had a negative impact on fine root standing crop, which the authors attributed to increased N mineralization associated with the warming treatment (Zhou *et al.*, 2011).

Exposure to elevated CO<sub>2</sub> did not lead to a shift in RMF in either tree species and there was no effect of CO<sub>2</sub> enrichment on fine root mass. This result is in contrast to reports of an increased RMF under elevated CO<sub>2</sub> for montane *Picea abies* saplings (Hättenschwiler & Körner, 1998) and for glacier forefield vegetation (Inauen *et al.*, 2012). A decrease in leaf N concentration under elevated CO<sub>2</sub> occurred in these two previous studies, and the shift in RMF was interpreted as a response to increased nutrient demand associated with photosynthetic enhancement. Although both *Larix* and *Pinus* exhibited sustained stimulation of photosynthesis

over the 9 years of enrichment in our study (Streit *et al.*, 2014), N concentrations in needles and mineral N contents in soils showed no sign of a decline in N availability due to elevated CO<sub>2</sub> (Dawes *et al.*, 2013). We therefore attribute the unresponsiveness in biomass allocation under CO<sub>2</sub> enrichment in our tree line system to minimal changes in N dynamics with the treatment. As in our study, there was no evidence of sustained enhanced below-ground allocation in a mature deciduous-mixed forest (based on fine roots; Bader *et al.*, 2013) or in a desert (Newingham *et al.*, 2013), both systems where elevated CO<sub>2</sub> did not reduce N availability (Schleppi *et al.*, 2012; Newingham *et al.*, 2013).

In conclusion, our results suggest that enhanced productivity at the alpine tree line is likely to occur in the future, especially as a result of global warming. Further, individual species and functional groups at the tree line will probably respond differently to increasing temperatures and rising CO<sub>2</sub> concentrations, with *Pinus* more responsive to warmer soils, *Larix* more responsive to elevated CO<sub>2</sub> and a shift towards increasing dwarf shrub dominance in the understorey with both environmental changes. In addition, the lack of interactive effects between the two treatments suggests that increasing CO<sub>2</sub> concentrations and temperatures might largely have independent effects on tree line vegetation. Finally, our results indicate that changes in vegetation biomass allocation might occur with warmer soils, with larger tree root systems (at least of *Pinus*) but a decline in total fine root mass. As a whole, these changes in tree line vegetation productivity and community composition associated with ongoing shifts in environmental conditions are likely to have important consequences for the structure of these high-elevation ecosystems and the ecosystem services they provide.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Climatic conditions and effects of the soil heating treatment on soil temperature and moisture during each of the 6 years of treatment.

**Table S2.** Plant biomass per unit of land area, including total biomass and contributions from understorey above-ground parts and fine roots, for each combination of CO<sub>2</sub> level, soil warming treatment and plot tree species (raw values).

**Table S3.** Tree biomass (total, needles, above-ground wood and coarse roots) for each combination of CO<sub>2</sub> level, soil warming treatment and plot tree species (raw values).

**Figure S1.** Mass of fine roots separated into <0.5 mm and 0.5–2 mm diameter size classes for each combination of CO<sub>2</sub> level, soil warming treatment and plot tree species.